Fabrizio Bruschi *Editor*

Helminth Infections and their Impact on Global Public Health

Second Edition

Springer

Helminth Infections and their Impact on Global Public Health

Fabrizio Bruschi
Editor

Helminth Infections and their Impact on Global Public Health

Second Edition

Springer

Editor
Fabrizio Bruschi
Department of Translational Research, N.T.M.S.
Università di Pisa, Medical School
Pisa, Italy

ISBN 978-3-031-00302-8 ISBN 978-3-031-00303-5 (eBook)
https://doi.org/10.1007/978-3-031-00303-5

1st edition: © Springer-Verlag Wien 2014

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Foreword

This new edition of *Helminth Infections and their Impact on Global Public Health* is a welcome contribution to the canon of biomedical literature on the world's neglected tropical diseases. Written by my friend and colleague, Professor Fabrizio Bruschi, it is not lost on me that as a professor from the University of Pisa he continues a 400-year-old legacy, possibly the oldest in medical parasitology. In the 1600s, Francesco Redi armed with a doctoral degree from the same university began conducting studies on *Ascaris* nematodes and *Fasciola* trematodes. Redi demonstrated how parasitic worms were different from free-living earthworms, ultimately becoming one of the fathers of medical parasitology. He also conducted fundamental studies that refuted theories of spontaneous generation and blazed a path for modern experimental biology.

Now, centuries later, we have a far better understanding of the global health impact of parasitic helminths. This includes the latest information from the Global Burden of Disease from 2019 (GBD 2019) confirming how helminths rank among the leading pathogens in terms of human disease prevalence. For example, more than 900 million people in low- and middle-income countries are infected with the three major soil-transmitted helminths, including *Ascaris* worms but also hookworms and *Trichuris* whipworms (Loukas et al. 2021). In addition, strongyloidiasis is also a common and serious intestinal helminth infection, while almost 20% of the world's population have been exposed to *Toxocara* eggs through zoonotic transmission (dogs and cats) (Rostami et al. 2019). Lymphatic filariasis and onchocerciasis are major insect-transmitted nematode infections affecting almost 100 million people in Africa or Southeast Asia. Another 200 million people live with trematode infections, led by schistosomiasis and food-borne trematodiases (including fascioliasis), while millions also suffer from larval cestode infections, including cysticercosis and echinococcosis.

Together, human helminth infections comprise a huge burden of disease, in addition to exerting a tremendous economic toll that traps people in an endless cycle of poverty. Regarding the former, helminth infections rarely kill, but they exert devastating effects on child development, woman's reproductive health, and adult productivity. We are only beginning to understand the full impact of helminths in terms of illnesses that disproportionately affecting girls and women. One helminthiasis of increasing global concern is female genital schistosomiasis causing pain, bleeding, and social stigma in up to 40 million adolescent and young adult women on the African continent (Hotez et al. 2019). Yet another aspect is how helminths interact with other infections. For example, the same female genital schistosomiasis increases the risks of women acquiring HIV/AIDS (Hotez et al. 2019), while the anemias of hookworm and schistosomiasis may be additive to malaria anemia to cause profound hemoglobin deficiencies in some populations, including pregnant women (Ness et al. 2020). Through such mechanisms, helminth infections affect economic development. In so doing helminth infections reinforce poverty. Indeed, it might be argued that helminth infections represent a major reason why the 750 million people who live below the World Bank poverty level cannot hope to escape their economic fate unless they are treated (Kang et al. 2018).

Some good news is that mass drug treatment programs are underway for many populations living with helminth infections in Africa, Southeast Asia, and Central and South America (Molyneux et al. 2021). Less certain is how the COVID-19 pandemic, now in its third year will affect these programs. Prior to the pandemic, it was estimated that more than one billion people receive preventive treatments as organized by the World Health Organization. This was quite gratifying to me personally because I worked with colleagues such as Profs David Molyneux, Alan Fenwick, Don Bundy, Lorenzo Savioli, and others to advocate to the US and UK Governments to provide critical support for these programs (Hotez et al. 2021). However, now these programs are under threat due to the social disruptions from COVID-19 and even the diversion of essential funds to combat COVID-19. We may face a grim reality that helminth infections and other neglected tropical diseases might re-emerge or once increase in their prevalence. Still other factors include climate change, urbanization, and other social determinants.

I am extremely enthusiastic about this new edition, it comes at a time when the world too easily forgets that just because COVID-19 emerged this does not mean the other conditions disappeared. Helminth infections are as serious and important has they have ever been, and Prof. Fabrizio's new book will become an important guidebook.

Peter Hotez, MD PhD, is a Professor of Pediatrics and Molecular Virology and Microbiology at the Baylor College of Medicine, where he is also the Dean of the National School of Tropical Medicine, Texas Children's Hospital Chair in Tropical Pediatrics, and a Co-Director of the Texas Children's Center for Vaccine Development.

Peter Hotez

References

Hotez PJ, Engels D, Gyapong M, Ducker C, Malecela MN (2019) Female genital schistosomiasis. N Engl J Med 381(26):2493–2495. doi: https://doi.org/10.1056/NEJMp1914709. PMID: 31881137

Hotez PJ, Fenwick A, Molyneux D (2021) The new COVID-19 poor and the neglected tropical diseases resurgence. Infect Dis Poverty 10(1):10. doi: https://doi.org/10.1186/s40249-020-00784-2. PMID: 33509283; PMCID: PMC7843239

Kang S, Damania A, Majid MF, Hotez PJ (2018) Extending the global worm index and its links to human development and child education. PLoS Negl Trop Dis 12(6):e0006322. doi: https://doi.org/10.1371/journal.pntd.0006322. PMID: 29927931; PMCID: PMC6013015

Loukas A, Maizels RM, Hotez PJ (2021) The yin and yang of human soil-transmitted helminth infections. Int J Parasitol 51(13–14):1243–1253. doi: https://doi.org/10.1016/j.ijpara.2021.11.001. Epub 2021 Nov 10. PMID: 34774540

Molyneux DH, Asamoa-Bah A, Fenwick A, Savioli L, Hotez P (2021) The history of the neglected tropical disease movement. Trans R Soc Trop Med Hyg 115(2):169–175. doi: https://doi.org/10.1093/trstmh/trab015. PMID: 33508096; PMCID: PMC7842098

Ness TE, Agrawal V, Bedard K, Ouellette L, Erickson TA, Hotez P, Weatherhead JE (2020) Maternal hookworm infection and its effects on maternal health: a systematic review and meta-analysis. Am J Trop Med Hyg 103(5):1958–1968. doi: https://doi.org/10.4269/ajtmh.20-0503. PMID: 32840198; PMCID: PMC7646767

Rostami A, Riahi SM, Holland CV, Taghipour A, Khalili-Fomeshi M, Fakhri Y, Omrani VF, Hotez PJ, Gasser RB (2019) Seroprevalence estimates for toxocariasis in people worldwide: a systematic review and meta-analysis. PLoS Negl Trop Dis 13(12):e0007809. doi: https://doi.org/10.1371/journal.pntd.0007809. PMID: 31856156; PMCID: PMC6922318

Preface

The Global Burden of Disease from 2019 confirms the impact of helminths on the public health at a global level. Almost a billion people live harboring helminths in their intestine. A good news is represented by the almost complete eradication at a global level of *Dracunculus medinensis*, the Guinea worm.

Geohelminths (*Ascaris*, hookworms, and *Trichuris* whipworms) represent the most globally spread parasites, accounting for more than 1.5 billion infections (a fourth of the entire world's human population), and probably are more frequent than any other infectious agent, including bacteria and viruses. Waiting for valuable vaccines, the only way to control such infections relies on ameliorating hygiene conditions and on preventive chemotherapy (since 2010, some 3.3 billion treatments were delivered through schools).

As regards another helminthic disease such as schistosomiasis, the Global Burden of Disease Study 2016 estimated its global burden at 2.5 million disability-adjusted life years.

After the success of the first edition of the book, published in 2014, with more than 19,000 downloads to date, here it is the second edition which will update the previous one.

Unfortunately, the epidemiological situation since then is not significantly changed all over the world.

Helminth infections such as those soil transmitted, schistosomiasis, foodborne trematodiases, or onchocerciasis, and or filariasis, dracunculiasis, echinococcosis, taeniasis, and cysticercosis are included in the Neglected Tropical Disease list for which a new roadmap 2021–30 was launched last year with the aim to cover the last mile to achieve the control of such diseases.

In this new edition, the chapters about most of the parasites covered in the first one were updated by the previous authors, but of course, it was not possible to include all the helminths, it is the case of pinworm, *Angiostrongylus*, *Capillaria*, *Dicrocoelium*, *Diphyllobothrium*, *Gnathostoma*, *Hymenolepis, Paragonimus* just to mention the most important.

The book deals with two general chapters, one on the systematics and biology of helminths and the other on paleoparasitology of helminth infections which shows

how the mankind has encountered these pathogens at least 10,500 years ago when the domestication of household animals began.

Then, chapters of this new edition are specifically devoted to the most relevant helminths which affect the human population such as *Schistosoma*, *Fasciola*, *Opisthorchis*, among trematodes, *Echinococcus* and *Taenia* spp., among cestodes, and soil-transmitted helminths, *Trichinella*, *Toxocara*, *Anisakis*, *Strongyloides*, lymphatic and tissue as well as zoonotic filariae, among nematodes.

Nevertheless, two important contributions add significant informations: one is an overview of how the helminths have tailored the host immune system and its response against themselves, and not only, which was given by the renowned scientist Prof. R. Maizels. This chapter is very useful to understand in a general way how the host can face the helminth infections, giving the keys for a better comprehension of the immunology section in the chapters devoted to each parasite.

The other important new contribution is that by Bundy and colleagues who focused on the economical impact of helminth infections and over all that of mass deworming in endemic areas.

Finally, even in this new edition, a chapter on the possible exploitation of helminth-derived molecules for the possible treatment of human immune-mediated diseases is included.

Helminths not only represent a public health problem but they also undermine the future of next generations in endemic areas, then the control of such infections is strategic for the development of these geographic regions and the increase in the knowledge of the infections and diseases caused by them represent an important contribution to help the disadvantaged populations living in those areas.

Pisa, Italy — Fabrizio Bruschi
28 January 2022

Contents

Editor and Contributors

About the Editor

Fabrizio Bruschi is a Medical Doctor graduated at the Università di Pisa, Pisa, Italy, Hematology Specialist at the same University. He obtained the Certificat d'Etudes Superieures in Immunologie et Pathologie Parasitaires at the Université Pierre et Marie Curie, Paris, France. Dr. Bruschi is a Professor of Parasitology and Parasitic Diseases in the School of Medicine of Università di Pisa. He is a corresponding member of the National School of Tropical Medicine at Baylor's College, Houston, Texas, an author or co-author of over 130 publications, and an Academic Editor of journals and books for Elsevier, Springer, and Bentham Science Publishers.

Contributors

Claudio Bandi Department of Veterinary Sciences and Public Health, Università degli Studi di Milano, Milan, Italy
Department of Veterinary Science, Università degli Studi di Parma, Parma, Italy

M. D. Bargues Departamento de Parasitologia, Facultad de Farmacia, Universidad de Valencia, Burjassot, Valencia, Spain

E. Brunetti Infectious Diseases and Immunology Unit, WHO Collaborating Centre on Clinical Management of Cystic Echinococcosis, IRCCS San Matteo Hospital Foundation, University of Pavia, Pavia, Italy

Fabrizio Bruschi Department of Experimental Pathology, Medical Biotechnologies, Infectivology and Epidemiology, Università di Pisa, Medical School, Pisa, Italy
Department of Translational Research, N.T.M.S., Università di Pisa, Medical School, Pisa, Italy

Donald A. P. Bundy London School of Hygiene and Tropical Medicine, London, UK

Suzy J. Campbell SCI Foundation, London, UK

Serena Cavallero Department of Public Health and Infectious Diseases, Section of Parasitology, "Sapienza—University of Rome", Rome, Italy

Goylette F. Chami Nuffield Department of Population Health, University of Oxford, Oxford, UK

Kevin Croke Harvard T.H. Chan School of Public Health, Boston, MA, USA

Stefano D'Amelio Department of Public Health and Infectious Diseases, Section of Parasitology, "Sapienza—University of Rome", Rome, Italy

Jillian T. Detwiler Department of Biological Sciences, Faculty of Science, University of Manitoba, Winnipeg, MB, Canada

Jean Dupouy-Camet Medical Faculty, University of Paris, Paris, France

Rashika El Ridi Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt

Sara Epis Department of Veterinary Sciences and Public Health, Università degli Studi di Milano, Milan, Italy
Department of Veterinary Science, Università degli Studi di Parma, Parma, Italy

Elizabeth Ferrer Instituto de Investigaciones Biomédicas "Dr. Francisco J. Triana Alonso" (BIOMED) and Departamento de Parasitología, Facultad de Ciencias de la Salud, Universidad de Carabobo Sede Aragua, Maracay, Venezuela

Gino Fornaciari School of Specialization in Archaeological Heritage, Department of Civilisations and Forms of Knowledge, University of Pisa, Pisa, Italy

Albis Francesco Gabrielli Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland

Raffaele Gaeta Division of Paleopathology, Department of Translational Research and of New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy

Claudio Genchi Department of Veterinary Sciences and Public Health, Università degli Studi di Milano, Milan, Italy
Department of Veterinary Science, Università degli Studi di Parma, Parma, Italy

Clare M. Hamilton Moredun Research Institute, Edinburgh, UK

William Harnett Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK

Eric P. Hoberg Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI, USA
Department of Biology and Museum of Southwestern Biology, University of New Mexico, Albuquerque, NM, USA

Achim Hoerauf Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany

Celia V. Holland Department of Zoology, Trinity College Dublin, Dublin, Ireland

Marc P. Hübner Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany

Masataka Korenaga Department of Parasitology, Kochi Medical School, Kochi University, Kochi, Japan
Faculty of Health Sciences, Kochi Gakuen University, Kochi, Japan

Laura Kramer Department of Veterinary Sciences and Public Health, Università degli Studi di Milano, Milan, Italy
Department of Veterinary Science, Università degli Studi di Parma, Parma, Italy

Laura E. Layland Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany

Rick M. Maizels Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK

T. Manciulli WHO Collaborating Centre on Clinical Management of Cystic Echinococcosis, Pavia, Italy
Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy
Largo Giovanni Alessandro Brambilla, Florence, Italy

S. Mas-Coma Departamento de Parasitologia, Facultad de Farmacia, Universidad de Valencia, Burjassot, Valencia, Spain

Simonetta Mattiucci Department of Public Health and Infectious Diseases, Section of Parasitology, "Sapienza—University of Rome", Rome, Italy

Antonio Montresor Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland

Maria Angeles Gomez Morales Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy

Ahmad Othman Medical Parasitology Department, Faculty of Medicine, Tanta University, Tanta, Egypt

Marialetizia Palomba Department of Public Health and Infectious Diseases, Section of Parasitology, "Sapienza—University of Rome", Rome, Italy

María Jesús Perteguer Instituto de Salud Carlos III, Centro Nacional de Microbiología, Servicio de Parasitología, Majadahonda, Madrid, Spain

Elena Pinelli National Institute of Public Health and Environment, Bilthoven, Netherlands

Edoardo Pozio Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy

Justyna Rzepecka Concept Life Sciences, Edinburgh, UK

Linda Schultz London School of Hygiene and Tropical Medicine, London, UK

F. Tamarozzi Department of Infectious-Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Verona, Italy

Hugo C. Turner Imperial College London, London, UK

M. A. Valero Departamento de Parasitologia, Facultad de Farmacia, Universidad de Valencia, Burjassot, Valencia, Spain

D. A. Vuitton French National Reference Centre for Echinococcoses, University Bourgogne Franche-Comté and University Hospital, Besançon, France

Ayako Yoshida Centre for Animal Disease Control, University of Miyazaki, Miyazaki, Japan

Dante S. Zarlenga Animal Parasitic Diseases Lab, Agricultural Research Service, Beltsville, MD, USA

Chapter 1
Epidemiology and Economics of Deworming

Donald A. P. Bundy, Suzy J. Campbell, Goylette F. Chami, Kevin Croke, Linda Schultz, and Hugo C. Turner

Abstract Global access to deworming is one of the public health success stories of the twenty-first century and was the key catalyst for creating the neglected tropical disease (NTD) agenda. Human worm infections appear to have been with us since the domestication of household animals, some 10,500 years ago, and putative treatments are known from the earliest pharmacopoeias, but it has only been in the last 100 years that we have sought a public health solution and only in the last 5 years that real success at scale has been achieved. This is a success that depends on donated drugs and targeted treatment campaigns outside of the traditional health system. In this chapter, we explore the scientific foundations for this success and explore what this implies for the future management of soil-transmitted helminths (STHs) by health systems. This chapter describes the evolution of public health approaches to reduce the prevalence and morbidity of STH and the evidence of impact of mass drug administration on their target populations, and provides context for the debate that has surrounded these results. This chapter also details the costs of delivering these interventions as well as how future delivery approaches can align with Universal Health Care objectives.

Authors are listed in alphabetical order, with equal contributions from each author.

D. A. P. Bundy · L. Schultz (✉)
London School of Hygiene and Tropical Medicine, London, UK
e-mail: Donald.Bundy@lshtm.ac.uk; linda.schultz@lshtm.ac.uk

S. J. Campbell
SCI Foundation, London, UK

G. F. Chami
Nuffield Department of Population Health, University of Oxford, Oxford, UK

K. Croke
Harvard T.H. Chan School of Public Health, Boston, MA, USA

H. C. Turner
Imperial College London, London, UK

F. Bruschi (ed.), *Helminth Infections and their Impact on Global Public Health*,
https://doi.org/10.1007/978-3-031-00303-5_1

1.1 Introduction

Global access to deworming is one of the public health success stories of the twenty-first century and was the key catalyst for creating the neglected tropical disease (NTD) agenda. Human worm infections appear to have been with us since the domestication of household animals, some 10,500 years ago, and putative treatments are known from the earliest pharmacopoeias, but it has only been in the last 100 years that we have sought a public health solution and only in the last 5 years that real success at scale has been achieved. This is a success that depends on donated drugs and targeted treatment campaigns outside of the traditional health system. In this chapter, we explore the scientific foundations for this success and explore what this implies for the future management of soil-transmitted helminths (STHs) by health systems.

1.1.1 The Evolution of Deworming Programmes

Figure 1.2 tracks the evolution of deworming programmes from their start as large-scale projects before the 1970s to the global movement and national programmes that are the norm today.

The earliest public health programmes that would be recognizable to us today as community deworming were the Rockefeller hookworm campaigns of the early twentieth century, in the Southern United States and certain endemic countries (Ettling 1981; Stiles and Garrison 1906). Despite the toxicity and low efficacy of the drugs then available, recent re-analysis shows that these programmes may have been successful in supporting human development (see Bleakley (2007) for an analysis which finds positive long run effects and Roodman (2018) for a critical analysis), and they laid the conceptual foundation for much of what was to follow.

In tracking this process, there are three interrelated strands that developed together. Two of these strands were science-driven: one was the accumulation of evidence of the scale of health impact and the other the development of cost-efficient interventions, based on growing understanding of epidemiology, pharmaceuticals, and public health implementation science. The third strand depended upon the success of these two and was the slow and difficult evolution in public health policy.

1.1.1.1 The Importance of Scale

The scale of infection had long been recognized, but it took the global chaos of human movements during the Second World War to remind the public health community that worms were the most ubiquitous of chronic human infections, as memorably reported in “This Wormy World” (Stoll 1947). The ground-breaking work of Julia Walsh and Kenneth Warren (Walsh and Warren 1979) led to growing recognition that these

hundreds of millions of STH infections could add up to a huge public health burden even if each individual case was not a clinical priority. In the 1980s, the Rockefeller Foundation launched the "Great Neglected Diseases of Mankind Programme" and cited *Ascaris lumbricoides* infection as an exemplar of this principle (Warren et al. 1993). In 1993, the World Bank's World Development Report "Investing in Health" (World Bank 1993) explored the economic argument for investing in diseases based on their scale, impact, and cost and presented the use of DALYs (disability-adjusted life years) as a way of quantifying the impacts of disease beyond their immediate clinical consequences. Soil-transmitted helminths were cited as a specific example where the long-term developmental impacts, such as on life time educational achievement and future earnings, were potentially a greater contribution to the disease burden than short-term clinical disease (Warren et al. 1993).

1.1.1.2 Developing more Efficient Programmes

When it became clear that worms were a significant public health problem, there was greater incentive to find solutions. In the 1970s, several drugs developed originally for veterinary use were licenced for use in humans; worryingly, these remain the most commonly used treatments today. Almost at the same time, parasite epidemiology took an extraordinary leap forward by applying the principles of population dynamics, originally developed for whole organism ecology, to parasite populations (Anderson and May 1982). This showed that STH infections were regulated by the numbers of adult worms present and thus that reducing infection intensity would simultaneously reduce both morbidity and transmission (see Chap. 11 by Gabrielli (2022) in this volume for more details of the biology and epidemiology of STH). Since infection intensity was often age-related (Fig. 1.1), treating the most heavily infected age groups of the population should then disproportionately reduce infection and disease in the population as a whole. Applying this theory to practical programme design showed that targeting of treatment at school-aged children, who had the most intense infections, resulted in reduced infection in untreated adults (Bundy 1990) and later led to recognition of the economic corollary that there were essentially free additional benefits, or "externalities", to be gained from well-targeted interventions (Miguel and Kremer 2004). In parallel with the focus on targeting, there was a surge of implementation science that focused on minimizing treatment delivery costs. Since the cost of individual diagnosis was some ten times the cost of treatment, the policy game changer was the acceptance that once a community had been recognized as requiring treatment, this could be rolled out as mass drug administration (MDA) without further individual screening (Bundy 1990).

1.1.1.3 Towards a New Global Policy on Deworming

Global policy change tracked this convergence between recognizing the problem and finding a cost-effective solution. Interest in controlling helminth infections

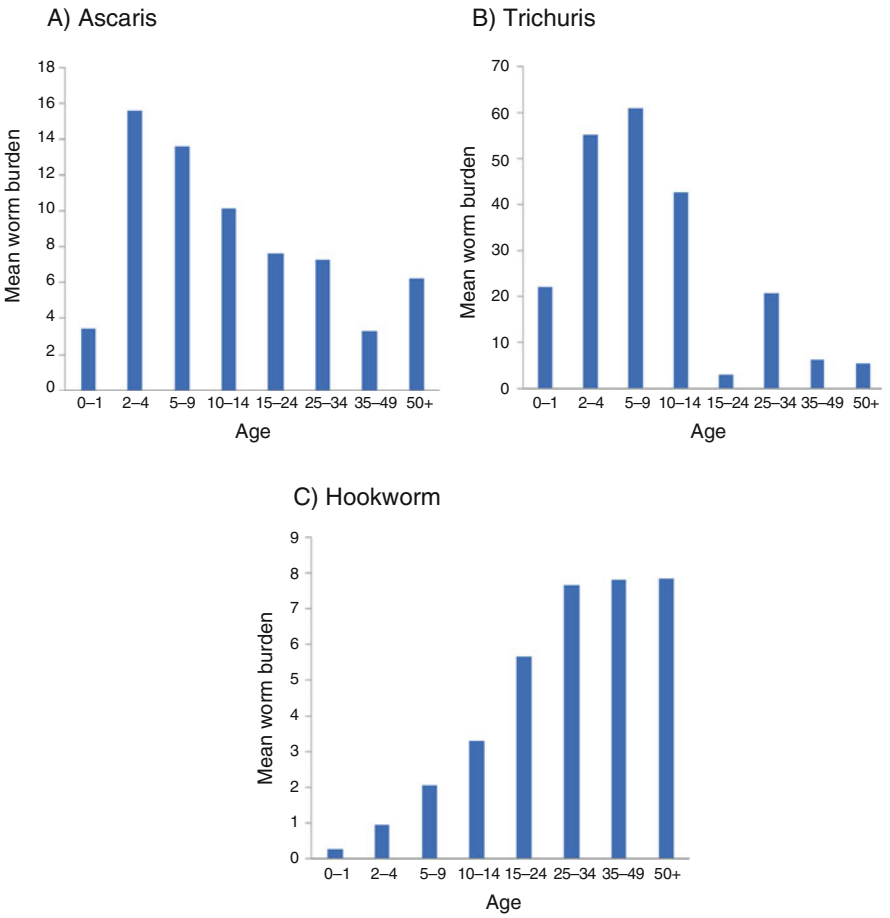

Fig. 1.1 Cross-sectional surveys of the mean intensity of infection in different age groupings for *Ascaris lumbricoides* (**a**), *Trichuris trichiura* (**b**), and hookworm (**c**) based on worm expulsion studies. Source: Adapted from Anderson et al. (2015)

surged after the decision by Merck in 1987 to donate the newly discovered veterinary drug ivermectin for use in controlling river blindness and pledging "as much as necessary for as long as necessary" (Sturchio 2001). This set a precedent for other major donations: from GlaxoSmithKline in 1997 (2012 for STH), Johnson & Johnson in 2006, and Merck KGaA in 2007 (Table 1.1). The value of school-based MDA was recognized by the education community as part of the FRESH (Focusing Resources on Effective School Health) framework launched at the World Education Forum in Dakar, 2000 (Bundy et al. 2006), which was followed a year later by a World Health Organization (WHO) declaration in support of school-based MDA (WHA 2011).

Table 1.1 Medicines donated by pharmaceutical companies to WHO for the control of preventive chemotherapy (PC-)NTDs

Company	Drug donated	Susceptible disease	Commitment
Merck & co.	Ivermectin (Mectizan)	Onchocerciasis and lymphatic filariasis	Since 1987: unlimited supply until onchocerciasis is eliminated
			Since 1997: unlimited supply until lymphatic filariasis is eliminated from Yemen and African continent in regions where lymphatic filariasis is co-endemic with onchocerciasis
			2018–2025: Up to 100 million treatments to eliminate lymphatic filariasis using WHO-recommended triple-therapy MDA in regions that are not co-endemic for onchocerciasis
GlaxoSmithKline (GSK)	Albendazole	Lymphatic filariasis	Since 1997: up to 600 million tablets annually until lymphatic filariasis is eliminated as a public health problem
		STH	2012–2020: 400 million tablets annually for the treatment of STH in school-aged children
Pfizer	Azithromycin	Trachoma	Since 1998–2025: unlimited quantity to eliminate trachoma as a public health problem
Johnson & Johnson	Mebendazole	STH	2006–2025: initially 50 million annual donation, revised to 200 million annual donation in 2010, for the treatment of STH in school-aged children. From 2020, Johnson & Johnson is donating its chewable paediatric formulation, which can be safely used by preschool-aged children
Merck KGaA	Praziquantel	Schistosomiasis	Since 2007: initially up to 200 million tablets to treat schistosomiasis in school-aged children; commitment revised to an unlimited donation in 2017, until schistosomiasis is eliminated as a public health problem

Source: Adapted from Bradley et al. (2021) with additional information from Johnson and Johnson (2019)

At the beginning of the 2000s, the epidemiology, economics, and policy components were in place for the rollout of mass treatment programmes to control STH, lymphatic filariasis, and onchocerciasis, providing a model approach to addressing some of the most common infections of low-resourced communities. Activists looked back to the analogous "*Great Neglected Diseases*" programme launched more than 20 years previously and adopted the new "brand" of *Neglected Tropical*

Diseases (WHO 2004, 2006b; Molyneux et al. 2005; Hotez et al. 2006a, 2006b). A new NTD department was opened at WHO in 2005.

In 2012, a coalition of development partners made a global call, "the London Declaration", to support the WHO NTD 2012–2020 Road Map (WHO 2012) and to continue and expand access to drug donations. Among other pledges, 13 pharmaceutical companies collectively committed to donate 14 billion treatments to control and eliminate 10 NTDs, including the 5 preventive chemotherapy (PC-)NTDs, over a 10-year period (Table 1.1) (Uniting to Combat NTDs 2012). This $18 billion pharmaceutical donation circumvented the scarcity of domestic resources to secure sufficient quality-assured drugs to achieve NTD targets. The London Declaration attracted additional investors and stakeholders to strengthen country capacity to deliver drugs at scale (Espinal et al. 2021). By 2016, more than a billion treatments were being delivered every year, the majority by school-based MDA for STH (Uniting to Combat NTDs 2016), and in the following year, the *Guinness World Records* recognized the largest drug donation in a single day, with 200 million doses arriving to distribution facilities across six countries (Guinness World Records 2017). In 2021, a second NTD Road Map was launched, charting a path to 2030 (WHO 2020b).

To summarize, Fig. 1.2 shows that it took a combination of new approaches to launch the ultimately successful movement towards making deworming universally accessible. The first 30 years (1970–2000) were largely focused on demonstrating the previously unrecognized development burden and on creating a control approach, based on the "new" safer anthelmintics, which was also good value for money because it was focused only on a subsection of the population, did not require individual diagnosis, and was delivered through the existing education system and subsidized by donated treatment. 2000 was the watershed moment when there was formal normative acceptance by both the education and health sectors. It then took another 10 years to reach the status of a global movement (perhaps delayed by the "worm wars"; see below), and it is only in the last 5 years that national programmes at scale have become the new normal. The next section considers how these policy changes were rolled out in practice by countries and development partners.

1.2 Global Evidence of Deworming

School-aged children are the cohort with the highest infection burden for STH, and WHO set a global target of 75% treatment coverage of school-aged children (WHO 2012). Focusing treatment on this cohort is anticipated to reduce the greatest burden of attributable morbidity while also holding potential anthelmintic resistance in check (Campbell et al. 2018). Utilizing the existing school infrastructure to deliver periodic MDA for STH and SCH is efficient and cost-effective as it reaches 575 million school-aged children in low-income countries (Bundy 2011) and serves a population that often has little contact with the formal health system (Bundy et al. 2017). Moreover, treatment with anthelmintics offers educational and nutritional

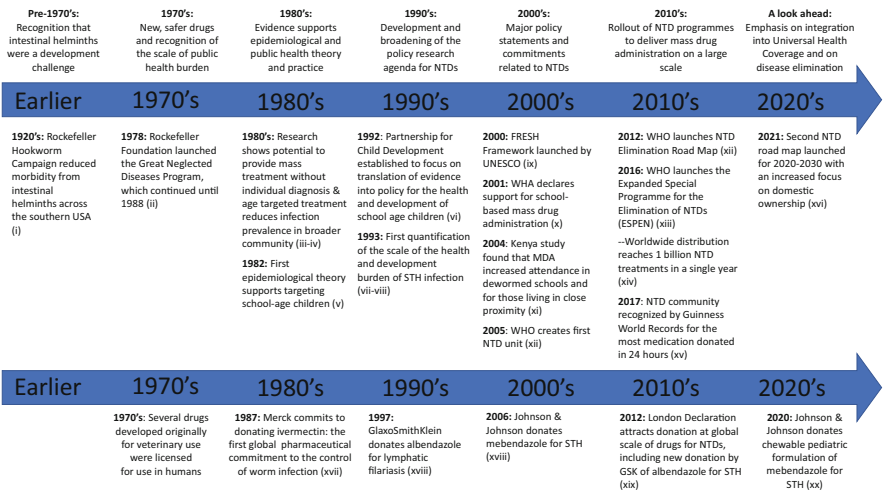

Fig. 1.2 Timeline of milestones related to the epidemiological understanding of helminth infections, relevant policy measures, and pharmaceutical donations. Source: Ettling 1981, Keating (2014), Bundy (1990), Bundy et al. (1990), Anderson and May (1982), Partnership for Child Development https://www.imperial.ac.uk/partnership-for-child-development/about-us/, Warren et al. (1993), World Bank (1993), Bundy et al. (2006), WHA (2011), Miguel and Kremer (2004), WHO (2012), WHO Africa (2015), Uniting to Combat NTDs (2016), Guinness World Records (2017), WHO (2020), Sturchio (2001), Bradley et al. (2021), Uniting to Combat NTDs (2012), Johnson and Johnson (2019)

benefits precisely at the time when children are physically and cognitively maturing (Miguel and Kremer 2004; Baird et al. 2016). Moderate- to heavy-intensity (MHII) STH infections are associated with malnutrition, lethargy, stunting, and impaired physical and cognitive growth (Crompton and Whitehead 1993; Hall et al. 2008; Stoltzfus et al. 1997).

1.2.1 Coverage Achieved to Date

Since 2010, some 3.3 billion treatments have been delivered through schools for the control of STH infection (Montresor et al. 2020). There is some indication that the number of school-aged children living with STH infections was reduced by half between 2010 and 2015 (Bundy et al. 2018); however, the relative contributions of large-scale sustained deworming, improvements to mapping estimates and disease predictions, and socioeconomic development are not possible to elucidate. As of 2018, treatment coverage for STH exceeded 60% of school-aged children in endemic countries, with 28 (of 96) endemic countries reaching effective treatment coverage for 5 or more years ($\geq$75%) (Montresor et al. 2020). Of the 28 countries, Burkina Faso and Mali have since stopped MDA and are conducting regular surveillance to detect disease resurgence (Montresor et al. 2020).

Following the London Declaration, most endemic countries scaled up deworming programmes. Many conducted prevalence surveys to determine programmatic area (s) and endemicity level and develop a treatment strategy per WHO recommendations (WHO 2011). This baseline becomes useful for measuring resultant programmatic effectiveness. Although deworming drugs are coordinated by WHO free of charge to countries, extensive within-country distribution is required, necessitating budget and personnel commitments. Most countries use national to local "cascade" distribution; the "reverse cascade" is advantageous for monitoring and evaluation (M&E), to effectively transmit local numbers treated to national levels.

Performance tracking is essential; thus, effective M&E is integral to program success. Existing M&E guidance (WHO 2011), geared towards scaling up, prioritizes process and performance monitoring, including independent MDA monitoring and coverage evaluation surveys (WHO 2016) of participants' receipt of deworming tablets. Results guide programmatic improvements, ideally before the next MDA. While country programmes tend to plan monitoring processes, few establish performance evaluation at inception to determine, via reassessment, programmatic effectiveness in reducing disease severity after achieving five rounds of sustained, high-coverage MDA (WHO 2011). Reassessments are critically important for revising treatment frequency in accordance with WHO decision trees (WHO 2011) and, eventually, to determine whether elimination as a public health problem (EPHP) (WHO 2020b) has been achieved. Yet these surveys are expensive, technically complex (requiring epidemiological oversight), and rarely done without external assistance.

1.2.2 Looking Towards the Endpoint

Some programmes provide outstanding examples of success. Kenya's National School-Based Deworming Programme was established with extensive M&E, including pre- and post-MDA surveys and reassessment surveys, enabling assessment of yearly treatment impact and overall reductions in prevalence and intensity of infection (Mwandawiro et al. 2019). India's National Deworming Day, reaching over 226 million children (WHO n.d.), is the world's largest single-day public health campaign. With substantial government investment and political commitment, India is the exemplar of domestic contribution to deworming. Reassessments have enabled India to conceptualize a 5-year road map, further optimizing domestic deworming investment.

Preventive chemotherapy's oft-discussed limitation is its inability to prevent reinfection. However, it was never meant to. Preventive chemotherapy for STH was intended as sustained, regular drug provision, for the goal of controlling morbidity from MHII STH infection (WHO and WHO Expert Committee on the Control of Schistosomiasis 2002). It was always recommended that STH programs be accompanied by water, sanitation, and hygiene (WASH) and health education (WHA 2011) although these often receive inadequate NTD programme attention

(Campbell et al. 2016). Long-term, community-wide sanitation infrastructure and hygiene behaviours are believed necessary to sustain disease reductions in endemic settings (Anderson et al. 2014; Campbell et al. 2014).

There may also be important lessons to learn from countries that were among the first and most successful in achieving control. Japan and South Korea both made a very intentional effort to eliminate STH infections. They both used school-based selective drug administration, combined with regular mass screening, health education, night soil treatment, improved WASH infrastructure, and specific legislation, such as the Parasitosis Prevention Law in Japan (Hasegawa et al. 2020; Hong et al. 2006). In both cases, the decline in infection was accompanied by economic development and socioeconomic improvements. This is also true for Kenya and India and can only help reinforce the sustainability of the interventions. The economic trajectory of most countries where STHs are present today is also upwards, and the World Bank estimates that a third of low-income countries in Africa will be middle-income countries by 2030.

1.2.3 Elimination as a Public Health Problem or Interruption of Transmission?

Since 2012, public health policy has gradually shifted from morbidity control to EPHP, and more recently, some programmes express plans to achieve "interruption of transmission" (IOT). In 2030 targets, EPHP is defined as achieving $<2\%$ proportion of MHII STH infections (WHO 2020b). While this may represent achievement of IOT, this is as yet unproven; however, analyses suggest attainment is not uniformly possible (Brooker et al., 2015). If EPHP attainment becomes used to then stop preventive chemotherapy, there will need to be more direct evidence and consideration of other control aspects; otherwise, analyses indicate likely resurgence of STH if solely preventive chemotherapy to SAC is provided. Countries must be enabled for success; yet currently, major evidence shortfalls for IOT include metrics, diagnostic techniques, resources, country capacity, survey methodologies, and validation criteria. Interruption of transmission will likely require increased domestic and donor funding, intensified mapping (including methodologies and diagnostics), increased treatments to more cohorts, community-based augmentation of school-based platforms, WASH, lower administrative implementation units, analyses of undifferentiated infections, increased monitoring, tracking of MDA compliance, and monitoring of anthelmintic resistance. Current donations and resourcing do not extend to this. Pharmaceutical companies aim to reduce drug donations and focus on eliminating lymphatic filariasis, onchocerciasis, and trachoma (considered possible by preventive chemotherapy). Unless evidence-based, implementable prospects for IOT are forthcoming, there may be reduced donor interest in maintaining long-term STH preventive chemotherapy.

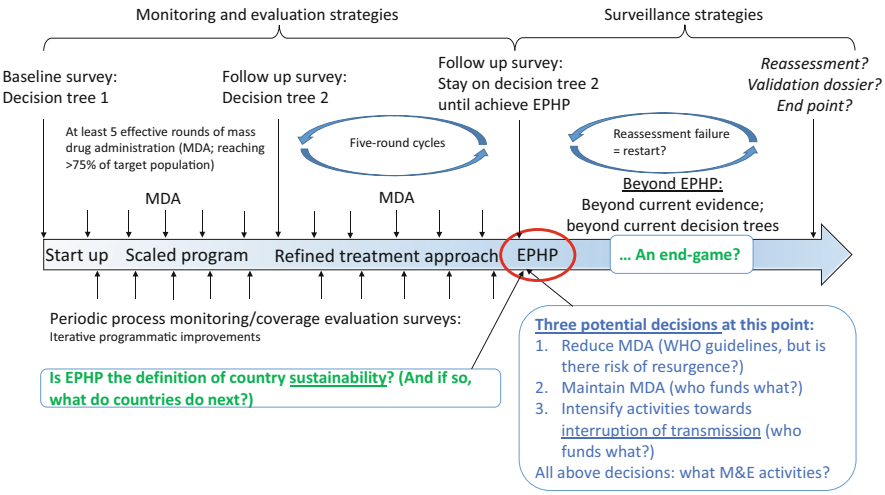

Fig. 1.3 The need for an evidence-informed monitoring and evaluation framework

Ground-breaking trials include the WASH Benefits randomized controlled trials (RCTs) of WASH and nutrition interventions on outcomes including STH in Kenya and Bangladesh (Luby et al. 2018; Null et al. 2018), the TUMIKIA RCT of community-wide versus school-based treatment in Kenya (Pullan et al. 2019) and the multi-country DeWorm3 RCT which will provide evidence towards feasibility of IOT using biannual MDA to all ages (Ásbjörnsdóttir et al. 2018). A programmatic effort to achieve IOT, using tailored strategies, is the Deworming Innovation Fund, aiming to achieve national IOT in Rwanda and Zimbabwe, county IOT in Kenya, and acceleration towards national IOT in Ethiopia (The END Fund 2020; CIFF 2020).

Looking forward, attention should shift to programmatic and technical requirements of country deworming programmes now that they are scaled up. There are now few STH-endemic landscapes without a control programme (some exceptions include conflict zones). Many countries have reached a "tipping point" of successive years of MDA; every country can anticipate needing at least one and, the majority, two reassessments before 2030. Crucially, robust guidance is needed regarding what countries do when they achieve EPHP, the basis of the 2030 targets (WHO 2020b). At that point, countries are at a decision-making nexus, with scarce evidence for every scenario. They can (1) reduce treatment frequency (per WHO decision trees (WHO 2011)), (2) retain treatment frequency, or (3) intensify activities (increasing treatment frequency and/or incorporating other activities, possibly towards IOT) (Fig. 1.2). Programmatic, technical, sustainability, and resource-based parameters for each decision vary dramatically. Thus, the need for evidence-informed, global M&E frameworks for STH has become acute (Fig. 1.3).

With increased focus on self-reliance and domestic financing towards 2030, the WHO supplemental Sustainability Framework for Action (WHO 2021) aims to

guide governments on embedding NTD programmes within national health strategies. In this context, it is worth noting that the India National Deworming Day programme is almost entirely supported from domestic funds and domestically produced anthelmintics. If the largest deworming programme in the world can be self-reliant, then there is hope for programmes everywhere.

In the next section, we consider the evidence that these programmes have had an impact on their target populations and discuss the debate that has surrounded these results.

1.3 Assessing the Impact: The "Worm Wars" and Beyond

The previous sections have described the long trajectory of deworming programmes and the growing global progress in scaling up these programmes, lowering STH burdens, and reducing associated morbidity. However, despite this progress, there has been some disagreement about the evidence base for health gains at population level from MDA for deworming. This section discusses these debates.

1.3.1 Reviewing the Statistical Evidence from Meta-Analyses

Perhaps the most important disagreement relates to how to aggregate and interpret over 40 years of randomized trials of MDA. Many individual MDA trials have been underpowered to detect meaningful changes in health and nutrition outcomes, so meta-analytic approaches are important tools. Nonetheless, implementation of meta-analysis and interpretation of findings on this topic remain contested. A series of Cochrane systematic reviews and meta-analyses, most recently in 2015 (Taylor-Robinson et al. 2015) and in 2019 (Taylor-Robinson et al. 2019), and a Campbell Collaboration review (Welch et al. 2016) found no average impact of MDA for deworming on nutritional and educational outcomes. By contrast, other meta-analyses have aggregated a larger set of studies and found significant effects, most notably for weight gain, which is the nutritional outcome most commonly measured in STH trials (Croke et al. 2017).

Early versions of these systematic reviews differed significantly in their point estimates of average effects of MDA (Taylor-Robinson et al. 2015; Croke et al. 2017). However, over time, and with scientific exchanges between the review teams, the reviews have begun to converge in the population of included studies and accordingly in the point estimates for key outcomes. For example, the most recent Cochrane review (Taylor-Robinson et al. 2019) estimates an average effect of MDA on weight of 0.11 kg (95% confidence interval (CI) −0.01, 0.24), while the Croke et al. (2017) review estimates an effect of 0.13 kg (95% CI 0.03, 0.24). Notably, these meta-analyses also agree in their finding that trials which dewormed children with confirmed worm infections produced large benefits. Taylor-Robinson et al.

(2019) do not separately analyse "test and treat" studies, but the 2015 Cochrane review (Welch et al. 2016) found significant gains in nutritional outcomes (e.g., weight, height, middle-upper arm circumference) from these studies.

Indeed, while the various systematic reviews continue to differ in inclusion/exclusion and data extraction decisions regarding individual studies, the convergence of point estimates, and the agreement that deworming treatment brings nutritional benefits to infected populations, suggests that the most pressing question about MDA relates to the cost-effectiveness and the spatial and temporal targeting of the intervention.

1.3.2 Debates over Long-Run Results of Deworming

Separately from debates over the short-run health effects of MDA, a separate literature focuses on long-term benefits to health and broader socioeconomic outcomes. Outside of deworming, a growing literature has demonstrated the importance of early life health and nutrition to adult health and well-being. Thus, an important element of the potential cost-benefit ratio of deworming in childhood is consideration of benefits over the life course. As with MDA meta-analyses, debates have also emerged over the interpretation of the evidence on this topic. These debates have focused on several influential studies which examine short- and long-run socioeconomic benefits of deworming, notably the Miguel and Kremer (2004) study of school-based deworming in western Kenya, and findings from longitudinal tracking of this original trial population (Baird et al. 2016; Hicks et al. 2021). In a setting where STH infection was almost universal, Miguel and Kremer (2004) found that mass deworming increased attendance at dewormed schools as well as for those living in close proximity. A re-analysis of this study found an error which reduced estimates of the geographic distance over which beneficial effects of deworming were detected (Aiken et al. 2015). However, subsequent studies have compared the more intensively and less intensively treated cohorts over the longer term, focusing on education, health, and economic outcomes. Baird et al. (2016) found that a decade after treatment, dewormed men worked 17% more hours per week and had higher living standards, while women were more likely to have passed primary school leaving exams and attended secondary school (Baird et al. 2016). Following the same cohorts after 20 years, Hicks et al. (2021) found that the more intensively treated groups had per capita household consumption expenditure that was 14% higher ($p = 0.06$) than less treated groups (Hicks et al. 2021). This leads the authors to estimate 37% annual social internal rate of return for MDA in this setting. Detailed scrutiny of the longitudinal studies (Roodman 2016, 2017) has largely supported the validity of these findings.

However, even as these studies from the western Kenya cohort demonstrate large benefits over the life course, other recent trials in lower prevalence settings have not found educational or cognitive gains to MDA. For example, Liu et al. (2017) do not find any nutritional or test score gains from MDA in schools in rural China, while

Croke and Atun (2019) do not find significant literacy or numeracy gains 10 years after early childhood deworming in rural eastern Uganda, although effects on nutrition had been observed soon after treatment. As with studies of short-run health outcomes, heterogeneous effects are likely mediated by environmental conditions; both the China and eastern Uganda settings were low to medium prevalence and light intensity settings, whereas, as Hicks et al. (2021) note, the high prevalence and heavy intensity of infection around Lake Victoria circa 1998 could explain the large gains that deworming has generated over the life course in that part of Kenya.

1.3.3 Reframing the Debates over Evidence as Policy Decisions

How, then, should this complex body of evidence be interpreted by policymakers? We can reframe the debates over statistical evidence in the form of a policy decision problem: how should a policymaker with a given level of helminth prevalence think about deworming policies, taking into account expected value, cost, and equity? Decisions about whether or not to support MDA in a given setting require both synthesis and interpretation of the global evidence base but also local knowledge and a decision theory perspective. Some analysts have focused on a "hypothesis testing" approach (i.e., whether or not MDA has a zero-average effect across all settings) to inform a binary decision about whether MDA programmes are justified on a global level. However, this approach to evidence synthesis does not match the decision problem facing health policymakers. Perhaps a more relevant policy question in the case of deworming MDA is rather where MDA can be expected to be cost-effective relative to other health interventions in a given setting.

As mentioned above, there is a consensus clinically and in the meta-analyses that infected children should be treated. All recent meta-analyses find that there is large heterogeneity in impact, with significant effects in some trials and settings and minimal effects in others (consistent with the clinical understanding of STH infection). Since treatment of infected children is uncontroversial, it seems likely to be uncontroversial to presumptively treat high prevalence populations (i.e., where average infection rates are 80–100%). Conversely, it is also likely consensus that it does not make sense to conduct MDA in populations with low prevalence and very few to no highly infected children. The policy question globally is where to place the threshold: WHO guidelines place the threshold for annual deworming at 20% prevalence (WHO 2017), and a recent modelling study (Lo et al. 2016) supported this 20% threshold.

A decision theory approach faced by a specific policymaker should integrate global evidence with knowledge of local conditions to generate an expectation of benefit, net of costs, that MDA is likely to generate. A reasonable interpretation of the global evidence base is that deworming has population-level impact on children's nutrition but that the impacts are likely concentrated in heavily infected individuals,

so population benefits (and the statistical power of trials to detect them) will vary by population infection prevalence and especially intensity. There is also some probability, in high prevalence settings, that MDA can benefit individuals over the life course. Equity considerations are also relevant since infection is correlated with poverty and disadvantage. Expected benefits in a given epidemiological context should therefore consider both short- and long-run benefits in a probabilistic framework and should be compared to the modest costs of MDA and the cost/benefit profile of other health interventions.

The quality of data and analysis of the impact of deworming programmes have improved with time, and current evidence suggests that, in some settings, the impacts are substantial and long term. In the next section, we consider the costs of achieving these impacts.

1.4 The Economics of Interventions

One of the first key areas of health economic analysis related to deworming was whether selective treatment should be used, that is, where only those that are tested positive for infection (or suspected to be infected) are treated or mass treatment. Although selective treatment uses fewer drugs relative to mass treatment, due to the costs associated with conducting the testing and the test's sensitivity, mass treatment is less costly and more effective strategy for deworming (Warren et al. 1993). Consequently, mass treatment became the standard strategy.

1.4.1 Costs Related to Deworming Programmes

The cost of deworming varies between different settings depending on several factors, such as the implementation method, the salaries of healthcare personnel, and the size of the targeted population (Goldman et al. 2007; Gedge et al. 2018; Turner et al. 2021).

1.4.1.1 Delivery Cost Benchmarks and Drivers

Current benchmarks of the delivery costs of mass deworming are generally quoted to be around US$0.50 per treatment (Turner et al. 2021; Fitzpatrick et al. 2016). However, delivery costs vary across different settings and are positively correlated with local GDP (Fitzpatrick et al. 2016). Therefore, there are settings with higher delivery costs. Importantly, deworming delivery costs show economies of scale, and therefore, as the number of people treated increases, the cost per treatment tends to decrease (Turner et al. 2018; Conteh et al. 2010). The costs of deworming will

Table 1.2 Hypothetical case study of the estimated financial costs of using different treatment strategies within the Kenyan national STH control programme

Strategy	Number treated	Assumed cost per treatment (US$)	Estimated total financial cost per year (US$)
School-based treatment	6 million children (Hodges 2017)	0.30[a]–0.56[b]	1.8–3.4 million
Community-wide treatment	14 million individuals[c]	0.32[d]–0.46[e]	4.4–6.4 million

Source: Adapted from Turner and Bundy (2020)
[a]Based on the WHO MDA cost benchmark model (Fitzpatrick et al. 2016)
[b]Estimate from Evidence Action (a programmatic estimate for 2015) (Hodges 2017)
[c]Approximated based on demographic data from the World Bank (n.d.)
[d]Based on the estimate from the TUMIKIA trial (Pullan et al. 2019): routine scenario (excluding the research costs) relating to whole county (i.e., estimate at scale). US$0.025 per treatment was added for the cost of albendazole
[e]Based on the estimate from the TUMIKIA trial (Pullan et al. 2019)—routine scenario (excluding the research costs) relating to trial areas only. US$0.025 per treatment was added for the cost of albendazole

therefore depend on the size of the targeted population, and the cost per treatment can be much higher for small programmes.

When comparing different cost estimates, it is important to note if these are financial or economic costs. Financial costs represent the actual monetary expenditure for the goods, resources, and services that are purchased (i.e., the amount of money paid) for an intervention. Economic costs conceptualize costs more broadly and represent the full value of the resources utilized in providing an intervention, including the economic value of donated resources (such as unpaid time of health personnel). Economic costs of deworming programmes are therefore typically higher than financial costs.

The precise relative cost of different deworming implementation methods (such as school-based vs community-wide treatment) is currently unknown. It is important to note that even if community-wide treatment has a lower cost per treatment compared to school-based strategies, the total annual cost of community-wide treatment will typically be higher because more individuals are targeted (case study in Table 1.2) (Turner and Bundy 2020). That said, it has been shown that leveraging existing delivery platforms (such as child health days or antenatal clinics) is cheaper than providing the treatment through a dedicated deworming programme (Turner et al. 2021; Bangert et al. 2019; Chami and Bundy 2019). For example, Boselli et al. (2011) estimated that the delivery costs of adding deworming into an existing immunization and vitamin A supplementation campaign cost less than US$0.01 per treatment (2009 prices) when targeting children 1–5 years of age and women of childbearing age.

1.4.1.2 Cost of the Drugs

The drugs used for deworming are often donated, and when this is the case, they are not a financial cost to the programmes (Turner et al. 2021). Their economic value can, however, be included as an economic cost, depending on the study's perspective *i.e. the viewpoint from which the intervention's costs and consequences are evaluated*. The value of donated medicines can be a notable economic cost to deworming programmes (Turner et al. 2021). GlaxoSmithKline valuation of donated albendazole is US$0.045 per tablet (which was reduced from a valuation of US$0.19 per tablet in 2009 (Goldman et al. 2011). It should be noted that it is difficult to estimate the true economic cost of these deworming drugs (Turner et al. 2017, 2019b; Hernando et al. 2016). In some cases, the value of the drugs reported by donating companies can be higher than the cost of generic versions, and the correct value to use is debatable (Turner et al. 2021; Hernando et al. 2016). The market price of albendazole and mebendazole can be as low as US$0.02–0.03 per tablet and as high as several hundred dollars within US markets (Pullan et al. 2019; Boselli et al. 2011; Shahriar and Alpern 2020). If and how the donated drugs are valued causes variation in cost-effectiveness estimates of deworming. In addition, some countries do not use donated drugs and purchase their own drugs. Such variation needs to be considered when comparing costing and cost-effectiveness analyses (Turner et al. 2021).

1.4.2 Cost-Effectiveness of Deworming

A number of cost-effectiveness analyses have been performed on STH deworming (reviewed in more detail in the Turner et al. (2021) paper). The estimated cost-effectiveness of annual school-based deworming for STH has been found to be favourable but varies across different studies (with the cost per DALY averted varying between US$8 and 1077 (Table 1.3)). This variation is at least partly due to two key factors. The first is the local pre-control endemicity: the higher the endemicity, the higher the level of morbidity. Therefore, as the pre-control endemicity increases so does the cost-effectiveness of deworming. The second factor is the methods used to estimate the DALYs and how these are changed over time (Turner et al. 2021). For example, cognitive impairment was removed as a quantifiable sequela of STH infection for Global Burden of Disease Study 2010. Although this was justified by a perceived lack of evidence of causation (Taylor-Robinson et al. 2012), it is an area of debate within the field (Owada et al. 2017; Bundy et al. 2009; Campbell et al. 2016).

The generalizability of the reported cost-effectiveness estimates of deworming depends on multiple factors, including the epidemiological setting and drivers that influence the delivery costs (Turner et al. 2021). It is important to consider these when comparing and interpreting different studies for informing policy decisions

Table 1.3 The cost per disability-adjusted life year (DALY) averted estimates relating to annual school-based deworming for STH

Study	Publication year	Intervention and setting	Approach used to estimate the effectiveness and time horizon	Assumed average costs of preventive chemotherapy	Average cost-effectiveness ratio per DALY averted	Cost year
Soil-transmitted helminthiases (STH)						
Chan et al. (Chan 1997)	1997	Mass treating SAC against *A. lumbricoides*—Within a high prevalence community (timeframe for the intervention, 10 years)	Dynamic transmission model (time horizon, 10 years)	US$1600 to treat the schoolchildren per 100,000 population in China	Us$8	Unclear
Miguel and Kremer (Miguel and Kremer 2004)	2004	Biannual mass school-based treatment—Given within a project in Kenya (timeframe for the intervention, 1 year)	Based on project data (time horizon, 1 year)	Based on US$0.49 per pupil per year (removing the costs related to praziquantel)	US$280 (per STH related DALY averted)	Unclear
Hotez et al. (DCP2) (Hotez, Bundy et al. 2006)	2006	Annual mass school-based treatment—Hypothetical setting (timeframe for the intervention, not clearly stated)	Back of the envelope (time horizon, not clearly stated)	Not stated	US$326.43 (note that within the report, the results were reported as US$3.41 but there were errors within the calculation (GiveWell 2011b))	Unclear
GiveWell (GiveWell 2011a)	2011	Annual mass school-based treatment—Hypothetical setting (timeframe for the intervention, one treatment round)	Back of the envelope (time horizon, one treatment round)	US$0.085 per treatment	US$82.54	Unclear
Lo et al. (Lo et al. 2016)	2016	Annual mass school-based treatment—Hypothetical setting (timeframe for the intervention, 5 years)	Dynamic transmission model (time horizon, 5 years)	US$0.53 per treatment (including drug costs)	20% prevalence in SAC: US$1077 60% prevalence in SAC: US$298 85% prevalence in SAC: US$174	2015

(continued)

Table 1.3 (continued)

Study	Publication year	Intervention and setting	Approach used to estimate the effectiveness and time horizon	Assumed average costs of preventive chemotherapy	Average cost-effectiveness ratio per DALY averted	Cost year
Schistosomiasis, lymphatic filariasis, and STH						
De Neve et al. (De Neve et al. 2018)	2018	Annual mass school-based treatment—Based on the PC programme in Madagascar (timeframe for the intervention, one treatment round)	Static model (time horizon, unclear)	Not directly reported	US$125 (95% uncertainty range, 65–231)	2013
Schistosomiasis and STH						
Warren et al. (DCP1) (Warren et al. 1993)	1993	Hypothetical setting (timeframe for the intervention, 10 years)	Static calculation (time horizon, 10 years)	US$0.8–1.80 per child per year (including drug costs)	US$6–33	Unclear
Miguel and Kremer (Miguel and Kremer 2004)	2004	Annual mass school-based treatment for schistosomiasis and biannual mass school-based treatment for STH—Given within a project in Kenya (timeframe for the intervention, 1 year)	Based on project data (time horizon, 1 year)	US$0.49 per pupil per year (including drug costs)	US$5 (99% of the benefit was due to averted schistosomiasis)	Unclear
Lo et al. (Lo et al. 2015)	2015	Annual mass school-based treatment—Within four communities in Côte d'Ivoire (timeframe for the intervention, 15 years)	Dynamic transmission model (time horizon, 15 years)	US$0.71 per treatment (including drug costs)	US$118 (US$87–140) (92% of the disability resulted from *Schistosoma* spp. infections)	2014

It was not possible to adjust the different studies for inflation and they are reported in their original cost year (Turner et al. 2019a)
DALY, disability-adjusted life year; DCP1, disease control priorities in developing countries (first edition); DCP2, disease control priorities in developing countries (second edition); SAC, school-aged children; STH, soil-transmitted helminthiases
Source: Adapted from Turner et al. (2021)

(Turner et al. 2021). The majority of the estimates are below the cost-effectiveness thresholds commonly used for low-income countries (Turner et al. 2021), and the highest estimate relates to a low endemicity setting (20% prevalence, below which mass treatment is not recommended (Table 1.3)). A further consideration is that the cost-effectiveness of deworming is greater when considering integrated control, such as the cost-effectiveness of deworming against both schistosomiasis and STH in one programme, as opposed to separate control programmes (Table 1.3).

It is important to note that it is debatable whether the DALY averted metric (which focuses on losses of optimum health) is truly capturing all the long-term benefits and value for money of deworming against STH (Turner et al. 2021). Consequently, the broad benefits of deworming against STH may not be fully captured by the conventional approaches to cost-effectiveness analysis. For example, Hicks et al. (2021) recently demonstrated significant long-term economic benefits of deworming children, such as on household income. Additionally, the DALY framework fails to acknowledge the implications of socioeconomic context; the burden of disease will vary within at-risk groups based on poverty-related factors.

Box 1.1 School-Based vs Community-Wide Deworming for STH

A key research gap is the relative benefits and cost-effectiveness of switching from school-based to community-wide MDA for STH. On the plus side, using community-wide MDA for STH could reduce infection overall (by treating currently untreated adults and perhaps children not reached through the school-based programme) generating additional health benefits, and in addition, mathematical models suggest that community-wide MDA may contribute to the interruption of transmission (Anderson et al. 2014; Truscott et al. 2014, 2016), which could potentially be cost-saving in the long term (Turner et al. 2015a). On the minus side, however, community-wide MDA would very significantly increase the number of treatments required, potentially more than doubling costs in the example here (Table 1.2).

The potential benefits of switching to community-wide MDA are highly dependent on the local setting (Anderson et al. 2015). This is illustrated in Fig. 1.1, which shows the different age profiles of infection for the different STH species. Based on these age profiles, the benefits could be notable for hookworm but small for the other species (Truscott et al. 2016; Anderson et al. 2015; Turner et al. 2015a). The benefits will also be smaller in settings that have a low baseline level of endemicity and for settings that have had past community-wide MDA for lymphatic filariasis. Consequently, the health gains from switching will vary depending on which STH species are endemic, the treatment history, and the baseline level of endemicity (Turner and Bundy 2020; Turner et al. 2015a).

1.4.3 Economic Benefits of Deworming

In addition to their impact as measured by averting DALYs, NTDs are known to cause financial hardship among affected individuals, which can exacerbate the cycle of poverty (Fitzpatrick et al. 2017). Therefore, deworming can also have important socioeconomic benefits (as discussed in the "Global Evidence of Deworming" section and summarized by Ahuja et al. (2017)).

Some studies have estimated the monetary value of the benefits of deworming programmes (Turner et al., 2015b, Turner et al., 2020). For example, Redekop et al. (2017) estimated large economic benefits from preventive chemotherapy. Typically within these studies, the majority of the economic benefits are due to the estimated monetary value of productivity gains, and these are highly dependent on several assumptions, such as the number of disease cases averted due to deworming, the effect of clinical disease on productivity, the number of years of productive life lived with clinical disease, employment rates and wage rates (Turner 2021). Furthermore, most of the studies used the human capital approach where all potential production not performed by a person due to morbidity or early mortality is counted as the productivity loss (Gedge et al. 2018; Turner 2021; Turner et al. 2016). Consequently, the estimated monetized economic benefits being quoted in many studies are generally based on potential rather than experienced productivity gains. That said, the overall conclusion that the deworming programmes generate notable economic benefits appears robust, and some studies have looked at the actual economic benefits experienced by treated populations.

The evidence suggests that deworming has achieved impact at remarkably low cost. But the programmes are often stand-alone efforts reliant to a large extent on external funding. Is the success sustainable with the present model? In the next section, we envisage future approaches that are more aligned to the aspirations of access to Universal Health Care (UHC).

1.5 Health System Issues

Mass drug administration depends on the large-scale donation of medicines, which cannot go on indefinitely if there are donor fatigue, changes in industry leadership, fluctuations in international aid commitments, and global insecurity (Glenn et al. 2020). In this section, we rethink the current approach to deworming, with less emphasis on MDA and school children and more emphasis on adults and UHC.

1.5.1 Reliance on External Support and Donations and MDA Instability

A pressing example of the potential vulnerability of MDA to external shocks was shown by the aid cuts which took place when the United Kingdom's Department for International Development (DFID) was merged with the Foreign and Commonwealth Office to form the Foreign, Development, and Commonwealth Office, as of 2 September 2020. By January 2021, UK overseas development assistance for low-income countries was cut by US$1.69 billion (£1.2bn) (House of Commons: Foreign Affairs Committee 2020; Mitchell et al. 2021). Prior to these changes, DFID was a key donor to NTD programmes, for example, pledging US$271 million (£195 m) towards MDA implementation during the London Declaration (Watts 2017).

In addition to political changes, global insecurity has highlighted weaknesses in the current model of MDA implementation. With the SARS-CoV2 pandemic, WHO, which manages the drug donations, recommended halting MDA as of 1 April 2020 (WHO 2020a). Mass drug administration is a vertical campaign that bypasses existing health systems (Chami and Bundy 2019). Although a cadre of volunteers, lay health workers, and primary school teachers are trained through MDA to distribute preventive chemotherapies, these medicines are often unavailable within peripheral primary healthcare (PHC) facilities. Without donated medicines available in local health systems, individuals infected with one or more STH were left untreated when MDA was halted since alternative options were unavailable. The COVID-19 pandemic has shown clearly the vulnerability of MDA alone because of its restricted access to medicines outside of scheduled campaigns.

Increasing country ownership of STH programmes is an important way forward to establishing a more sustainable treatment programme. The recently launched WHO 2021–2030 Road Map for NTDs emphasizes increasing country ownership, in particular exploring options for domestic financing (WHO 2020b). In line with bold new visions in the Road Map, this implies a need to (1) switch from treating specific diseases to treating people, (2) integrate treatment within local healthcare systems, and (3) increase country decision-making for STH treatment regimens/strategies. To achieve these objectives, alternatives to MDA are needed that align more closely with the principles of UHC, which aspires to make essential services always available and to ensure that when they are used, they do not result in financial hardship.

1.5.2 MDA Does Not Equate with UHC

Mass drug administration often is used as a proxy indicator of UHC (Fitzpatrick et al. 2018). By providing essential health services at no cost to the end patient, MDA is one step towards providing essential health services for NTDs (Chami and Bundy

2019). However, using MDA as a proxy indicator of UHC may be misleading and masks the inequities present in the distribution of preventive chemotherapies and lack of control for the patient over their own treatment options (Dean et al. 2019). Endemic country financing options still need to be developed for medicine purchase and delivery through PHC facilities.

With current approaches to MDA, the choice of when and where to receive treatment is not made by the patient. In many endemic settings, there are often no on-demand treatment options for STH. However, it may now be possible to develop strategies for on-demand treatment for STH as prevalence decreases worldwide due to the successes of MDA (Montresor et al. 2020). The placement of medicines in PHC facilities to enable on-demand treatment raises a number of challenges and, in turn, future research opportunities. Open questions remain as to whether the donated medicines should be provided for use outside of vertical campaigns. In addition to promoting UHC and in-country ownership, there is a need to assess whether the individuals most in need of treatment would be reached through PHC facilities and whether this strategy is cost-effective, including the willingness to pay of participants for preventive chemotherapies or the willingness of national health systems to pay for diagnostics (Storey et al. 2019).

1.5.3 Rethinking Infection Mapping Strategies

At a minimum, to make progress towards the placement of medicines in PHC facilities, new infection mapping strategies are needed that require a rethinking of the overall strategy. For example, they need not be reliant, as they are now, on administrative units (e.g., districts) or sampling of children in primary schools. The target population for on-demand treatment access, focusing on the users of PHC, might arguably be primarily adults. Adults can have heavy infections with STH, especially hookworm, can play a major role in sustaining transmission in endemic communities, and, perhaps most importantly, are in charge of the decisions and financial resources for bringing children to health facilities for treatment (Chami et al. 2015, 2018). This is in contrast to the current focus on school-aged children within primary schools for STH treatment (WHO 2006a).

This shift in thinking implies a need for new prevalence mapping strategies to measure STH prevalence within the catchment of the PHC facility. A list of communities served by a health centre, a spatial buffer such as a predefined radius from the health centre, or spatially regulated sampling (Fronterre et al. 2020) may be used to define catchment areas. After defining catchments, random sampling of eligible communities and individuals within those communities may be used to estimate STH prevalence. Cutting-edge approaches for spatial modelling, yet to be used by the NTD field, such as gravity models also may be applied to incorporate healthcare access within catchment definitions (Apparicio et al. 2008). Remarkably, Travel times to a majority of government health centres across sub-Saharan Africa already have been estimated and are publicly available (Weiss et al. 2020). These

revised implementation units for treatment programmes may guide the placement of preventive chemotherapies.

1.5.4 Towards Patient-Led, On-Demand Treatment

If STH treatment was available in PHC facilities, the next step to ensuring equitable access to treatment is to increase patient awareness. Campaigns have been underway to clearly communicate the definition of UHC, establish its purpose within countries, and provide technical knowledge (Holtz et al. 2018). In addition to the existing challenges of creating a common understanding and platform for UHC, STHs face the barrier of informing those who need treatment that on-demand options are available within PHC facilities. Health education campaigns will be needed to share the principles of UHC and to inform individuals of their right to request treatment for STH (Ediriweera et al. 2019; Montresor and Mupfasoni 2019). There is preliminary support for the demand for preventive chemotherapies outside of MDA (Dhakal et al. 2020). In Bangladesh, where only school-aged children were targeted for treatment, adults were found to experience a similar decline in prevalence when compared to treated children over a 10-year period. The authors speculate that this decline may be due to adults actively purchasing deworming medicines or improved WASH. To improve patient-led demand, other initiatives such as child health days, women's reproductive health clinics, and vaccine campaigns conducted through health centres might be coupled with the provision of deworming medicines to reach at-risk individuals.

Importantly, in STH-endemic areas, individuals who seek care from government health centres have been shown to differ in terms of socioeconomic status and WASH behaviours than individuals who seek care from traditional healers or private clinics or seek no care at all (Chami et al. 2018). This implies that those most likely to be infected with STH are also less likely to seek treatment. This has the potential to undermine a patient-led process. Working with local communities and working with community leaders to advocate on-demand treatment should be investigated as a method to increase patient awareness and address inequalities related to who seeks treatment (Valente and Pumpuang 2007).

1.5.5 Improved Health Information Systems to Support UHC

Health management information systems (HMIS) are improving in low-income countries. For example, the MalariaCare Electronic Data System used across Africa has enabled data entry at the district level with guided software platforms to reduce data entry time and errors (Burnett et al. 2019). A similar platform might be developed for (1) STH catchment mapping, (2) tracking of medicines delivered from national medical stores, and (3) record keeping of medicines administered to

patients. At the very least, results from NTD registers that are used to track STH campaigns should be shared with HMIS, particularly by training existing HMIS staff to handle MDA data (as is being done with ESPEN).

Digital health approaches have shown promise for improving data management of STH in PHC facilities. Mobile notifications already have been widely used for lymphatic filariasis (and the distribution of albendazole) (Stanton et al. 2016; Tilahun et al. 2021). Text messaging and mobile applications have assisted in tracking albendazole medicine supplies, confirming patient treatment receipt, and providing healthcare information. Biometric technology, for example, fingerprint scanning, in the Geshiyaro study in Ethiopia has been used to verify treatment receipt in MDA campaigns for STH (Mekete et al. 2019). In addition to data management and patient outreach, a move towards systems thinking may assist in strengthening local health systems.

1.5.6 Future Research Is Needed for UHC Integration

Such an approach would require acknowledging that "quick hit" solutions to STH are no longer plentiful (having been achieved by MDA) and that STH treatment can no longer be reduced to only MDA. Instead, complexity should be embraced by acknowledging the changing international landscape, patient needs, and dynamic health systems within endemic countries. For on-demand treatment in PHC facilities, there is a need to revise the understanding of the epidemiology of STH. Repeated MDA has been shown to miss individuals systematically, thereby introducing heterogeneity into the known distribution of infections within endemic communities (Basáñez et al. 2012; Chami et al. 2017, 2016). In particular, community-based distribution of albendazole in the context of areas co-endemic with lymphatic filariasis and STHs has been shown to miss the most marginalized individuals of low socioeconomic status and with limited access to adequate sanitation and safe water. These individuals are the most likely to be infected with STH. As these characteristics also represent individuals who also are less likely to seek care from government health centres (Chami et al. 2018), the need to monitor these characteristics in PHC facilities is twofold. Observable characteristics of poor socioeconomic status and inadequate WASH may be used to redefine at-risk groups for STH within PHC facilities. Simple characteristics such as home quality, drinking water source, and latrine ownership might be used to identify the groups for treatment through blanket or test-and-test strategies. One step towards inclusion of these social determinants of treatment would be to trial the collection of different indicators across countries where MDA is ongoing. The feasibility and applicability of observable characteristics could be systematically identified in future research to assess the evidence for UHC integration by piloting the collection of this information in NTD registers, holding focus groups in endemic communities, and conducting key informant interviews (e.g., with district health officers or primary school teachers).

1.6 Conclusions

This chapter has undertaken a sequential analysis of the global development of deworming programmes. We have discussed the evolution of policy, the translation of policy into programming, and the measurement of the impact and cost of the programmes and explored what might come next. In this final section, we offer some concluding thoughts on each of these topics.

The evolution of policy: There has been recognition of worms as a health issue for thousands of years, but it is only in the last 100 years that there have been concerted public health responses. The "modern" approach to deworming, with a focus on specific treatments delivered at large scale to at-risk populations, first emerged in the 1980s, some 40 years ago, and reached the status of broad consensus in the mid-2000s. The consensus was around mass drug administration, with effective pharmaceuticals delivered through schools to school-aged children without individual diagnosis, in communities shown by prior screening to have infection prevalence greater than 20%. While many countries went ahead with their own programmes, it was only 8 years ago, in 2012, that a global effort was launched, and only in the last 5 years that programmes have been implemented at global scale.

This then is a story of success. We would remark on two points. First, this seems like a long time for the rollout of a seemingly very simple intervention; change in global health policy comes slowly. Second, and perhaps worryingly, the main pharmaceuticals used are based on products first discovered for veterinary applications, and there has been no breakthrough deworming drug for human or veterinary use discovered in the last 30 years.

The translation of policy into programmes: The 2012 "London Declaration on NTDs" was a watershed moment in the global rollout of the deworming programmes, driven by the availability of donated treatments by the pharmaceutical industry. This has become the largest public health donation programme in human history, and the mobilization of drugs during 2018 was recognized as such by the *Guinness Book of World Records*. School-based MDA has been adopted by nearly all the countries where STH infection is endemic at levels considered to be of public health consequence. Some 3.3 billion treatments have been delivered to school-aged children through schools since 2010. Some countries have stopped treatment, but for a majority of the worst affected, the focus now is on identifying a threshold, based on "Elimination as a Public Health Problem" or "Interruption of Transmission", that will allow countries to scale back their programmes and to transition to sustainable, self-reliant programmes supported by domestic financing.

Measuring the impact of programmes: For many public health practitioners, their awareness of deworming may be largely as spectators of the "worm wars". Today, the apparently conflicting interpretations of the evidence seem to have converged in some sense: There is a consensus clinically and in the meta-analyses that infected children should be treated. All recent meta-analyses find that there is large heterogeneity in impact, with significant effects in some trials and settings and minimal effects in others (consistent with the clinical understanding of STH infection). There

appears to be common ground around the justification for presumptively treating high prevalence populations (i.e., where average infection rates are 80–100%). Conversely, it is also likely consensus that it does not make sense to conduct MDA in populations with low prevalence and very few to no highly infected children. The policy question globally is where to place the threshold. This helps reframe the debate over statistical evidence into a policy decision problem: how should a policymaker with a given level of helminth prevalence think about deworming policies, taking into account expected value, cost, and equity?

Assessing the costs and benefits of programmes: Analyses show that the current deworming strategies are cost-effective and value for money. This appears to hold even if the cost of procuring treatment is included. The programmes also appear to be cost-beneficial, although the current framework for estimating DALYs does not fully summarize the disease burden, potentially underestimating the returns. There is a need to more comprehensively capture the health benefits of deworming, including quantifying if they are associated with excess mortality, and evaluate the non-health-related benefits of deworming, such as improved educational and economic outcomes. This will be particularly important in considering the potential costs and benefits of broadening MDA coverage to whole communities. As programmes evolve away from stand-alone vertical programmes, decision-makers need to consider the cost-effectiveness of integrated NTD control programme packages and should account for the potential returns from building on established health system platforms and primary healthcare (PHC) facilities to deliver treatments, particularly to adults.

Rethinking deworming as an integrated part of health systems: Deworming programmes have become among the most ubiquitous and cost-effective public health programmes worldwide. This has taken a long time to happen, in terms of conceptualizing the problem, developing the solution, and mobilizing resources, but in the end has become a success story benefitting billions of children. Looking forward, however, it is clear that the current reliance on MDA, whether school based or not, presents major concerns about vulnerability to external shocks, such the cessation of the donations, and the consequences of such stand-alone programmes as countries strive to achieve Universal Health Care. It took some 40 years to develop and roll out the MDA approach; it would be timely to start thinking now about what should replace it in the context of UHC.

References

Ahuja A, Baird S, Hicks JH, Kremer M, Miguel E (2017) Economics of mass deworming programs. In: Bundy DAP, de Silva N, Horton S, Jamison DT, Patton GC (eds) Disease control priorities, third edition (volume 8): child and adolescent health and development, 3rd edn. World Bank Group, Washington, DC, pp 413–422. https://doi.org/10.1596/978-1-4648-0423-6_ch29

Aiken AM, Davey C, Hargreaves JR, Hayes RJ (2015) Re-analysis of health and educational impacts of a school-based deworming programme in Western Kenya: a pure replication. Int J Epidemiol 44(5):1572–1580. https://doi.org/10.1093/ije/dyv127

Anderson R, May R (1982) Population dynamics of human helminth infections: control by chemotherapy. Nature 297(5867):557–563. https://doi.org/10.1038/297557a0

Anderson R, Truscott J, Deirdre T, Hollingsworth (2014) The coverage and frequency of mass drug administration required to eliminate persistent transmission of soil-transmitted helminths. Philos Trans R Soc no. 369. https://doi.org/10.1098/rstb.2013.0435

Anderson RM, Turner HC, Truscott JE, Déirdre Hollingsworth T, Brooker SJ (2015) Should the goal for the treatment of soil transmitted helminth (STH) infections be changed from morbidity control in children to community-wide transmission elimination? Edited by Serap Aksoy. PLoS Negl Trop Dis 9(8):e0003897. https://doi.org/10.1371/journal.pntd.0003897

Apparicio, Philippe, Mohamed Abdelmajid, Mylène Riva, and Richard Shearmur. 2008. Comparing alternative approaches to measuring the geographical accessibility of urban health services: distance types and aggregation-error issues. doi:https://doi.org/10.1186/1476-072X-7-7

Ásbjörnsdóttir, Kristjana Hrönn, Sitara S. Rao Ajjampur, Roy M. Anderson, Robin Bailey, Iain Gardiner, Katherine E. Halliday, Moudachirou Ibikounle, et al. 2018. Assessing the feasibility of interrupting the transmission of soil-transmitted helminths through mass drug administration: the DeWorm3 cluster randomized trial protocol. Edited by Amadou GarbaPLoS Negl Trop Dis 12 (1): e0006166. doi:https://doi.org/10.1371/journal.pntd.0006166

Baird S, Hicks JH, Kremer M, Miguel NE, Audi K et al (2016) Worms at work: long-run impacts of a child health investment. Q J Econ 131(4):1637–1680

Bangert M, Bancalari P, Mupfasoni D, Mikhailov A, Gabrielli AF, Montresor A (2019) Provision of deworming intervention to pregnant women by antenatal services in countries endemic for soil-transmitted helminthiasis. PLoS Negl Trop Dis 13(5). https://doi.org/10.1371/journal.pntd.0007406

Basáñez M-G, French MD, Walker M, Churcher TS (2012) Paradigm lost: how parasite control may alter pattern and process in human helminthiases. Trends Parasitol 28(4):161–171. https://doi.org/10.1016/j.pt.2012.02.004

Bleakley H (2007) Disease and development: evidence from hookworm eradication in the American south. Q J Econ 122(1):73–117. https://doi.org/10.1162/qjec.121.1.73

Boselli G, Yajima A, Aratchige PE, Feldon KE, Xeuatvongsa A, Phounphenghak K, Sihakhang K et al (2011) Integration of deworming into an existing immunisation and vitamin a supplementation campaign is a highly effective approach to maximise health benefits with minimal cost in Lao PDR. Int Health 3:240–245. https://doi.org/10.1016/j.inhe.2011.08.002

Bradley M, Taylor R, Jacobson J, Guex M, Hopkins A, Jensen J, Leonard L et al (2021) Medicine donation programmes supporting the global drive to end the burden of neglected tropical diseases. Trans R Soc Trop Med Hyg 115(2):136–144. https://doi.org/10.1093/trstmh/traa167

Brooker SJ, Nikolay B, Balabanova D, Pullan RL (2015) Global feasibility assessment of interrupting the transmission of soil-transmitted helminths: a statistical modelling study. Lancet Infect Dis 15(8):941–950. Available from: http://dx.doi.org/10.1016/

Bundy D (1990) Control of intestinal nematode infections by chemotherapy: mass treatment versus diagnostic screening. Trans R Soc Trop Med Hyg 84(5):622–625. https://doi.org/10.1016/0035-9203(90)90126-Y

Bundy DAP, Wong MS, Lewis LL, Horton J (1990) Control of geohelminths by delivery of targeted chemotherapy through schools. Trans R Soc Trop Med Hyg 84(1):115–120

Bundy D (2011) In: Bundy DAP (ed) Rethinking school health: a key component of education for all. IBRD/World Bank, Washington, DC. https://doi.org/10.1596/978-0-8213-7907-3

Bundy D, Shaeffer S, Jukes M, Beegle K, Gillespie A, Drake L, Lee A-MF, Hoffman S-h et al (2006) School-based health and nutrition programs. In: Jamison D, Brenanm JG, Measham AR, Alleyne G, Claeson M, Evans D, Jha P, Mills A, Musgrove P (eds) Disease control priorities in developing countries, 2nd edn. The World Bank/Oxford University Press, New York, pp 1091–1108. http://files.dcp2.org/pdf/expressbooks/prevent.pdf#page=78

Bundy DAP, Kremer M, Bleakley H, Jukes MCH, Miguel E (2009) Deworming and development: asking the right questions, asking the questions right. Edited by Gavin Yamey. PLoS Negl Trop Dis 3(1):e362. https://doi.org/10.1371/journal.pntd.0000362

Bundy D, Schultz L, Sarr B, Banham L, Colenso P, Drake L (2017) The school as a platform for addressing health in middle childhood and adolescence. In: Bundy DAP, de Silva N, Horton S, Jamison DT, Patton G (eds) Disease control priorities, third edition (volume 8): child and adolescent health and development, 3rd edn. IBRD/World Bank, Washington, DC, pp 269–285. https://doi.org/10.1596/978-1-4648-0423-6_ch20

Bundy D, Appleby LJ, Bradley M, Kevin C, Deirdre Hollingsworth T, Pullan R, Turner HC, de Silva N (2018) 100 years of mass deworming Programmes: a policy perspective from the World Bank's disease control priorities analyses. Adv Parasitol 100:127–154. https://doi.org/10.1016/bs.apar.2018.03.005

Burnett SM, Wun J, Evance I, Davis KM, Smith G, Lussiana C, Tesha G et al (2019) Introduction and evaluation of an electronic tool for improved data quality and data use during malaria case management supportive supervision. Am J Trop Med Hyg 100(4):889–898. https://doi.org/10.4269/ajtmh.18-0366

Campbell SJ, Savage GB, Gray DJ, Atkinson J-AM, Soares Magalhães RJ, Nery SV, McCarthy JS et al (2014) Water, sanitation, and hygiene (WASH): a critical component for sustainable soil-transmitted helminth and schistosomiasis control. Edited by Xiao-nong Zhou. PLoS Negl Trop Dis 8(4):e2651. https://doi.org/10.1371/journal.pntd.0002651

Campbell SJ, Nery SV, McCarthy JS, Gray DJ, Soares Magalhães RJ, Clements ACA (2016) A critical appraisal of control strategies for soil-transmitted helminths. Trends Parasitol. https://doi.org/10.1016/j.pt.2015.10.006

Campbell SJ, Osei-Atweneboana MY, Stothard R, Koukounari A, Cunningham L, Armoo SK, Biritwum N-K et al (2018) Tropical medicine and infectious disease the COUNTDOWN study protocol for expansion of mass drug administration strategies against schistosomiasis and soil-transmitted helminthiasis in Ghana. Trop Med Infect Dis 3:10. https://doi.org/10.3390/tropicalmed3010010

Chami GF, Bundy DAP (2019) More medicines alone cannot ensure the treatment of neglected tropical diseases. Lancet Infect Dis. https://doi.org/10.1016/S1473-3099(19)30160-4

Chami GF, Fenwick A, Bulte E, Kontoleon AA, Kabatereine NB, Tukahebwa EM, Dunne DW (2015) Influence of Schistosoma Mansoni and hookworm infection intensities on Anaemia in Ugandan villages. PLoS Negl Trop Dis 9(10). https://doi.org/10.1371/journal.pntd.0004193

Chami GF, Kontoleon AA, Bulte E, Fenwick A, Kabatereine NB, Tukahebwa EM, Dunne DW (2016) Profiling nonrecipients of mass drug Administration for Schistosomiasis and Hookworm Infections: a comprehensive analysis of Praziquantel and Albendazole coverage in community-directed treatment in Uganda. Clin Infect Dis 62(2):200–207. https://doi.org/10.1093/cid/civ829

Chami GF, Kontoleon AA, Bulte E, Fenwick A, Kabatereine NB, Tukahebwa EM, Dunne DW (2017) Community-directed mass drug administration is undermined by status seeking in friendship networks and inadequate Trust in Health Advice Networks. Soc Sci Med 183:37–47. https://doi.org/10.1016/j.socscimed.2017.04.009

Chami GF, Kabatereine NB, Tukahebwa EM, Dunne DW (2018) Precision Global Health and comorbidity: a population-based study of 16 357 people in rural Uganda. J R Soc Interface 15. https://doi.org/10.1098/rsif.2018.0248

Chan MS (1997) The global burden of intestinal nematode infections—fifty years on. Parasitol Today 13(11):438–443. https://doi.org/10.1016/S0169-4758(97)01144-7

CIFF (2020) Deworming Innovation Fund:2020. https://ciff.org/grant-portfolio/deworming-innovation-fund/

Conteh L, Engels T, Molyneux DH (2010) Socioeconomic aspects of neglected tropical diseases. Lancet 375:239–247. https://doi.org/10.1016/S0140-6736(09)61422-7

Croke, Kevin, and Rifat Atun. 2019. The long run impact of early childhood deworming on numeracy and literacy: evidence from Uganda. doi:https://doi.org/10.1371/journal.pntd.0007085

Croke K, Hicks JH, Hsu E, Kremer M, Miguel E (2017) Should the WHO withdraw support for mass deworming? PLoS Negl Trop Dis 11(6). https://doi.org/10.1371/journal.pntd.0005481

Crompton DWT, Whitehead RR (1993) Hookworm infections and human iron metabolism. Parasitology 107(S1):s137–S145. https://doi.org/10.1017/S0031182000075569

Dean, Laura, Kim Ozano, Oluwatosin Adekeye, Ruth Dixon, Ebua Gallus Fung, Margaret Gyapong, Sunday Isiyaku, et al. 2019. Neglected tropical diseases as a 'litmus test' for universal health coverage? Understanding who is left behind and why in mass drug administration: lessons from four country contexts. Edited by Arianna R. Means. PLoS Negl Trop Dis 13 (11): e0007847. doi:https://doi.org/10.1371/journal.pntd.0007847

Dhakal S, Mohammad Jahirul KID, Al Kawsar A, Jasmine IID, Rahman M, Cara TID, Kabir A, Rubina IID (2020) Post-intervention epidemiology of STH in Bangladesh: Data to Sustain the Gains. https://doi.org/10.1371/journal.pntd.0008597

Ediriweera DS, Gunawardena S, Gunawardena NK, Iddawela D, Kannathasan S, Murugananthan A, Yahathugoda C, Pathmeswaran A, Diggle PJ, de Silva N (2019) Reassessment of the prevalence of soil-transmitted helminth infections in Sri Lanka to enable a more focused control Programme: a cross-sectional National School Survey with spatial modelling. Lancet Glob Health 7(9):e1237–e1246. https://doi.org/10.1016/S2214-109X(19)30253-0

Espinal M, Kruk ME, Cisse M, Mohamed M, Wainwright E (2021) Considerations for a sustainability framework for neglected tropical diseases programming. Trans R Soc Trop Med Hyg 115:176–178. https://doi.org/10.1093/trstmh/traa196

Ettling J (1981) The germ of laziness. In: Rockefeller philanthropy and public health in the new south. Harvard University Press, Cambridge, MA, p 263

Fitzpatrick, Christopher, Fiona M. Fleming, Matthew Madin-Warburton, Timm Schneider, Filip Meheus, Kingsley Asiedu, Anthony W. Solomon, Antonio Montresor, and Gautam Biswas. 2016. Benchmarking the cost per person of mass treatment for selected neglected tropical diseases: an approach based on literature review and meta-regression with web-based software application. Edited by Hélène Carabin. PLOS Negl Trop Dis 10 (12): e0005037. doi:https://doi.org/10.1371/journal.pntd.0005037

Fitzpatrick C, Nwankwo U, Lenk E, de Vlas SJ, Bundy DAP (2017) An investment case for ending neglected tropical diseases. In: Disease control priorities, 3rd edn (vol 6): Major infectious diseases. The World Bank, pp 411–431. https://doi.org/10.1596/978-1-4648-0524-0_ch17

Fitzpatrick C, Bangert M, Mbabazi PS, Mikhailov A, Zouré H, Rebollo MP, Correia MR, e Silva, and Gautam Biswas. (2018) Monitoring equity in universal health coverage with essential Services for Neglected Tropical Diseases: an analysis of data reported for five diseases in 123 countries over 9 years. Lancet Glob Health 6(9):e980–e988. https://doi.org/10.1016/S2214-109X(18)30307-3

Fronterre C, Amoah B, Giorgi E, Stanton MC, Diggle PJ (2020) Design and analysis of elimination surveys for neglected tropical diseases. J Infect Dis 221(Supplement_5):S554–S560. https://doi.org/10.1093/infdis/jiz554

Gabrielli AF (2022) Soil-transmitted helminthiasis. In: Bruschi F (ed) Helminth infections and their impact on global public health. Springer

Gedge LM, Bettis AA, Bradley MH, Déirdre Hollingsworth T, Turner HC (2018) Economic evaluations of lymphatic Filariasis interventions: a systematic review and research needs. Parasites Vectors 11(75). https://doi.org/10.1186/s13071-018-2616-z

GiveWell. 2011a. Cost-effectiveness in $/DALY for deworming interventions. https://www.givewell.org/international/technical/programs/deworming/cost-effectiveness

GiveWell. 2011b. Errors in DCP2 cost-effectiveness estimate for deworming. https://blog.givewell.org/2011/09/29/errors-in-dcp2-cost-effectiveness-estimate-for-deworming/

Glenn J, Kamara K, Umar ZA, Chahine T, Daulaire N, Bossert T (2020) Applied systems thinking: a viable approach to identify leverage points for accelerating Progress towards ending neglected tropical diseases. Health Res Policy Syst 18(56). https://doi.org/10.1186/s12961-020-00570-4

Goldman AS, Guisinger VH, Aikins M, Maria Lourdes E, Amarillo VY, Belizario BG et al (2007) National mass drug administration costs for lymphatic Filariasis elimination. PLoS Negl Trop Dis 1(1):e67. https://doi.org/10.1371/journal.pntd.0000067

Goldman AS, Brady MA, Direny A, Desir L, Oscard R, Vely JF, Linehan M, Baker M (2011) Costs of integrated mass drug administration for neglected tropical diseases in Haiti. Am J Trop Med Hyg 85(5):826–833. https://doi.org/10.4269/ajtmh.2011.10-0635

Guinness World Records. 2017. Most medication donated in 24 hours. https://www.guinnessworldrecords.com/world-records/439520-most-medication-donated-in-24-hours

Hall A, Hewitt G, Tuffrey V, De Silva N (2008) A review and meta-analysis of the impact of intestinal worms on child growth and nutrition. Maternal Child Nutr 4:118–236

Hasegawa M, Pilotte N, Kikuchi M, Means AR, Papaiakovou M, Gonzalez AM, Maasch JRMA et al (2020) What does soil-transmitted helminth elimination look like? Results from a targeted molecular detection survey in Japan. Parasites Vectors 13(1):6. https://doi.org/10.1186/s13071-019-3875-z

Hernando Y, Colwell K, Wright BD (2016) Doing well while fighting river blindness: the alignment of a corporate drug donation programme with responsibilities to shareholders. Trop Med Int Health 21(10):1304–1310. https://doi.org/10.1111/tmi.12759

Hicks JH, Miguel E, Walker M, Kremer M, Baird S (2021) Twenty-year economic impacts of deworming. PNAS 118(14). https://doi.org/10.1073/pnas.2023185118/-/DCSupplemental.y

Hodges, Liam Chan. 2017. Costs of school-based deworming: a best-buy development intervention—evidence action evidence action. https://www.evidenceaction.org/deworm-cost-per-child/

Holtz J, Cox B, Cico A (2018) Building momentum for universal health coverage, Washington, DC. https://lhssproject.org/building-momentum-for-universal-health-coverage

Hong ST, Chai JY, Choi MH, Huh S, Rim HJ, Lee SH (2006) A successful experience of soil-transmitted helminth control in the Republic of Korea. Korean J Parasitol 44(3):177–185. https://doi.org/10.3347/kjp.2006.44.3.177

Hotez PJ, Bundy DAP, Beegle K, Brooker S, Drake L, de Silva N, Montresor A et al (2006a) Helminth infections: soil-transmitted helminth infections and schistosomiasis. In: Jamison DT, Breman JG, Measham AR (eds) Disease control priorities in developing countries, 2nd edn. The International Bank for Reconstruction and Development / The World Bank, Washington, DC, pp 467–482. http://www.ncbi.nlm.nih.gov/pubmed/21250326

Hotez, Peter J., David H. Molyneux, Alan Fenwick, Eric Ottesen, Sonia Ehrlich Sachs, and Jeffrey D. Sachs. 2006b. Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, and malaria: a comprehensive pro-poor health policy and strategy for the developing world. PLoS Med. Public Library of Science. doi:https://doi.org/10.1371/journal.pmed.0030102

Keating C (2014) Ken Warren and the Rockefeller foundation's great neglected diseases network, 1978–1988: the transformation of tropical and global medicine. Mol Med 16(20):S24–S30

House of Commons: Foreign Affairs Committee (2020) Merging success: bringing together the FCO and DFID, London. https://publications.parliament.uk/pa/cm5801/cmselect/cmfaff/809/80902.htm

Johnson & Johnson (2019) Johnson & Johnson extends long-standing donation program to support global effort to tackle intestinal worms. Johnson & Johnson. https://www.jnj.com/johnson-johnson-extends-long-standing-donation-program-to-support-global-effort-to-tackle-intestinal-worms

Liu C, Louise L, Zhang L, Luo R, Sylvia S, Medina A, Rozelle S, Smith DS, Chen Y, Zhu T (2017) Effect of deworming on indices of health, cognition, and education among schoolchildren in rural China: a cluster-randomized controlled trial. Am J Trop Med Hyg 96(6):1478–1489. https://doi.org/10.4269/ajtmh.16-0354

Lo NC, Bogoch II, Blackburn BG, Raso G, N'Goran EK, Coulibaly JT, Becker SL, Abrams HB, Utzinger J, Andrews JR (2015) Comparison of community-wide, integrated mass drug administration strategies for schistosomiasis and soil-transmitted helminthiasis: a cost-effectiveness

modelling study. Lancet Glob Health 3(October):e629–e638. https://doi.org/10.1016/S2214-109X(15)00047-9

Lo NC, Lai YS, Karagiannis-Voules DA, Bogoch II, Coulibaly JT, Bendavid E, Utzinger J, Vounatsou P, Andrews JR (2016) Assessment of global guidelines for preventive chemotherapy against schistosomiasis and soil-transmitted helminthiasis: a cost-effectiveness modelling study. Lancet Infect Dis 16(9):1065–1075. https://doi.org/10.1016/S1473-3099(16)30073-1

Luby SP, Rahman M, Arnold BF, Unicomb L, Ashraf S, Winch PJ, Stewart CP et al (2018) Effects of water quality, sanitation, handwashing, and nutritional interventions on Diarrhoea and child growth in rural Bangladesh: a cluster randomised controlled trial. Lancet Glob Health 6(3): e302–e315. https://doi.org/10.1016/S2214-109X(17)30490-4

Mekete K, Ower A, Dunn J, Sime H, Tadesse G, Abate E, Nigussu N et al (2019) The Geshiyaro project: a study protocol for developing a scalable model of interventions for moving towards the interruption of the transmission of soil-transmitted helminths and Schistosome infections in the Wolaita zone of Ethiopia. Parasit Vectors 12(503). https://doi.org/10.1186/s13071-019-3757-4

Miguel E, Kremer M (2004) Worms: identifying impacts on education and health in the presence of treatment externalities. Econometrica 72(1):159–217. https://doi.org/10.1111/j.1468-0262.2004.00481.x

Mitchell I, Hughes S, Ritchie E (2021) An overview of the impact of proposed cuts to UK aid | center for global development. Center for Global Development. https://www.cgdev.org/publication/overview-impact-proposed-cuts-uk-aid

Molyneux DH, Hotez PJ, Fenwick A (2005) 'Rapid-impact interventions': how a policy of integrated control for Africa's neglected tropical diseases could benefit the poor. PLoS Med 2(11):e336. https://doi.org/10.1371/journal.pmed.0020336

Montresor, Antonio, and Deniose Mupfasoni. 2019. Achievements of the deworming programme in Sri Lanka. Lancet Glob Health. Elsevier Ltd.. doi:https://doi.org/10.1016/S2214-109X(19)30309-2

Montresor A, Mupfasoni D, Mikhailov A, Mwinzi P, Lucianez A, Jamsheed M, Gasimov E et al (2020) The global progress of soil-transmitted helminthiases control in 2020 and World Health Organization targets for 2030. PLoS Negl Trop Dis 14(8):1–17. https://doi.org/10.1371/journal.pntd.0008505

Mwandawiro C, Okoyo C, Kihara J, Simiyu E, Kepha S, Campbell SJ, Freeman MC, Brooker SJ, Njenga SM (2019) Results of a national school-based deworming programme on soil-transmitted helminths infections and schistosomiasis in Kenya: 2012–2017. Parasites Vectors 12(76):1–18. https://doi.org/10.1186/s13071-019-3322-1

Neve JW, De RL, Andriantavison KC, Krisam J, Rajoela VH, Rakotoarivony RA, Rambeloson V, Schultz L, Qamruddin J, Verguet S (2018) Health, financial, and education gains of investing in preventive chemotherapy for schistosomiasis, soil-transmitted helminthiases, and lymphatic Filariasis in Madagascar: a modeling study. PLoS Negl Trop Dis 12(12):1–17. https://doi.org/10.1371/journal.pntd.0007002

Null C, Stewart CP, Pickering AJ, Dentz HN, Arnold BF, Arnold CD, Benjamin-Chung J et al (2018) Articles effects of water quality, sanitation, handwashing, and nutritional interventions on Diarrhoea and child growth in rural Kenya: a cluster-randomised controlled trial. Lancet Glob Health 6:e316–e329. https://doi.org/10.1016/S2214-109X(18)30005-6

Owada K, Nielsen M, Lau CL, Clements ACA, Yakob L, Soares RJ, Magalhães. (2017) Measuring the effect of soil-transmitted helminth infections on cognitive function in children: systematic review and critical appraisal of evidence. Adv Parasitol 98:1–37. https://doi.org/10.1016/bs.apar.2017.05.002

Pullan RL, Halliday KE, Oswald WE, Mcharo C, Beaumont E, Kepha S, Witek-McManus S et al (2019) Effects, equity, and cost of school-based and community-wide treatment strategies for soil-transmitted helminths in Kenya: a cluster-randomised controlled trial. Lancet 393(10185): 2039–2050. https://doi.org/10.1016/S0140-6736(18)32591-1

Redekop, William K., Edeltraud J. Lenk, Marianne Luyendijk, Christopher Fitzpatrick, Louis Niessen, Wilma A. Stolk, Fabrizio Tediosi, et al. 2017. The socioeconomic benefit to individuals of achieving the 2020 targets for five preventive chemotherapy neglected tropical diseases. Edited by Paul J Brindley PLOS Negl Trop Dis 11 (1): e0005289. doi:https://doi.org/10.1371/journal.pntd.0005289

Roodman, David. 2016. Why I mostly believe in worms. The GiveWell Blog December 6, 2016. https://blog.givewell.org/2016/12/06/why-i-mostly-believe-in-worms/

Roodman, David. 2017. How thin the reed? Generalizing from 'Worms at work.' The GiveWell Blog. January 4, 2017. https://blog.givewell.org/2017/01/04/how-thin-the-reed-generalizing-from-worms-at-work/

Roodman D (2018) Comment: the impacts of hookworm eradication in the American south, San Francisco

Shahriar AA, Alpern JD (2020) Antiparasitic drugs in the United States—two roads to high prices. Front Sociol 5(October):540478. https://doi.org/10.3389/fsoc.2020.540478

Stanton M, Molineux A, Mackenzie C, Kelly-Hope L (2016) Mobile technology for empowering health workers in underserved communities: new approaches to facilitate the elimination of neglected tropical diseases. JMIR Public Health Surveill 2(1). https://doi.org/10.2196/publichealth.5064

Stiles CW, Garrison PE (1906) A statistical study of the prevalence of intestinal Worms in man. US Government Printing Office

Stoll NR (1947) This wormy world. J Paristol 33:1–18

Stoltzfus RJ, Albonico M, Tielsch JM, Chwaya HM, Savioli L (1997) School-based deworming program yields small improvement in growth of Zanzibari school children after one year. J Nutr 127(11):2187–2193. https://doi.org/10.1093/jn/127.11.2187

Storey HL, Agarwal N, Cantera J, Golden A, Gallo K, Herrick T, Belizario V et al (2019) Formative research to inform development of a new diagnostic for soil-transmitted helminths: going beyond the laboratory to ensure access to a needed product. Edited by Ana Lourdes Sanchez. PLoS Negl Trop Dis 13(5):e0007372. https://doi.org/10.1371/journal.pntd.0007372

Sturchio JL (2001) The case of Ivermectin: lessons and implications for improving access to care and treatment in developing countries. Community Eye Health 14(38):22–23. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1705916/pdf/jceh_14_38_022.pdf

Taylor-Robinson DC, Maayan N, Soares-Weiser K, Donegan S, Garner P (2012) Deworming drugs for soil-transmitted intestinal Worms in children: effects on nutritional indicators, Haemoglobin and school performance (review). Cochrane Database Syst Rev 11:143. http://www.thecochranelibrary.com

Taylor-Robinson DC, Maayan N, Soares-Weiser K, Donegan S, Garner P (2015) Deworming drugs for soil-transmitted intestinal Worms in children: effects on nutritional indicators, Haemoglobin, and school performance (review). Cochrane Database Syst Rev 7:160. https://doi.org/10.1002/14651858.CD000371.pub6

Taylor-Robinson DC, Maayan N, Donegan S, Chaplin M, Garner P (2019) Public health deworming Programmes for soil-transmitted helminths in children living in endemic areas (review). Cochrane Database Syst Rev 9:172. https://doi.org/10.1002/14651858.CD000371.pub7

The END Fund (2020) The END Fund announces the deworming innovation Fund in Four African Countries on the inaugural world NTD day. https://www.prnewswire.com/news-releases/the-end-fund-announces-the-deworming-innovation-fund-in-four-african-countries-on-the-inaugural-world-ntd-day-300996147.html

Tilahun B, Gashu KD, Mekonnen ZA, Endehabtu BF, Angaw DA (2021) Mapping the role of digital health technologies in the case detection, management, and treatment outcomes of neglected tropical diseases: a scoping review. Trop Med Health 49(17). https://doi.org/10.1186/s41182-021-00307-1

Truscott JE, Déirdre Hollingsworth T, Brooker SJ, Anderson RM (2014) Can chemotherapy alone eliminate the transmission of soil transmitted helminths? Parasites Vectors 7(1):266. https://doi.org/10.1186/1756-3305-7-266

Truscott JE, Turner HC, Farrell SH, Anderson RM (2016) Soil-transmitted helminths: mathematical models of transmission, the impact of mass drug administration and transmission elimination criteria. Adv Parasitol 94:133–198. https://doi.org/10.1016/bs.apar.2016.08.002

Turner H (2021) Health economic analyses of the global Programme to eliminate lymphatic Filariasis. Int Health 13(Suppl.1):S71–S74. https://doi.org/10.1093/inthealth/ihaa095

Turner HC, Bundy DAP (2020) Programmatic implications of the TUMIKIA trial on community-wide treatment for soil-transmitted helminths: further health economic analyses needed before a change in policy. Parasites Vectors 12(102). https://doi.org/10.1186/s13071-020-3977-7

Turner HC, Truscott JE, Bettis AA, Shuford KV, Dunn JC, Déirdre Hollingsworth T, Brooker SJ, Anderson RM (2015a) An economic evaluation of expanding hookworm control strategies to target the whole community. Parasites Vectors 8(570). https://doi.org/10.1186/s13071-015-1187-5

Turner HC, Truscott JE, Hollingsworth TD, Bettis AA, Brooker SJ, Anderson RM (2015b) Cost and cost-effectiveness of soil-transmitted helminth treatment programmes: systematic review and research needs. Parasites Vectors 8(355). https://doi.org/10.1186/s13071-015-0885-3

Turner HC, Bettis AA, Chu BK, McFarland DA, Hooper PJ, Ottesen EA, Bradley MH (2016) The health and economic benefits of the global programme to eliminate lymphatic Filariasis (2000-2014). Infect Dis Poverty 5(54). https://doi.org/10.1186/s40249-016-0147-4

Turner HC, Bettis AA, Chu BK, Mcfarland DA, Hooper PJ, Mante SD, Fitzpatrick C, Bradley MH (2017) Investment success in public health: an analysis of the cost-effectiveness and cost-benefit of the global programme to eliminate lymphatic Filariasis. Clin Infect Dis 728(6):728–763. https://doi.org/10.1093/cid/ciw835

Turner HC, Jaspreet Toor TD, Hollingsworth, and Roy M. Anderson. (2018) Economic evaluations of mass drug administration: the importance of economies of scale and scope. Clin Infect Dis 66(8):1298–1303. https://doi.org/10.1093/cid/cix1001

Turner HC, Lauer JA, Tran BX, Teerawattananon Y, Jit M (2019a) Adjusting for inflation and currency changes within health economic studies. Value Health 22(9):1026–1032. https://doi.org/10.1016/j.jval.2019.03.021

Turner HC, Walker M, Pion SDS, McFarland DA, Bundy DAP, Basáñez M-G (2019b) Economic evaluations of onchocerciasis interventions: a systematic review and research needs. Trop Med Int Health 24(7):788–816. https://doi.org/10.1111/tmi.13241

Turner HC, French MD, Montresor A, King CH, Rollinson D, Toor J (2020) Economic evaluations of human schistosomiasis interventions: a systematic review and identification of associated research needs. Wellcome Open Res 5(45). https://doi.org/10.12688/wellcomeopenres.15754.2

Turner HC, Stolk WA, Solomon AW, King JD, Montresor A, Molyneux DH, Toor J (2021) Are current preventive chemotherapy strategies for controlling and eliminating neglected tropical diseases cost-effective? BMJ Glob Health 6(8):e005456. https://doi.org/10.1136/bmjgh-2021-005456

Uniting to Combat NTDs. 2012. London declaration on neglected tropical diseases. https://unitingtocombatntds.org/wp-content/uploads/2017/11/london_declaration_on_ntds.pdf

Uniting to Combat NTDs. 2016. Executive summary: beating 10 diseases, reaching a billion people. https://unitingtocombatntds.org/reports/5th-report/executive-summary/

Valente TW, Pumpuang P (2007) Identifying opinion leaders to promote behavior change. Health Edu Behav 34(6):881–896. https://doi.org/10.1177/1090198106297855

Walsh JA, Warren KS (1979) Selective primary health care. N Engl J Med 301(18):967–974. https://doi.org/10.1056/nejm197911013011804

Warren KS, Bundy DAP, Anderson RM, Davis AR, Henderson DA, Jamison DT (1993) Helminth infections. In: Disease control priorities in developing countries, 1st edn. Oxford University Press, Oxford, pp 131–160

Watts C (2017) Neglected tropical diseases: a DFID perspective. PLoS Negl Trop Dis 11(4). https://doi.org/10.1371/journal.pntd.0005492

Weiss DJ, Nelson A, Vargas-Ruiz CA, Gligorić K, Bavadekar S, Gabrilovich E, Bertozzi-Villa A et al (2020) Global maps of travel time to healthcare facilities. Nat Med 26(12):1835–1838. https://doi.org/10.1038/s41591-020-1059-1

Welch VA, Ghogomu E, Hossain A, Awasthi S, Bhutta Z, Cumberbatch C, Fletcher R et al (2016) Deworming and adjuvant interventions for improving the developmental health and Well-being of children in low- and middle-income countries: a systematic review and network meta-analysis. Campbell Syst Rev 12(1):1–383. https://doi.org/10.4073/csr.2016.7

WHA (2011) Resolution 54.19 schistosomiasis and soil-transmitted helminth infections, Geneva

WHO (2004) Intensified control of neglected diseases report of an international workshop, Geneva. www.who.int

WHO (2006a) Preventive in human helminthiasis chemotherapy, coordinated use of anthelmintic drugs in control interventions: a manual for health professionals and Programme managers. Switzerland, Geneva

WHO (2006b) Strategic and technical meeting on intensified control of neglected tropical diseases, Geneva. http://www.who.int/neglected_diseases/en/

WHO (2011) Helminth control in school-age children: a guide for managers of control Programmes, 2nd edn. Switzerland, Geneva. http://www.who.int/neglected_diseases/en

WHO (2012) Accelerating work to overcome the global impact of neglected tropical diseases: a roadmap for implementation. Switzerland, Geneva

WHO (2016) Coverage evaluation surveys for preventive chemotherapy: field guide for implementation. Switzerland, Geneva. http://www.who.int/about/licensing/copyright_form/en/index.html

WHO (2017) Preventive chemotherapy to control soil-transmitted helminth infections in at-risk population groups. WHO, Geneva. http://apps.who.int/bookorders

WHO (2020a) COVID-19: WHO issues interim guidance for implementation of NTD Programmes. WHO World Health Organization. http://www.who.int/neglected_diseases/news/COVID19-WHO-interim-guidance-implementation-NTD-programmes/en/

WHO (2020b) Ending the neglect to attain the sustainable development goals. World Health Organization, Geneva. https://doi.org/10.1596/978-1-4648-0524-0/ch17

WHO (2021) A sustainability framework for action against neglected tropical diseases 2021–2030. Ending the neglect to attain the sustainable development goals, Geneva. https://www.who.int/publications/i/item/9789240019027

WHO. n.d. Soil-Transmitted Helminths: Number of Children (Pre-SAC and SAC) Requiring Preventive Chemotherapy for Soil-Transmitted Helminthiases: 2019. Accessed April 7, 2021. https://apps.who.int/neglected_diseases/ntddata/sth/sth.html

WHO, and WHO Expert Committee on the Control of Schistosomiasis (2002) Prevention and control of schistosomiasis and soil-transmitted helminthiasis: report of a WHO expert committee, Geneva. https://apps.who.int/iris/handle/10665/42588

World Bank (1993) World development report 1993: investing in health. World development report 1993. Oxford University Press, New York. https://doi.org/10.1596/0-1952-0890-0

World Bank. n.d. World Bank Indicators. https://data.worldbank.org/indicator

Chapter 2
History and Diversity: Establishing a Context for Helminth Biology

Dante S. Zarlenga, Eric P. Hoberg, and Jillian T. Detwiler

Abstract Over the years, we have come to recognize that evolution is a dynamic process and a fundamental organizing principle for exploring diversity and the biosphere. Basic knowledge of systematics and phylogenetics within an evolutionary context is essential for gaining a flexible understanding of contemporary parasite diversity and developmental pathways and assessing how these are influenced by environmental perturbation and anthropogenic forcing. Further, an appreciation for historical processes as determinants of modern-day geographic patterns and host associations is needed to explore the outcomes of environmental change on parasite evolution and parasite faunal structure. Collectively, these data lead to a better predictive capacity for future changes in the distribution patterns and roles that parasites play in animal and human health. We provide a powerful alternative to a century of coevolutionary thinking that has dominated parasitology, in a succinct outline of the recently proposed Stockholm Paradigm which explores diversity, evolution, and biogeography of complex parasite-host assemblages. In this chapter, we highlight how insights from the past and knowledge of current parasite assemblages expose the impacts that accelerated climate warming, habitat perturbation, erosion of biodiversity, and changes in host adaptation have had on the ebb and flow of zoonotic infectious diseases. We further look at how molecular and biochemical studies have advanced systematics, taxonomic stability, and diagnostic capability and are guiding future progress toward understanding parasites, parasitism, and their relationships to global public health.

D. S. Zarlenga (✉)
Animal Parasitic Diseases Lab, Agricultural Research Service, Beltsville, MD, USA

E. P. Hoberg
Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI, USA

Department of Biology and Museum of Southwestern Biology, University of New Mexico, Albuquerque, NM, USA

J. T. Detwiler
Department of Biological Sciences, Faculty of Science, University of Manitoba, Winnipeg, MB, Canada

F. Bruschi (ed.), *Helminth Infections and their Impact on Global Public Health*,
https://doi.org/10.1007/978-3-031-00303-5_2

2.1 Introduction

Systematics is the foundation for biology. It provides a basic evolutionary map to discover, characterize, and interpret global diversity and our place in the biosphere. It also allows us to explore questions related to host associations, life histories, genetics, and patterns of infection and disease, the cornerstones of epidemiology. Systematics reflects the intersection of phylogeny (evolutionary or genealogical relationships of organisms) and taxonomy (a standard nomenclature, the process of species delimitation, and the theory and practice of classification). Significantly, it brings history to the table and links evolution, ecology, and biogeography.

Many operational definitions or "species concepts" have arisen over time, and these concepts have become more convoluted as the number and types of characters and methodologies have increased, including the use of biochemical and genetic information. Scientists seeking to establish and validate helminth systematics are seemingly disadvantaged in that they lack fossil records to support present-day classifications. Instead, they must reconstruct evolutionary history that gave rise to assemblages of considerable diversity predicated on examining characters in a continuum from comparative morphology to whole genome sequences and based on informatics and insights from extant organisms only. With concepts of phylogenies in hand, host and parasite associations can be explored over time and across a global arena that is under dynamic change. Episodic change or perturbation at all levels of history, linking evolutionary and ecological time, introduces uncertainty but also drives the overall structure of complex biological systems including those represented by hosts and parasites (Brooks et al. 2019).

Heisenberg, a German theoretical physicist, conveyed pioneering insight on quantum mechanics, uncertainty, and indeterminacy. In his 1927 paper on the Uncertainty Principle, he commented on the relationship between the position and the momentum of photons and the future behavior of an atomic particle. In his discussion, he indicated that ". . .it is not the conclusion that is wrong (determining future behavior) but the premise (predictability). . . ." He determined that our observations have a direct effect on perceived behavior of quanta or, more generally, on outcomes. His discussion of observer effects on measuring and conveying scientific data is most applicable when trying to understand the concepts of classification, evolution, and the ever-changing role that the environment plays on diversification.

Many philosophies have been put forth on the natural order of things. J. J. C. Smart (1959) suggested that biology is a dynamic entity that neither creates nor refutes but is a manifestation or technological application of the laws of physics and chemistry:

> . . .in the (physical type sciences) we are interested in laws, whereas in (the biological type sciences) we are interested in the natural history of structure . . . and in the explanation of why things with this natural history function as they do.

Smart's premise was there could be no "laws of nature" that guide biological species because any biological laws like the laws of physics would, by definition, disallow deviation. Michael Ghiselin (1974) further suggested that biological species and

monophyletic taxa are not nominal classes but actual individuals where multiplicity is not required to define a class. Thus, it behooves us to think of and investigate evolution, speciation, and outcomes represented in taxonomy as ongoing processes, producing a more fluid and mutable understanding of species in both space and time. Consequently, we deal with snapshots or slices in contemporary time that do not immediately reflect the dynamic oscillations and complexity of past events and associations, nor do they always anticipate future patterns of diversity (e.g., Hoberg and Zarlenga 2016). Stable classifications and taxonomy with considerable predictive power to reveal structure, history, and connectivity in the biosphere require ample field-based sampling, a valid comparative (phylogenetic) context, and inclusive consideration of environmental mosaics in which we reside. Critical insights emerge directly from a foundation of specimen-based archives and information systems developed across networks of natural history museums (e.g., Dunnum et al. 2017; Cook et al. 2020; Colella et al. 2021). These pathways, a creative nexus in science, are prone to human interpretation, frailty, and change, much as Heisenberg discovered in the movement of subatomic particles (Hull 1988).

A wealth of reviews, chapters, and articles has been written on the taxonomy and systematics of helminths. Since specific topics and detailed presentations of the biology of each parasite group constitute other portions of this book, we offer a look at helminth phylogeny from a different perspective. We endeavor to examine why "history matters." A deeper understanding of the historical arena on global to landscape scales contributes to our knowledge of complex host-parasite assemblages. Geographic patterns, host associations, and historical determinants (abiotic and biotic) are foundations for examining the outcomes of perturbation and allow us to predict and anticipate future changes in the distribution of parasites through niche modeling (Peterson 2011) and, by extension, their potential impact on human and animal health, including the realm of food security on landscape to global scales (Brooks and Hoberg 2013; Hoberg and Brooks 2015; Brooks et al. 2019; Brooks et al. 2022; Trivellone et al. 2022). We highlight how past and current evidence provides a window from which to explore a future of dynamic change caused by accelerated climate warming, habitat perturbation, erosion of biodiversity, dissemination of invasive species, changes in host adaptation, and emergence of zoonotic infectious diseases. Highlighted are recent advancements in molecular identification and population genetics to underscore the value of well-engineered population research to advancing sound taxonomy. Finally, we consider how studies on genomics and phylogenomics have begun to better inform us on the broader "Tree of Life." In so doing, we hope to help advance and guide future progress in understanding parasitology and its relationship to global public health.

2.2 Complexities Surrounding Helminth Systematics

Helminths that typically utilize humans either as primary or intermediate hosts are represented by roundworms (phylum Nematoda), flukes (Digenea), and tapeworms (Eucestoda), the latter two belonging to the phylum Platyhelminthes. There are no members of the phylum Acanthocephala that commonly parasitize humans. Body form and symmetry among the primary helminths vary widely, although each major phylum is characterized by a general plan and structure. The nematodes are dioecious (either male or female). Those which infect people commonly include gastrointestinal parasites such as hookworms and ascarids (geo-helminths) and lymphatic parasites such as those that cause onchocerciasis and filariasis. All nematodes possess cylindrical bodies, a fluid-filled pseudocoelom, and a complete tubular digestive system in all stages of development. The body is relatively impervious to the external environment because of an outer cuticle that is synthesized at the end of each larval stage and just prior to molting. In contrast, the Platyhelminthes or flatworms have a soft, solid-tissue body circumscribed by a plasma membrane or tegument. Unlike other bilaterians, the flatworms have neither a coelom nor a complete digestive tract. Specialized organs for circulatory or respiratory systems are to some extent reduced. In the absence of circulatory and respiratory systems, oxygen and other nutrients must diffuse across a permeable tegument. All platyhelminths are hermaphroditic except for blood flukes (schistosomes), which are dioecious. In their adult, sexually mature stage, all helminths are macroparasites and relatively large ($>$ 1 mm long), though some adult tapeworms can be measured in meters rather than millimeters. Although there are numerous biological and developmental characters that link these groups, faunal and morphological diversity abounds.

Revealing the complex tapestry of human helminths and infection relies on clear definitions of faunal diversity, species, and populations. Diversity, encompassing spatial, ecological, and genealogical aspects, can be considered in hierarchical patterns relative to scale. It extends from regional faunas to species, cryptic diversity (e.g., cryptic morphospecies), and populations (Hoberg 1997; Pérez-Ponce de Léon and Nadler 2010; Hoberg et al. 2015). As a gateway to fine-scale relationships, variation in local haplotype diversity is ephemeral and serves as an indicator for landscape epidemiology (analyses of ecosystems) and regional processes. It is the foundation for understanding patterns of emergence (Thompson 2005). Landscape evaluations remain essential for understanding basic determinants for occurrence, emergence, and disease. It is critical to recall the axiom that the distribution of disease is often heterogeneous and local and occurs in circumscribed islands within a more extensive spatial range for a parasite or host-parasite assemblage (Audy 1958; Hoberg 2010; Brooks et al. 2019). Thus, it becomes important to use molecular phylogenetic and phylogeographic methods to understand the history of genetic variation within populations, the genetic differentiation between populations, and the extent of gene flow among populations (Avise 2000; Criscione et al. 2005; Huyse et al. 2005; Nieberding et al. 2008; Davies et al. 2020).

These relationships for spatial and temporal scale highlight the importance of phylogeny and hierarchical order in framing hypotheses and constraining explanations for species diversity, faunal structure, and history. In recent years, we have seen a transformation in how we view the nature of species and a shift from typological and authoritative approaches that characterized much of the twentieth century (Brooks and McLennan 2002; Brooks et al. 2019) to those that involve hypothesis testing. Modern-day hypotheses emerged from an evolutionary species concept (historical and phylogenetic definitions of species) followed by secondary evaluation relying on aspects of biogeography, ecology, and reproductive isolation (aspects of the biological species concept) (see Brooks and McLennan (2002) and Wiley and Lieberman (2011)). Species delineation and the process of speciation (the mechanisms involved in the origins of species) are linked, where history (phylogeny) allows the recognition of species followed by testable hypotheses based upon the biological species concept (Brooks and McLennan 2002; Nadler 2002; see also Sect. 2.3.1). Recent examples are analyses that explore species diversity within Taeniidae and among species of *Taenia* and *Echinococcus* (Nakao et al. 2007, 2009, 2013a, b; Lavikainen et al. 2008, 2016; Terefe et al. 2014; Yanagida et al. 2014; Lee et al. 2016; Ito et al. 2017) and studies of species richness in *Trichinella* (Zarlenga et al. 2006; Pozio et al. 2009; Korhonen et al. 2016; Sharma et al. 2020). Insights from molecular sequences and genomic data have led to substantial reorganization within the Taeniidae including establishment or resurrection of several generic-level taxa (e.g., Lavikainen et al. 2008, 2016) and refinements in our understanding of historical biogeography and diversification (e.g., Yanagida et al. 2014; Brooks et al. 2019).

2.2.1 Helminth Parasites and the World Stadium

The impact of parasites occurs at the junction of human populations and behavior, ecosystem structure, and globalization in a matrix increasingly determined by climatological forces of anthropogenic origin and environmental perturbation (Patz et al. 2000, 2007, 2008; Brooks and Hoberg 2007, 2013; Weaver et al. 2010; Brooks et al. 2019). Despite thousands of years of medical and veterinary intervention, helminth parasites remain a considerable regional and global concern for people, their domestic food animals, and free-ranging vertebrate species. Over the past 10,000–15,000 years, the evolution of agriculture, animal domestication, urbanization, transformation of natural habitats, and globalization have all been driving forces for the emergence of helminth and other diseases (Daszak et al. 2000; Patz et al. 2008; Rosenthal et al. 2008; Hoberg 2010; Kuris 2012). These historical processes are equivalent to those in a present-day regime of accelerating environmental change (Brooks and Ferrao 2005; Hoberg and Brooks 2008, 2013; Brooks et al. 2019). Tipping points represented by a burgeoning human population, the development of agriculture, animal domestication, and our expanding global footprint (Barnosky et al. 2012; Pecl et al. 2017) have had a direct influence on the

occurrence of parasites in humans; however, many host-parasite associations for extant parasites have considerably deeper origins extending into the Pliocene and Pleistocene (synthesis in Brooks et al. 2019).

Parasites are often obscure, although collectively they represent more than 40–50% of the organisms on Earth. They are integral components of all ecosystems and have considerable involvement in at least 75% of trophic links within food webs (Dobson et al. 2008; Lafferty et al. 2008; Kuris et al. 2008). Significantly, 61% of all pathogens are zoonotic, derived primarily through interactions with free-ranging vertebrate species (Daszak et al. 2000). Human pathogens (primarily the microparasite assemblage of viruses and bacteria) are often associated with wildlife (Taylor et al. 2001; Cleaveland et al. 2001; Wolfe et al. 2007; Jones et al. 2008). Emergent and pandemic disease as evidenced by the H1N1 influenza outbreak of 1918 and most recently the SARS CoV-2 coronavirus outbreak of 2020 indicates the scope and nature of circulation among vertebrate reservoirs, the importance of interfaces, and the potential for host colonization (e.g., Mollentze and Streicker 2020; Brooks et al. 2019; Boeger et al. 2022; Hoberg et al. 2022). This intricate web of interactions establishes the significance of human parasites as mediators of health and well-being, food sustainability, food and water security, availability and safety, socioeconomic development, and, more broadly, ecological structure and services that contribute to continuity and connectivity in the biosphere (Patz et al. 2007, 2008; Polley 2005; Whitmee et al. 2015; Trtanj et al. 2016; Watts et al. 2017a, b).

Based on global estimates, between 75,000 and 300,000 species of helminths infect terrestrial and aquatic vertebrates (Dobson et al. 2008). More recent estimates, based on specimens in the former US National Parasite Collection, suggest a range of 100,000 to 300,000 species of helminths of which 85-95% remain undiscovered (Carlson et al. 2020). Among these, 287 are known to occur in humans, 95% of which are zoonotic (Cleaveland et al. 2001; Taylor et al. 2001). An alternative estimate places this number at 305 helminth species in humans, with 83 identified as prevalent and 39 able to cause substantial morbidity or mortality (Ashford and Crewe 2003; Kuris 2012) (Table 2.1). Among this larger assemblage, 39 species have patterns of circulation and transmission that are solely dependent on human hosts. Overall, only 44% of the most prevalent (and known) micro- and macroparasites are considered zoonotic. This more conservative estimate denotes parasites as zoonotic because they cannot be sustained in humans as definitive hosts (consistent with $R_0 < 1$) and includes helminths for which humans may be infected by larval stages such as the metacestodes of certain taeniid tapeworms (species of *Taenia* and *Echinococcus*) or larvae of nematodes such as *Baylisascaris*, *Anisakis* and *Pseudoterranova*, and *Toxascaris* and *Toxocara* (Polley 2005; Kuris 2012). Consequently, species of *Taenia* utilizing people as definitive hosts (*T. saginata*, *T. solium*, and *T. asiatica*), but requiring domestic ungulates for transmission, are not regarded as zoonotic; however, they are among the most important of recognized foodborne parasites (e.g., Robertson et al. 2013, 2014). Among these, only *T. solium* is considered highly pathogenic as the causative agent of human neurocysticercosis.

Table 2.1 Helminth species characteristic of people across the world including those dependent on humans for transmission and some prominent zoonotic parasites (based on Ashford and Crewe (2003) with modifications from Jenkins et al. (2013), Nakao et al. (2013b), McBurney-Lin et al. (2018), Deplazes et al. (2019), Otranto and Deplazes (2019), and Sapp and Bradbury (2020))

Platyhelminthes
Digenea (11 human-dependent species)
Schistosomatidae-
Schistosoma haematobium[a]
Schistosoma intercalatum[a]
Schistosoma japonicum[b]
Schistosoma mansoni?[a]
[+ species of *Schistosoma* (8), *Gigantobilharzia* (2), *Trichobilharzia* (4)][c]
Echinostomatidae-
Echinostoma echinatum[b]
[+ species of *Acanthoparyphium* (2), *Artyfechinostoma* (2), *Echinocasmus* (5), *Echinostoma* (11), *Hypoderaeum* (1)][c]
Gymnophallidae-
[*Gymnophalloides seoi*][c]
Fasciolidae-
Fasciolopsis buski[b]
[+ species of *Fasciola* (2)][c]
Gastrodiscidae-
Gastrodiscus hominis[b]
Heterophyidae-
Heterophyes heterophyes?[b]
[+ species of *Apophallus* (1), *Centrocestus* (5), *Cryptocotyle* (1), *Haplorchis* (5), *Heterophyes* (5), *Metagonimus* (4), *Stictodora* (3)][c]
Opisthorchidae-
Clonorchis sinensis?[a]
Opisthorchis fellineus?[b]
[+ species of *Metorchis* (2)][c]
Paragonimidae-
Paragonimus westermanii[b]
[+ species of *Paragonimus* (8)][c]
Troglotrematidae-
[*Nanophyetus salmincola*][c]
Eucestoda (6 human-dependent species)
Diphyllobothriidae-
Diphyllobothrium latum[b]
[+ species of *Diphyllobothrium* (15), *Diplogonoporus* (3), *Pyramicocephalus* (1), *Schistocephalus* (1)][c]
[*Spirometra* (4), as plerocercoid or sparganum][c]
Anoplocephalidae-
Inermicapsifer madagascariensis[b]
[+ species of *Bertiella* (2), *Raillietina* (3)][c]

(continued)

Table 2.1 (continued)

Dilepididae-
[*Dipyllidium caninum*][c]
Hymenolepididae-
Rodentolepis nana[a]
[+ *Hymenolepis diminuta*][c]
Taeniidae-
Taenia asiatica[a]
Taenia saginata[a]
Taenia solium[a]
[+ species of *Echinococcus* (6), *Taenia* (6) as metacestodes][c]
[*Versteria* cf. *mustelae* [+ *Versteria* sp. as metacestodes][c]
Mesocestoididae-
[species of *Mesocestoides* (2)][c]
Nematoda (22 human-dependent species)
Strongyloididae-
Strongyloides fuelleborni fuelleborni[b]
Strongyloides fuelleborni kellyi[a]
Strongyloides stercoralis[a]
Ancylostomatidae-
Ancylostoma duodenale[a]
[+ species of *Ancylostoma* (4)][c]
Necator americanus[a]
Chabertiidae-
Oesophagostomum bifurcum[b]
Ternidens deminutus[b]
Trichostrongylidae-
Trichostrongylus colubriformis[b]
Trichostrongylus orientalis[b]
Angiostrongylidae
Angiostrongylis costaricensis[c]
Angiostrongylis cantonensis[c]
Oxyuridae-
Enterobius gregorii[a]
Enterobius vermicularis[a]
Ascarididae-
Ascaris lumbricoides[a]
[+ species of *Baylisascaris* (1), *Toxocara* (2), *Toxascaris* (1)][c]
Anisakidae-
[species of *Anisakis* (2), *Pseudoterranova* (1)][c]
Dracunculidae-
Dracunculus medinensis[a]
Thelazidae-
Thelazia callipaeda[c]

(continued)

Table 2.1 (continued)

Thelazia californiensis[c]
Gnathostomatidae-
[species of *Gnathostoma* (6)][c]
Gongylonematidae-
[*Gongylonema pulchrum*][c]
Onchocercidae-
Brugia malayi[b]
Brugia timori[a]
Loa loa[a]
Mansonella ozzardi[a]
Mansonella perstans[a]
Mansonella streptocerca[a]
Onchocerca volvulus[a]
Onchocerca cervicalis[c]
Onchocerca dewitti[c]
Onchocerca lupi[c]
Wuchereria bancrofti[a]
Dirofilaria immitus [+ species of *Dirofilaria* (4)][c]
Dipetalonema arbuti, *D. sprenti*[c]
Trichuridae-
Trichuris trichiura[b]
[+ *Calodium hepaticum*, *Eucoleus aerophilus*, *Paracapillaria philippinensis*][c]
Dioctophymidae-
[*Dioctophyme renale*][c]
Trichinellidae-
Trichinella spiralis[b]
[+*T. britovi*, *T. murrelli*, *T. nativa*, *T. nelsoni*, *T. pseudospiralis*][c]
Acanthocephala (0 human-dependent species)
Macracanthorhynchus hirudinaceus[c]
Macracanthrohynchus ingens[c]
Moniliformis moniliformis[c]

[a]Parasites completely dependent on human transmission
[b]Parasites that occur among humans and other definitive hosts, and for which people are not required for transmission, but may be involved in circulation
[c]Parasites that represent prominent regional to local zoonoses, and in which humans are not involved in transmission or circulation

Approximately 25% of the world's population is infected with helminth parasites. Among these, there are 100–150 million suffering substantial morbidity. Not all parasites exact equivalent costs in human health as they vary in virulence, prevalence, abundance, and pathogenicity (Kuris 2012). For example, the soil-transmitted nematodes *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus*, and *Ancylostoma duodenale* are cosmopolitan and cause greater morbidity in humans than any other parasitic diseases except malaria (Murray and Lopez 1996; Weaver

et al. 2010). Further, the distribution posed by diseases and the impact of parasitic helminths are often heterogeneous. Local factors related to history, climate, land use, food habits, demographics, human behavior, and sanitation play prominent roles as determinants of human infections (Patz et al. 2007; Weaver et al. 2010; Kuris 2012). An emerging challenge can be seen in the disruption of socioeconomic controls over the occurrence and impact of infection. Rapidly accelerating climate change, environmental perturbation, biodiversity loss, and armed conflict with displacement and movement of refugees, all lead to challenges for food and water security and sustainability. A concomitant breakdown in medical infrastructure interacts with these abiotic and biotic drivers as synergistic threat multipliers that facilitate new patterns of infection and disease (e.g., Patz et al. 2007, 2008; Brooks and Hoberg 2013; Whitmee et al. 2015; Watts et al. 2017a, b; synthesis in Brooks et al. 2019).

2.2.2 Host Colonization Drives Helminth Evolution

History is a defining factor in exploring and understanding contemporary distributions and risk space posed by helminth parasites in human populations. Traditionally, explanations for host occurrence, biogeography, and diversity have been linked to coevolutionary histories (cospeciation/association by common descent of host and parasite lineages) (e.g., Brooks and McLennan 1993, 2002; Brooks and Hoberg 2013) wherein parasite faunas of humans and other vertebrates are largely derived from historical ancestor-descendant relationships with other primates (Kuris 2012). Considering the broader dimensions of diversity, a prevailing, and largely continuing, assumption describing complex host-parasite assemblages has been that parasites coevolve with their hosts (e.g., Brooks and Ferrao 2005; Brooks et al. 2015; Nylin et al. 2018, Brooks et al. 2019; and earlier papers cited therein). This coevolutionary/cospeciation perspective continues to be omnipresent, extending to contemporary assumptions about the difficulty for host-specific parasites to undergo host switching across vertebrate lineages (Brooks and Ferrao 2005; Brooks et al. 2015). The expected interdependence of these phenomena (cospeciation and specificity) discounts host colonization by otherwise narrowly distributed helminths and creates the *parasitological paradox* (Agosta et al. 2010).

Observations across the biosphere define the paradox: (i) parasites are considered extreme ecological specialists, especially with respect to co-adaptation and persistence limited to a small range of hosts, and therefore should rarely switch hosts and (ii) host colonization among related or unrelated hosts is commonly revealed in phylogenetic studies (Hoberg and Brooks 2008; Nylin et al. 2018). It behooves us to explore the apparent paradox posed by cospeciation, host specificity, and colonization (Hoberg and Brooks 2008, 2013; Agosta et al. 2010; Brooks and Hoberg 2013; Brooks et al. 2019). The paradox is significant because it relates directly to faunal assembly and outcomes for episodic ecological collision, invasion, and mixing, which define the arena for emergent pathogens in space and time. Conceptually and operationally, this is important given that specificity has been regarded as an

historical bulwark creating a protective bubble limiting the potential for colonization by pathogens. The cospeciation bubble thus stands as the basis for erroneous assumptions about the rarity and unpredictability of emergent pathogens and diseases in space and time (e.g., Brooks and Ferrao 2005; Brooks et al. 2015, 2019, 2022; Boeger et al. 2022).

A challenge to an orthodoxy of association by descent is an empirical record clearly demonstrating that host colonization is common across all scales of Earth history (e.g., Hoberg and Brooks 2008). Episodes of host colonization have directly influenced parasite faunal structure across the biosphere in space and time, including associations with humans (we are not exceptional!), and are the basis for what we recognize as emerging infectious diseases in humans, food animals, and crops (Brooks et al. 2019, 2022; Trivellone et al. 2022). Although cospeciation as the driving force behind complex host-parasite associations and faunas has had limited explanatory power, it has remained convenient and provides a simple, elegant view of faunal diversification and assembly through time (Nylin et al. 2018). Such a simplified view of a dynamic biosphere has hindered studies of global complexity and the outcomes of geographic expansion, ecological perturbation, and host colonization as prominent processes (e.g., Hoberg and Brooks 2010). Biotic expansion including anthropogenic invasion is pervasive; episodes leading to the breakdown of ecological isolation and barriers to host colonization have important implications for the distribution and evolution and assembly of helminth faunas and more generally emerging infectious diseases (Wolfe et al. 2007; Hoberg 2010; Brooks and Hoberg 2013; Hoberg and Brooks 2013; Cook et al. 2017).

An alternative view, proposed as the *Stockholm Paradigm*, resolves the paradox and more completely accommodates the apparent discordance for empirical findings about cospeciation and colonization (Hoberg and Brooks 2015; Araujo et al. 2015; Brooks et al. 2019). The Stockholm Paradigm is an evolutionary/ecological synthesis integrating four core processes, each with an extensive history of discussion: (1) the *Ecological Fitting* (EF) (Janzen 1985; Agosta et al. 2010); (2) the *Oscillation Hypothesis* (OH) (Janz and Nylin (2008); (3) the *Geographic Mosaic Theory of Coevolution* (GMC) (Thompson 2005); and (4) the *Taxon Pulse Hypothesis* (TP) (Erwin 1985).

As summarized by Hoberg et al. (2015), "EF refers to the ability of ecological specialists to host switch easily and without prior evolution of novel host-use capabilities, when the host resource upon which they are specialized is phylogenetically conservative and widespread. The OH postulates that large-scale evolutionary diversification of interspecific ecological associations involves an initial phase (permitted by EF) in which host-range increases, setting the stage for the parasite to become an ecological generalist, which in turn sets the stage for the generalist parasite to become fragmented into new specialists. The GMC describes the microevolutionary co-adaptive dynamics among new combinations of interacting species, explaining the emergence of new specialists from ancestral generalists. The relative ease with which host switches, oscillations, and new co-adapted associations can arise, reflects that even ecological specialists exist in a 'sloppy' rather than a tightly optimized fitness space (Agosta and Klemens 2008; Agosta and Brooks 2020;

Agosta et al. 2010). The TP dynamic postulates that species-level biodiversity results from alternating episodes of biotic expansion and biotic isolation. This appears to be largely responsible for altering geographic and trophic ecological contexts, leading to opportunity for new arrays of associations to arise often manifested in mosaic structure that relates to faunal assembly over space and time (Hoberg et al. 2012). In conjunction with EF, host colonization is maximized during phases of biotic expansion (disruption), whereas stasis promotes emergence of new specialists during episodes of geographic isolation (Hoberg and Brooks 2008, 2010). The Stockholm Paradigm provides a new way to understand emerging pathogens and diseases in the biosphere, including a shift in emphasis from reactive to proactive and anticipatory policies of management."

The salient points are that episodes of ecological disruption lead to movement and it is that response which ultimately creates opportunity. Movement, breakdown in ecological isolation, and faunal mixing (creating mosaic faunas) establish new interfaces and opportunities. Opportunity meeting capacity for resource exploitation by pathogens in the arena of ecological fitting leads to colonization and establishes new patterns of diversification and faunal assembly and structure. Colonization does not require the origin of a magic or novel mutation for exploitation of a new host group. Events of host colonization are more likely to occur among phylogenetically proximate host groups and often with limited disease apparent (e.g., consistent with cophylogenetic histories among rodent or lagomorph groups and helminths – Galbreath and Hoberg 2015; Haukisalmi et al. 2014, 2016; Bell et al. 2016; Haas et al. 2020). When colonization occurs across widely disparate host groups, the process is complex and may involve a stepping-stone dynamic (e.g., Araujo et al. 2015) as exemplified in the emergence of SARS-CoV2 which resulted from sequential colonization events apparently from chiropterans to humans through an array of mammals including some carnivores (e.g., Brooks et al. 2020; Morens and Fauci 2020). Further, colonization across broad phylogenetic gulfs appears associated with heightened consequences for disease (Guth et al. 2019) and may reflect the degree to which such host assemblages are marginal fitness space for pathogen development and persistence (Brooks et al. 2019).

Whereas coevolutionary history can explain some helminth faunas in Great Apes and humans (e.g., pinworms, species of *Enterobius*, and hookworms, species of *Oesophagostomum*) (Brooks and Ferrao 2005), the reality is considerably more complex and fascinating. A contemporary helminth fauna in humans has been cumulative, serving to indicate the rich temporal, spatial, and ecological connectivity that *Homo sapiens* have had across the biosphere in space and time (e.g., Hoberg 2006; Terefe et al. 2014; Yanagida et al. 2014; Brooks et al. 2019). The diverse helminth fauna among humans denotes dynamic and episodic shifts in climate, habitat, and ecological structure during the Late Pliocene and Quaternary (Hoberg et al. 2012; Cook et al. 2017). These changes occurred in migratory/dispersal capacity and in foraging behavior among our initial hominoid (Brooks and Ferrao 2005; Folinsbee and Brooks 2007) and immediate human ancestors (Hawdon and Johnston 1996; Jenkins et al. 2013) and among our contemporary worldwide population. Emphasized are the importance of history and scale and the connectivity

of processes for geographic and host colonization in evolutionary and ecological time. Many human parasites have origins linked to shared trophic relationships and host switching among carnivorans, other mammals and birds that are either carnivores or piscivores (Hoberg et al. 2001; Ashford and Crewe 2003; Kuris 2012; Brooks et al. 2019). As such, geographic proximity, ecological structure, and connectivity among foraging guilds, as reflected in the capacity of ecological fitting, are key drivers of parasite faunal assembly and diversification. Events such as these account for numerous host-specific parasites in humans such as species of *Taenia.*

Shared trophic resources are also the basis for many contemporary zoonotic infections and less involved in the process of parasite speciation (Kuris 2012). For example, species of *Diphyllobothrium* and diphyllobothriid tapeworms known to parasitize marine mammals and assemblages of *Trichinella* among mammalian hosts at high latitudes of the Nearctic also parasitize humans (Jenkins et al. 2013). The considerable diversity of heterophyid, echinostomatid, and other trematodes transmitted through freshwater and marine fishes and crustaceans promotes their circulation among assemblages of vertebrates, including humans, throughout the world (Marty and Andersen 2000).

2.2.3 *Anthropogenic Translocation of Parasitic Helminths*

Sorting out which parasites are our coevolutionary legacies (distributed out of Africa or other regions with hominid expansion) and which were acquired through ecological dynamics provides a nuanced understanding of the mechanisms involved in faunal assembly. Contemporary global expansion (from Africa and Eurasia into North America) has led to a breakdown of geographic and ecological isolation and an increasingly broad exposure of humans to "exotic" helminths and other parasites (Daszak et al. 2000; Harcourt 2012; Jenkins et al. 2013). Global invasion and secondary distribution of parasites (anthropogenic translocation) coincided with early Eurasian trade routes, European expansion, colonial occupations, and slave trade. As a result, a rich temporal (chronological) and spatial (geographic from landscape to regions) mosaic for acquisition, introduction, and establishment of helminth assemblages has emerged (Hoberg 2010; Hoberg et al. 2012; McMichael et al. 2017; Brooks et al. 2019). In a contemporary setting, anthropogenic drivers increasingly influence invasion and distribution of parasites and pathogens with attendant threats across a matrix linking environments, economies, and societies (Pimentel et al. 2005). The character and evolution of geographic expansion for both free-living and parasitic species have also been influenced by a series of thresholds and tipping points in human history beginning with multiple episodes of our expansion out of Africa, mediated by climate and environmental oscillations, extending to nearly 200 Kya (de Menocal and Stringer 2016; Groucutt et al. 2018). Further, the advent of agriculture and animal husbandry 10 to 11 Kya, the age of European exploration ensuing around the year 1500, and the industrial revolution have all represented irreversible points of change for people and our

interface with the environment (e.g., Riccardi 2007; Harcourt 2012; McMichael et al. 2017). Today, human influence is a pervasive force in evolution as seen in natural systems and in the diverse assemblages of pathogens in both free-ranging and domesticated hosts (Palumbi 2001; Pecl et al. 2017; Barnosky et al. 2012). These emerged from a burgeoning population and a transition from a slow and large world dominated by isolation and local effects to a rapid and small world resulting from globalization, homogenization, and integration of fragmented environmental networks (Hoberg 2010; Hoberg and Brooks 2013; Brooks et al. 2019).

2.3 Defining Diversity

In exploring the history of the biosphere, information on biodiversity is only as useful as the conceptual universe in which it is explained. Accurate definitions of diversity are essential to understanding the role of parasites in human and animal diseases. These definitions are also critical to studying epidemiology, developing management practices to limit transmission, and designing treatment regimens to reduce, mitigate, or eliminate infections. Over the past 200 years, species-level identification of specimens has relied on comparative morphology and is often dependent on examining fully developed adult worms. This is best exemplified by the challenges in diagnosing zoonotic helminths in human infections (Jenkins et al. 2013). In the absence of mature or gravid specimens, authoritative morphological identification has often not been possible due to the absence of reliable structural attributes in other parasitic stages. It was not until the advent and application of reliable and rapid molecular-based diagnostic methodologies (Polley and Thompson 2009; Jenkins et al. 2013) that these problems began to resolve themselves. Molecular-based diagnostics can now supplant preparation and microscopic examination of whole specimens, although such approaches remain directly tied to definitive identification of adult parasites through linkage to a morphospecies name and concept. Validation of molecular data from multiple authoritatively identified adults, held as archival vouchers in museum collections, is the gateway for application of sequences and appropriate molecular markers for diagnostics of life history stages including eggs and larvae.

Reliance on archival museum collections as resources for biodiversity informatics and our study of the biosphere, including history and structure, remains a fundamental but underappreciated foundation (Cook et al. 2013, 2020; Dunnum et al. 2017). Museum collections and specimens are the self-correcting records for biodiversity that document the geographic occurrence and host associations for parasites derived from repeated sampling events in the biosphere. As such, they remain especially relevant to understanding diversity and changing patterns of distribution over time. Deposition and full documentation of specimens (parasites and fully identified host specimens or tissues) and characterization of their environmental context in appropriate archives should be the expectation from ongoing programs for host-parasite surveys and strategic monitoring for particular spectrums of pathogens

(Hoberg 2010; Cook et al. 2013, 2017; Brooks et al. 2014). In this manner, the influence of accelerated climate change, ecological perturbation, human activities and invasion, and other factors that determine the dynamic distributions of pathogens and disease may be tracked in space and time through the application of comparative baselines. Specimens combined with molecular protocols as described below have become the foundation for exploring patterns of cryptic diversity (Pérez-Ponce de Léon and Nadler 2010) and for understanding the nature and structure of emergent infectious diseases (Thompson 2005; Hoberg et al. 2012; Brooks et al. 2014, 2020).

The Stockholm Paradigm can promote a nuanced view of the biosphere and the distribution of biodiversity including pathogens. Increasingly sustainable and cost-effective approaches to anticipating, mitigating, and managing emergent diseases in space and time are possible and in direct contrast to reactive modes associated with current epidemiological foundations (Brooks et al. 2014, 2019, 2020; Cook et al. 2020). As we outlined recently (Hoberg et al. 2015):

> In an increasingly proactive stance, the basic tenets of the Stockholm Paradigm direct attention to emerging infectious diseases, before they happen, in the context of ecological perturbation, using knowledge of biodiversity, past environments, and equivalence of biological processes to anticipate the future in a world of rapid change. Host switching and emergence do not occur in an ecological and/or historical vacuum, but are linked to specific processes, most predictably to breakdown in ecological isolation and increasing opportunity. Central to understanding the implications of global change is a firm foundation based on biodiversity discovery, emphasizing the need to re-engage a considerable infrastructure and history of integrative research in parasitology. A recent proposal for such a pathway, termed '*DAMA*' for 'documentation–assessment–monitoring–action', would serve to build biodiversity informatics and capacity in parasitology to understand, anticipate, and respond to the outcomes of accelerating environmental change (Brooks et al. 2014, 2019). Such a path would lead to synergy linking museums and their broad-based biodiversity data, genomics, and geographic systems in descriptions of a biosphere in transition.

2.3.1 Molecular Epidemiology, Diversity, and Helminth Systematics

The application of molecular taxonomy, phylogeny, and population genetics to epidemiological problems has become known as molecular epidemiology (Foxman and Riley 2001). For human helminths, recognizing genetically based variation has helped identify species or populations of epidemiological concern, recognize factors that promote transmission to human hosts, and trace the evolution and spread of physiological characters such as drug resistance (Steinauer 2009; Norton et al. 2010; Blanton et al. 2011). Unique DNA sequences or molecular markers are identified using state-of-the-art methodologies and then used to characterize neutral genetic variation. These markers can be employed to study population-based demographic parameters and processes such as dispersal, mating systems, and effective population size. In addition, markers can be developed for regions of the genome that respond to

selective forces stemming from interactions with the environment, hosts, or other parasites. Both sets of markers have been used to study the relationship between mass drug administration programs and the evolution of drug resistance (Lustigman et al. 2012). Increasingly, neutral and adaptive variation of human helminths is being assessed across the entire genome or transcriptome using next-generation sequencing technologies, leading to the development of genomic epidemiology. Genome-wide analyses have been used to determine the population structure of transmission zones and predict the efficacy of mass drug administration programs for some filarial worms (e.g., *Onchocerca volvulus* and *Wuchereria bancrofti*, Hedtke et al. 2020). Further, genomic epidemiology is improving our understanding of the genetic basis for anthelminthic resistance (Doyle and Cotton 2019).

As suggested above, the molecular epidemiology of human helminths has been strongly influenced by advances in biotechnology, especially DNA sequencing technologies. DNA sequencing platforms are continuously being developed to produce higher quantities of data and with better quality that ultimately improve our ability to accurately and precisely measure genetic variation (Pareek et al. 2011). In human helminths, early work employed a few isoenzyme markers to detect whether genetic variation existed among geographic isolates of single species or between species (e.g., Coles (1970) with *Schistosoma mansoni* and Flockhart et al. (1982) with *Trichinella* spp.). Recently, more informative DNA approaches (gene sequences, single-nucleotide polymorphisms (SNPs), and microsatellites) have become widely used to infer how different aspects of parasite biology influence the population genetics of human helminths. In human *Ascaris* (Peng and Criscione 2012), *S. mansoni* (Steinauer et al. 2010), and *Trichinella* (Rosenthal 2008; Rosenthal et al. 2008), population genetic approaches using DNA-based genetic variation have elucidated transmission cycles and the role of hosts and geography in structuring populations. New sequencing technologies have and will continue to facilitate marker discovery at the genomic scale, wherein the cost and time associated with developing and using markers will continue to decline. For example, 61,547 microsatellite loci were found in the *Brugia malayi* genome using modern techniques (Castagnone-Sereno et al. 2010), whereas only 2 microsatellite loci were identified using older sequencing approaches (Underwood et al. 2000). Such a large number of loci distributed across the genome can be used for a variety of population genetic applications including linkage mapping, which can identify genes associated with phenotypic traits such as virulence, drug resistance, or host specificity. Despite the utility and epidemiological significance of these applications, to date, the only human helminth with a linkage map is *S. mansoni* (Criscione et al. 2009). However, that situation may change as methods, such as extreme QTL and exome capture, make generating linkage maps more feasible for parasitic helminths (Chevalier et al. 2014).

In concert with sequencing technologies, advances in molecular biological methodologies such as whole genome amplification, in particular, multiple displacement amplification (MDA), have allowed us to generate microgram quantities of DNA from small amounts of tissue (Dean et al. 2002). MDA has been validated as providing unbiased whole genome amplification in single *B. malayi* and

W. bancrofti microfilaria (McNulty et al. 2008; Small et al. 2019) and in single *S. mansoni* miracidia (Valentim et al. 2009). This advancement has enabled hundreds of microsatellites or SNPs to be genotyped from single parasites, which is especially relevant when only helminth larval stages can be sampled for molecular epidemiological studies. For instance, only the zoophilic strain of *B. malayi* can be maintained in the laboratory; thus, to understand the population genetics of the anthropophilic strains, larval parasites (e.g., microfilariae 200–275 um in length) must be sampled directly from human blood, tissues, or insect vectors with the luxury of culturing (McNulty et al. 2008). Prior to MDA, sufficient amounts of DNA could not be obtained from a single individual for multilocus genotyping, and therefore, several thousand microfilariae had to be pooled into a single extraction to amplify just two microsatellite loci (Underwood et al. 2000). Using aggregates of individuals in population genetic analyses has several drawbacks, in particular, the inability to characterize the tremendous genetic variation that can and does exist between individual organisms even within a single population. Perhaps most significantly for taxonomy, pooling precludes several analyses which help estimate the genetic differentiation between populations such as linkage disequilibrium and Hardy-Weinberg-based *F* statistics (Silva et al. 2006; Steinauer et al. 2010). Other drawbacks have been described in more detail by Steinauer et al. (2010). Although MDA amplifies small amounts of human helminth DNA, criticisms include its expense, time, and the possibility of technical artifacts (reviewed in Doyle et al. (2019)). Alternative approaches, including the use of Whatman® FTA® cards to store DNA from eggs or larvae, followed by whole genome sequencing, have shown promise for broad assessments of the genetic variation of some human helminths (Doyle et al. 2019).

As molecular biology and technology have advanced, they changed our ability to assess the genetic variation of helminths relating not only to taxonomy and phylogeny but also to individual populations at ever-increasing genetic scales (i.e., from enzymes to whole genomes). Despite these advances, many molecular studies of human helminths are often phylogeographic in nature. Among the 39 human helminths recognized by Ashford and Crewe (2003), there is a clear predominance of studies that utilize only a few genes to assess genetic variation across a large geographic scale (see below under "Genomics, Systematics, and Parasitic Worms" for examples). This is likely driven in part because of the difficulties associated with recognizing parasite species. Parasites are small and live in or on hosts making aspects of their biology not directly observable. In addition, there is currently no consensus as to what constitutes appropriate discovery methods and analytical approaches for defining species, particularly in the context of cryptic diversity (Pérez-Ponce de Léon and Nadler 2010). Regardless of the interpretation, phylogeographic studies provide a first glimpse into the extent of genetic variation across broad geographic scales. In a review, Gorton et al. (2012) provided a flow diagram that describes the practical process of categorizing inter- and intraspecific variation for helminth parasites (Fig. 2.1).

For human helminths, the answer to the first question in Fig. 2.1 "Is there >1 parasite population or species?" has often been "yes" or "likely yes, but more

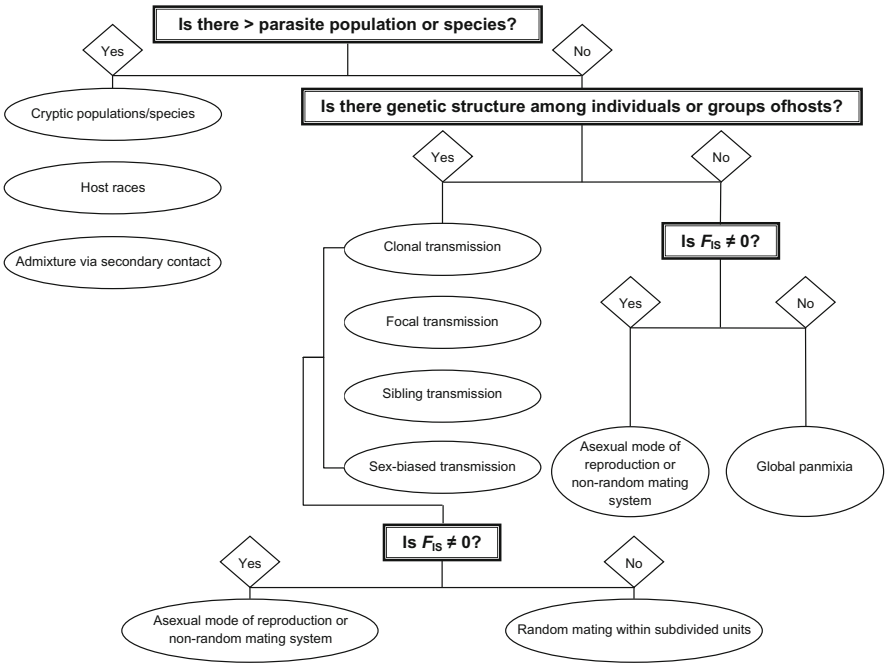

Fig. 2.1 Categorizing genetic variation among helminth parasites (From Gorton et al. 2012)

investigation is needed." Molecular phylogeny or phylogeographic studies using genetic distance data or reciprocal monophyly often reveal that organisms previously presumed to be one species are likely comprised of two or more, though usually not without some controversy. For example, discussions continue as to whether the cestode *T. asiatica* is a different species from *T. saginata* (Hoberg 2006; McManus 2006). As Yamane et al. (2012) summarized, all researchers have the same morphological and genetic data, but the taxonomic interpretations vary. Other species have been referred to as "species complexes" when a presumed single species is revealed to consist of multiple, closely related but genetically distinct organisms. Species complexes have been recognized among many of the 39 human helminths, including species of *Paragonimus*, *Schistosoma*, *Opisthorchis*, *Echinostoma*, *Fasciola*, and *Taenia*. As studies of the *P. westermani* complex show, members of parasite species complexes are often not formally described as species. For instance, geographic isolates of *P. westermani* from China and India exhibit nearly the same genetic distance (5.1% derived from partial cytochrome oxidase 1 [cox1], the nuclear ribosomal second internal transcribed spacer [ITS2], and partial 28S gene sequences) as observed between the sister species *P. harinasutai* and *P. ohirai* (5.2%). As such, isolates are currently referred to as genotypes or types 1–3 (Devi et al. 2013). It is not overly surprising that new genetic variants are initially described as strains, genotypes, lineages, or forms because the species concept has become more clouded in recent years with the use of molecular, immunological, and biological characters

(Edwards 2009; Pérez-Ponce de Léon and Nadler 2010; Nadler and Pérez-Ponce de León 2011). Further, since these variants are new to science, there is typically incomplete information regarding morphology, host use, life cycle, and distribution which could help address proper classification. Despite these difficulties, discovering and characterizing biodiversity is of high epidemiological importance. Ultimately, the relevant question for human helminths is "Are we engaged in a campaign to eliminate one species/population or a complex of many species/populations?" (McNulty et al. 2013). The crux of this question relates to identifying the limits of diversity for pathogens (species, populations, or variants) and having diagnostic capacities for known disease agents, as well as potential zoonoses that have so far eluded discovery. We can establish a context for threats represented by a global minefield of pathogens through phylogenetic triage and by applying new insights to anticipate and mitigate the outcomes of exposure and transmission on the distribution of infections and disease (Brooks et al. 2014, 2019; Mollentze and Streicker 2020).

2.3.2 Populations, Natural Hybrids, and Adequate Sampling

The presence of one or several populations can be detected using population genetic analyses. The same approach can be extended to address speciation. Many computer programs for population genetic analyses as well as packages in the R platform for statistical computing have been developed to estimate parameters that help infer genetically based differences between or within populations (Excoffier and Heckel 2006; Kamvar et al. 2017). For example, programs like FSTAT and GENEPOP estimate linkage disequilibrium (non-random association of alleles among loci), which, when present, may indicate cryptic species or distinct populations (Gorton et al. 2012). This and other population genetic parameters have revealed complex microevolutionary patterns that can help illuminate the reasons for taxonomic controversy. For example, questions regarding the species status of human (sensu stricto *A. lumbricoides*)- and pig (sensu stricto *A. suum*)-associated *Ascaris* have persisted in the literature and have prompted calls for population-level sampling (Nadler and Hudspeth 2000). Sequence and microsatellite-based analyses suggest that both geography and multiple host colonization events have influenced the evolutionary histories of human and pig *Ascaris* (Peng and Criscione 2012). Thus, Peng and Criscione (2012) reasoned that in addition to global population-level sampling, more genetic loci should be incorporated because both historical and contemporary dynamics need to be understood to resolve the taxonomy. For instance, mitochondrial DNA (mtDNA) haplotypes associated with *Ascaris* from pigs in China (Peng et al. 2005) were found in human *Ascaris* from Zanzibar (Betson et al. 2011). This geographically shared haplotype could be the result of two historical scenarios, either multiple host colonization or a historical introgression event. As such, it certainly does not confirm contemporary cross-transmission. To test for recent cross-transmission (from pig to human or vice versa), samples from

sympatric human- and pig-associated *Ascaris* should be sequenced or genotyped with several fast-evolving genetic loci such as microsatellites.

Cross-transmission of parasites between humans and other animals is important to detect because it can promote hybridization between helminth species. Hybridization has important consequences for taxonomy as it can generate intermediate phenotypes, which have historically fueled taxonomic uncertainty regarding the status of particular species (e.g., *Fasciola* spp. (Nguyen et al. 2009) and *T. asiatica/T. saginata*). Molecular genetic analyses have suggested historical and contemporary hybridization between species of *Paragonimus*, *Schistosoma*, *Echinococcus*, and *Taenia* (see Table 2.1 in Detwiler and Criscione (2010)). For example, historical introgression between the ruminant- and human-infecting *Fasciola hepatica* and *F. gigantica* has been inferred with parental taxa-specific markers and nuclear-mitochondrial discordance (see Table 2.1 in Detwiler and Criscione (2010)). Contemporary hybridization was detected between human- and pig-associated *Ascaris*, and between human *S. mansoni* and rodent *S. rodhaini* using microsatellites and Bayesian clustering (Criscione et al. 2007; Steinauer et al. 2008), and between sympatric species and genotypes of *Trichinella* that are freeze resistant (Dunams-Morel et al. 2012; La Rosa et al. 2003). Beyond affecting the morphology, hybridization may also impact infectivity, virulence, transmission, host specificity, and drug resistance in natural populations of human helminths. Few studies have investigated the epidemiological importance of hybrids, and much work remains to even understand the frequency of hybridization in natural populations of human helminths. Studies are also lacking on environmental and/or host factors that select for fit hybrids. However, the work that has been done suggests that hybridization could be an important factor that influences our interpretations of the systematics and biology of helminths.

2.4 Genomics, Systematics, and Parasitic Worms

Evolution is a branching process resulting from populations that diverge over time. It involves a gentle balance between diversification, fitness, and extinction. This process, often examined via research on extant organisms in the absence of fossil record, can be visualized in the construction of phylogenetic trees based upon the order in which these evolutionary events transpired. This in turn generates a historical pattern of species diversification from common ancestry. Once relegated to morphological and biological data, the onslaught of molecular and biochemical technologies has led to reassessing relationships within and among parasitic groups resulting in new insights across all helminth taxa. A holistic, molecular-based phylogenetic revision of the phylum Nematoda began in earnest back in 1998 when Blaxter et al. (1998) used sequencing data from a single gene, the nuclear small subunit ribosomal DNA (ssrDNA) from 53 nematode species to construct a tree that tested previous morphologically defined groups, Adenophorea (marine worms) and Secernentea (terrestrial worms). However, the ssrDNA data showed

that the Adenophorea was likely paraphyletic rather than monophyletic by incorporating some ancestors of the Secernentea and the Secernentea also did not coincide with classical taxonomy. The subdivision based upon ssrDNA resulted in five clades, Dorylaimia (Clade I), Enoplia (Clade II), and Chromadoria, which was further subdivided into Spirurina (Clade III), Tylenchina (Clade IV), and Rhabditina (Clade V).

Since that time, the tree has been refined with the addition of key ancestral taxa including nematodes from marine animals (Holterman et al. 2006; Meldal et al. 2007; van Megen et al. 2009). In contrast to Blaxter et al. (1998), new data proposed the most basal clade was dominated by Enoplia rather than the Dorylaimia. Further refinements to the phylogenetic tree incorporated the major clades originally defined by Blaxter et al. (1998), the minor clades that followed in 2006–2009 (Holterman et al. 2006; van Megen et al. 2009), as well as the inclusion of morphological data (De Ley and Blaxter 2002, 2004). These data collectively dispelled the Adenophorea and Secernentea classifications and proposed three major groupings, Enoplia (Clade I), Dorylaimia (Clade II) (Adenophorea), and Chromadoria (Secernentea). However, Smythe et al. (2019) pointed out that in all prior analyses, early diversification among the basal Nematoda lineages, i.e., Enoplia and Dorylaimia, had been poorly investigated because of inadequate sampling within the Enoplia subclass and because the preponderance of nematodes in prior studies was parasitic. This limited our understanding of early evolution and our glimpse into the transition from free-living to parasitic forms. Thus, by including the transcriptomes of nine free-living marine nematodes in their analysis of 108 free-living and parasitic nematodes, the data strongly supported a monophyletic Enoplia clade sister to all other nematodes that in turn was split into marine (Enoplida) and freshwater/terrestrial (Triplonchida) subclades. This was the first major molecular-based update of the Nematoda phylogeny since 2009.

Similar comprehensive studies have been performed on flatworms and cestodes to estimate the phylogeny of the Digenea (Olson et al. 2003; Bray et al. 2016) using the complete ssrDNA and partial data from the large subunit ribosomal DNA (lsrDNA) focusing on expansion segments D1–D3. The analysis by Olson et al. (2003) included 163 digenean taxa, whereas the study by Bray et al. (2016) examined 41 species of the family Opecoelidae. An increasingly robust phylogenetic backbone for the Eucestoda has emerged from explorations of both ssrDNA and lsrDNA in addition to more recent evaluations of large fragments (~4000 bp) of mtDNA (e.g., summarized in part in Caira and Jensen (2017) and Waeschenbach and Littlewood (2017)). Further, genome-scale data and next-generation methods are currently being applied to analyses of relationships among cyclophyllidean tapeworms and other eucestodes (Yuan et al. 2016). Among the parasitic flatworms, extensive analyses using both maximum parsimony and Bayesian inference have resulted in demonstrable changes in the membership of higher taxa and thus the construction of new revisions to previously accepted classifications.

Phylogenetics utilizes either biological, morphological, or developmental character states, single gene/protein sequences, or a small number of gene/protein sequences for revealing relationships among organisms. Phylogenetic applications

allow development of hypotheses for relationships of lineages, species, and higher taxa and are thus the foundation for exploring complex questions about the history of the biosphere. Single-gene or multigene studies have been utilized extensively to develop phylogenetic inference among members of Trichinella (Zarlenga et al. 2006), Schistosoma (Attwood et al. 2007), Taenia (Nakao et al. 2013a, b), and Anisakis (Mattiucci and Nascetti 2008), among many others; however, new approaches are being developed to increase the footprint of genes used to explore distantly related organisms. To date, this has been a bit more problematic given the varying rates of divergence and evolution.

Phylogenomics is a field of science where phylogenetics and genomics intersect and information drawn from whole genome sequencing is used to help decipher the bigger picture, i.e., the "Tree of Life" (Eisen 1998; Eisen and Fraser 2003; O'Brien and Stanyon 1999). By expanding comparisons to whole genomes or genomic features, variances encountered at the microscale can often be overcome by majority rule (Delsuc et al. 2005; Philippe et al. 2004; Jeffroy et al. 2006). Tree topologies in phylogenomics are less affected by rare genomic changes such as misalignments, horizontal gene transfer, and even missing data (Philippe et al. 2004) though horizontal gene transfer in large-scale analyses of prokaryotes has been problematic. As such, it becomes theoretically feasible to resolve deep evolutionary relationships using phylogenomics. One challenge to constructing higher-level phylogenies using vastly disparate organisms is the rarity of clearly defined homologous markers that span all sampled members (O'Malley and Koonin 2011). Today, with state-of-the-art next-generation sequencing technologies, the genomes of complex organisms can be completed in short order. However, even with genome-level sequence data, more shallow evolutionary relationships can pose challenges as well, as in the placement of the freeze-resistant genotypes of Trichinella. The phylogeny based on single-copy orthologous proteins/genes places Trichinella T8 and Trichinella britovi at the crown of the tree (Korhonen et al. 2016), whereas the phylogeny based upon whole mtDNA and multigene analyses suggests that the freeze-resistant genotypes Trichinella nativa and Trichinella T6 are the most recently diverged (Sharma et al. 2020; Zarlenga et al. 2006) even after applying better technologies to advance long read sequencing in this genus (Thompson et al. 2017). Surprisingly, both analyses generate well-supported trees and substantial equivalence elsewhere in the trees.

2.4.1 Phylogenomics and Evolutionary Inference

To date, the most extensive application of molecular data to phylogenetic inference remains with single- or multigene analyses. For more distantly related organisms, the ubiquitous ribosomal DNA subunits have been popular targets, whereas studies on more recently diverged organisms often focus on mtDNA or, more precisely, one or more genes within the mtDNA. With respect to phylogenomics studies, it is not examining the entire genome that best informs us but targeting the portions of the genome that eventually encode proteins, i.e., the transcriptome. Sequence-based

comparisons generally involve concatenating multitudes of data into a supermatrix and then evaluating these as a single evolving unit when performing comparisons between taxa. Tree construction can also be based upon individual gene/protein comparisons which are then combined to generate supertrees. These approaches are not unlike the more commonly performed phylogenetic studies but are executed on a much grander scale. Zhu et al. (2019) constructed a phylogeny from 10,575 evenly sampled bacterial and archaeal genomes and from these focused on 381 homologous markers to find that Archaea and Bacteria are more closely related than previous estimates. Phylogenomics can also be designed so as not to rely directly on sequence comparisons but on genomic features or the character makeup of a genome such as comparing the positions of introns or intervening sequences (Roy and Gilbert 2005) or commonality in the order in which genes appear in a genome, i.e., synteny, among others (Korbel et al. 2002).

As it relates to parasites, phylogenomics has been substantially relegated to studies on protozoans because of the dearth of whole genome sequence data from more complex parasite assemblages where the smaller protozoan genomes have made sequencing and genome assembly less problematic. This issue has begun to resolve itself with next-generation sequencing techniques. Still, some work has been performed reconstructing deep evolutionary relationships among nematodes, arthropods, and vertebrates: a question that has plagued those in evolutionary biology for many years. The two prevailing hypotheses suggest that larger clades can be defined either as Coelomata (animals with a coelom or internal body cavity that harbors key internal organs) or as Ecdysozoa (animals that shed their exoskeleton). The Coelomata hypothesis, which is based primarily on morphological and physiological parameters, maintains that chordates and arthropods are more closely related than either is to nematodes which do not possess a coelom. This contrasts with the Ecdysozoa hypothesis where tree topology is predicated upon shared developmental characters where arthropods and nematodes form a monophyletic clade independent of chordates because they undergo homologous molting processes.

In 1997, Aguinaldo et al., developed new algorithms to circumvent biases generated by long branch-length sequencing artifacts that surface when examining distant lineages. This occurs when sizable amounts of change among two lineages make them appear more similar because they share levels of change rather than common ancestry. From their data, Aguinaldo et al. (Aguinaldo et al. 1997) proposed that the Annelida-Mollusca lineage was sister to the arthropods and that molting animals form a clade, called the Ecdysozoa. In 2004, Wolf et al. (2004) used phylogenomics to address this question by examining greater than 500 protein sequences subgrouped into 8 macromolecular complexes. These complexes were then analyzed using both supermatrices from concatenated sequences and supertrees from optimized individual trees, as well as indels, gene content, and protein domain co-occurrence that are all less dependent upon direct sequence comparisons; however, only six eukaryotic species were used in this analysis. Surprisingly, all analyses converged on a coelomate topology. Using gene content, Dopazo et al. (2004) examined 25,000 amino acid sequences and corroborated the Coelomata hypothesis. In contrast, others showed that when extensive and well-documented character loss

was accounted for in the nematode Caenorhabditis elegans (Copley et al. 2004) (one of the six eukaryotes used in the analysis) or when fast-evolving sequences in C. elegans were removed from consideration (Dopazo and Dopazo 2005), the Ecdysozoa hypothesis was better supported. This work was corroborated by Philippe et al. (2005b). In Longhorn et al. 2007, Longhorn et al., used transcriptome data and protein alignments to create a robust Coelomata topology and dispel the arthropod-chordate relationship generated by phylogenomics which they believe resulted from biases in amino acid sequence composition of model organisms. In general, the biggest contribution to this long-standing controversy has been assuming that large datasets can counter deficiencies in sample size where a subset of transcriptome data can maximize taxonomic sampling but whole genome analysis maximizes gene sampling. Holton and Pisani (2010) bridged both barriers by assembling data from 43 whole Metazoan genomes and used supertree and supermatrix paradigms to analyze single- and multigene families. They concluded that the Coelomata topology was not supported.

Collectively, these studies show that even if datasets are demonstrably expanded, branch lengths and, in particular, long-branch attraction biases can substantially impact tree topology when comparing disparately related organisms and when using small sample sizes. Further, phylogenomics depends heavily upon the selection of core genes from sampled genomes that are evolutionarily stable. As the analysis spans more diverse lineages, the number of core genes and the number of homologous sites within those genes decrease. As an example, Ciccarelli et al. (2006) used phylogenomics to study the "Tree of Life" which encompassed the genomes of 191 species spanning the Archaea, Bacteria, and Eukarya. One might expect that this would be a robust study; however, among the tens of thousands of genes available, only 31 core sequences could be identified from the 191 species upon which this version of the "Tree of Life" could be constructed. The obvious question then is just how robust is such an analysis if one can only use but a fraction of available genes (Dagan and Martin 2006)?

Another factor that severely limits inferences from molecular phylogenies, in particular, those derived from diverse lineages and clades (phylogenomics), is the inability to estimate divergence rates from other than fossil record; only estimates of when changes occurred can be gleaned from common birth-death processes of evaluation (Quental and Marshall 2010; Morlon et al. 2011). Phylogenetic studies based upon molecular data often assume a constant rate of diversification even though many lineages are known to exhibit decreasing rates (Rabosky and Lovette 2008). Thus, time-dependent rate variations among taxonomic lineages must be accounted for in molecular-based phylogenies (Morlon et al. 2011). Also, a recent paper expressed compelling reasons why phylogenetic timetrees based upon extant data do not often follow those based upon fossil record (when available). Louca and Pennell (2020) portend that using the birth-death model, there exist an immeasurable number of potential diversification scenarios equally capable of generating an extant timetree that cannot be distinguished even in the presence of infinite data. If true, trees based only upon extant data are likely overinterpreted. However, Louca and Pennell (2020) offer a set of variables that can be easily applied to estimate historical,

underlying forces in diversification using data from extant phylogenetic trees scaled to time.

With this as a backdrop, one of the key points of contention between phylogenetics and phylogenomics is whether higher taxon sampling (phylogenetics) or greater gene sampling (phylogenomics) has a more profound impact on tree topology. These types of questions can be difficult to assess because molecular systematics remains an evolving and subjective science with few hardcore benchmarks. Large datasets used in phylogenomics may negate sampling errors, but systemic errors such as compositional biases, core gene selection, and misleading data still abound (Dagan and Martin 2006; Jeffroy et al. 2006; Philippe et al. 2005a). In a robust study of yeast phylogeny, whole genome data was analyzed via maximum likelihood (ML) and parsimony. One tree with 100% bootstrap support was created (Rokas et al. 2003); however, upon reanalyzing the same dataset using minimum evolution (ME), a different tree was created, also exhibiting 100% bootstrap support (Phillips et al. 2004). Recoding the nucleotides as purines or pyrimidines resulted in a new ME tree that aligned with the ML/parsimony tree. Philippe et al. (2005a) later suggested that putative discrepancies such as these should be tested by demonstrating that congruent trees can and will result from both taxon-poor and taxon-rich sampling. In the end, methods of data analysis are as influential as the data themselves. Also, as noted earlier, the breadth and divergence of sampled members in the analysis can adversely affect gene sampling.

The field of phylogenomics is still evolving. As with any new approach to problem-solving, new caveats and challenges will emerge (Philippe et al. 2005a). However, as it becomes easier and less costly to perform whole genome sequencing, the databases of more complex organisms will escalate which in turn should result in a coalescence of benefits from ample taxon sampling and gene sampling. Until that time, comparative genomics will continue to grow as a driving force for using large datasets to study distantly as well as closely related organisms in that comparative genomics looks at the presence or absence of protein sequences in conjunction with system biology to evaluate similarities, differences, and putative evolutionary links among organisms. As you will see below, much can be gleaned at both the micro- and macro-scales when studying evolutionary trends among organisms using comparative genomics. Thus, one may be able to study evolution inference without relying solely on phylogenetic trees.

2.4.2 Comparative Genomics, Transcriptomics, and Evolutionary Inference

In recent years, research on parasite genomes has come of age. In addition to being used to study evolutionary relationships and processes on grander scales (phylogenomics), they have come to better enlighten us on issues like host-parasite interactions and adaptation (Xu et al. 2019; Zarlenga et al. 2016) and have placed a

genetic face to the biological diversity that abounds in this group. At the time of writing, draft genome sequence data were available for at least 56 species of nematodes (parasitic and free-living) and 25 species of platyhelminths (Coghlan et al. 2019). Comparative analyses, i.e., comparative genomics, using genome information in conjunction with the transcriptome and proteome data that usually accompany these studies, have helped us understand the functions of genes and gene products. In essence, comparative genomics has helped link phenotypes to genotypes. The study by Coghlan et al. (2019) is the most complete assessment and comparison of nematode genomic data to date where the authors mined over 1.4 million genes to identify or predict pan-nematode targets for new forms of drug intervention and in so doing identifies gene families and biological pathways linked to the chief parasitic lineages. The significant variation in genome size among nematodes (42 Mb to 700 Mb) and among platyhelminths (104 Mb to 1259 Mb) provides anecdotal guidance as to the complexity of performing phylogenetic analyses with disparate lineages. However, comparative genomics is less focused on phylogeny and more interested on answering specific questions such as which genes link parasitic nematodes (parasitism), pathway similarities, and which genes are unique to subsets within this group and may have biological relevance.

A comparative analysis was performed by Tsai et al. (2013) that included the genomes of four tapeworms representing multiple genera, *E. multilocularis* (canine, humans, and rodents), *E. granulosus* (canine, humans, and ungulates), *T. solium* (swine and humans), and *Hymenolepis microstoma* (rodents and arthropods). This study showed extraordinary genetic plasticity among closely related organisms and how this plasticity contributed to the evolution of the group and provided insights into the acquisition of parasitism among cestodes. Identification of key heat shock proteins (HSP) in *Echinococcus* and *T. solium* and the massive independent but parallel expansion of this gene family in each species have given rise to theories on the role that the HSP genes play in the ability of cestodes to cope with change and therefore adapt to new environments and new hosts. Further, gene sets were identified that function to increase the ability of these flatworms to absorb needed nutrients rather than metabolize ingested foods: a genetic finding that corroborates their morphological structure.

A review by Lawton et al. (2011) used comparative genomics in an intragenus study of *Schistosoma* phylogeography. They targeted mtDNA genome organization, nuclear data, and existing cytogenetic information to gain better insight into the evolution of the genus particularly as it relates to opposing views of its African (Davis 1993) or Asian (Rollinson et al. 1997; Snyder and Loker 2000) descent. Based upon the genomic evidence, they concluded that the genus *Schistosoma* originated in Asia approximately 60–70 million years ago from an avian schistosomatid then switched to ungulates approximately 20 million years ago giving rise to the *S. japonicum* group. The *S. japonicum* group shares distinct genomic similarities with the avian parasites including but not limited to mtDNA gene order. Their analysis concluded that the genus then invaded Africa with the migration of mammals. It is believed this occurred as recently as 2–three million years ago (Attwood et al. 2007) and on two separate occasions, the second of which

gave rise to the *S. mansoni* and *S. haematobium* clades. This was followed by reinvasion of Asia and subsequent evolution of the *S. indicum* clade.

Comparative genomics is not relegated to intragenus studies. Numerous reports have surfaced using genomics to investigate more holistic questions such as parasite lifestyles, mechanisms constituting "parasitism" among nematodes, and identifying new targets for drug intervention (Blaxter et al. 2012; Coghlan et al. 2019; Heizer et al. 2013; Shinya et al. 2013; Strube et al. 2012; Tsai et al. 2013). Lu et al. (2020) performed a comparative, retrospective study on the developmental stages of 8 nematodes collectively encompassing 13 different stages. By examining the L3, they concluded that the parasitic stages were not inherited from common ancestors but evolved through common responses to similar selection pressures and that these life cycle patterns have been maintained among species that diverged many millions of years ago among different trophisms.

As one might expect, there is a large collection of genes that are conserved among metazoa because they harbor functions needed to sustain life in nearly all organisms. There is an equally large collection that constitutes genes archetypical of nematodes and still others that are non-conserved that uniquely define an organism or taxa; these are likely involved in functional diversification, speciation, and species adaptation. One study examining these subsets of genes was performed in conjunction with sequencing the genome of *T. spiralis* (Mitreva et al. 2011). In this study, the genome of *T. spiralis*, a member of a more ancestral clade in the Nematoda, i.e., Dorylaimia, was compared to other available nematode genomes in the hope of identifying pan-phylum-specific sequences and proteins. The ultimate goal of this type of work was to distinguish genes and proteins that can be evaluated as targets for broad-spectrum drug intervention. This is significant, principally because of the multitude of people worldwide requiring anthelmintics, the relatively small number of drugs available for this purpose, and the ever-increasing threat of resistance to those currently in use (Keiser and Utzinger 2010). Herein lies one very important intersection linking systematics, comparative genomics, and human health, namely, the use of pharmaco-phylogenomics in the development of prophylactic and therapeutic treatments for human parasites (Caffrey et al. 2009; Coghlan et al. 2019; Jex et al. 2011; Rufener et al. 2010; Swain et al. 2011; Taylor et al. 2013). However, the ultimate success in these types of studies and the breadth of the gene targets is predicated upon user-defined criteria for culling and grouping sequences.

2.5 Conclusions: Human Helminths in a World under Change

Parasites have been a fundamental component of the human landscape throughout our history. Contemporary parasite faunas, assembled across disparate time frames and sources, provide an intricate mosaic that reflects historical and ongoing interactions among ecology, evolution, and geographic colonization. Our understanding of

historical processes as determinants of faunal structure and parasite distribution is critical for mitigating their impact on human health and well-being in a world where dramatic changes in distribution and the interfaces for infection are being demonstrated and predicted (Hoberg and Brooks 2010; Brooks and Hoberg 2013).

Much of human parasitism has been linked to improper hygiene wherein 35% of the world's population (2.5 billion people) lacks access to improved sanitation. A similar percentage is infected by intestinal parasites as a direct result of poor hygiene and unwashed food. Within the span of just a few hundred years, we have seen the impact that human travel can have on the dissemination of once exotic parasites (Rosenthal et al. 2008; Hoberg 2010). Thus, interacting challenges between people and the biosphere are apparent and constitute a synergy for crises in biodiversity, climate change, and emerging infectious diseases. Since parasitism permeates our ecosystem, parasitological insights must be integrated into any discussion on the unfolding and accelerating effects of climate and ecological disruption given the potential for new and changing patterns of parasite/pathogen distribution among dynamic drivers for change on local, regional, and global scales. Within this discussion must come an appreciation for the fluidity rather than rigidity of helminth systematics and phylogenetics and the impact that environmental perturbation and anthropogenic forcing, through human behavior and globalization, impart on that plasticity.

References

Agosta SJ, Brooks DR (2020) The major metaphors of evolution: Darwinism then and now. Springer International Publishing. https://doi.org/10.1007/978-3-030-52086-1

Agosta SJ, Klemens SA (2008) Ecological fitting by phenotypically flexible genotypes: implications for species associations, community assembly and evolution. Ecol Lett 11:1123–1134

Agosta S, Janz N, Brooks DR (2010) How generalists can be specialists: resolving the "parasite paradox" and implications for emerging disease. Fortschr Zool 27:151–162

Aguinaldo AMA, Turbeville JM, Linford LS, Rivera MC, Garey JR, Raff RA, Lake JA (1997) Evidence for a clade of nematodes, arthropods and other moulting animals. Nature 387:489–493

Araujo SBL, Braga MP, Brooks DR, Agosta S, Hoberg EP, von Hathental F, Boeger WA (2015) Understanding host-switching by ecological fitting. PLoS One 10(10):e0139225. https://doi.org/10.1371/journal.pone.0139225

Ashford RW, Crewe W (2003) The parasites of Homo sapiens: an annotated checklist of the protozoa, helminths and arthropods for which we are home, 2nd edn. Taylor and Francis, New York

Attwood SW, Fatih FA, Mondal MM, Alim MA, Fadjar S, Rajapakse RP, Rollinson D (2007) A DNA sequence-based study of the Schistosoma indicum (Trematoda: Digenea) group: population phylogeny, taxonomy and historical biogeography. Parasitology 134:2009–2020

Audy JR (1958) The localization of disease with special reference to zoonoses. Trans R Soc Trop Med Hygiene 52:309–328

Avise JC (2000) Phylogeography: the history and formation of species. Harvard University Press, Cambridge

Barnosky AD, Hadly EA, Bascompte J et al (2012) Approaching a state shift in Earth's biosphere. Nature 486:52–58

Bell KC, Calhoun K, Hoberg EP, Demboski JR, Cook JA (2016) Temporal and spatial mosaics: deep host association and shallow geographic drivers shape genetic structure in a widespread pinworm, *Rauschtineria eutamii* (Nematoda: Oxyuridae). Biol J Linn Soc 119:397–413

Betson M, Halstead FD, Nejsum P, Imison E, Khamis IS, Sousa-Figueiredo JC, Rollinson D, Stothard JR (2011) A molecular epidemiological investigation of Ascaris on Unguja, Zanzibar using isoenyzme analysis, DNA barcoding and microsatellite DNA profiling. Trans R Soc Trop Med Hyg 105:370–379

Blanton RE, Blank WA, Costa JM, Carmo TM, Reis EA, Silva LK, Barbosa LM, Test MR, Reis MG (2011) Schistosoma mansoni population structure and persistence after praziquantel treatment in two villages of Bahia, Brazil. Int J Parasit 41:1093–1099

Blaxter ML, De Ley P, Garey JR et al (1998) A molecular evolutionary framework for the phylum Nematoda. Nature 392:71–75

Blaxter M, Kumar S, Kaur G, Koutsovoulos G, Elsworth B (2012) Genomics and transcriptomics across the diversity of the Nematoda. Parasite Immunol 34:108–120

Boeger WA, Brooks DR, Trivellone V, Agosta S, Hoberg EP (2022) Ecological super-spreaders drive host-range oscillations: omicron and risk-space for emerging infectious disease. Transbound Emerg Dis. https://doi.org/10.1111/tbed.14557. Online ahead of print

Bray RA, Cribb TH, Littlewood DTJ, Waeschenbach A (2016) The molecular phylogeny of the digenean family Opecoelidae Ozaki, 1925 and the value of morphological characters, with the erection of a new subfamily. Folia Parasitol 63:013. https://doi.org/10.14411/fp.2016.013

Brooks DR, Ferrao A (2005) The historical biogeography of coevolution: emerging infectious diseases are evolutionary accidents waiting to happen. J Biogeogr 32:1291–1299

Brooks DR, Hoberg EP (2007) How will global climate change affect parasite-host assemblages? Trends Parasitol 23:571–574

Brooks DR, Hoberg EP (2013) The emerging infectious disease crisis and pathogen pollution: a question of ecology and evolution. In: Rohde K (ed) The balance of nature and human impact. Cambridge University Press, Cambridge, pp 215–229

Brooks DR, McLennan DA (1993) Parascript: parasites and the language of evolution. Smithsonian Institution Press, Washington DC

Brooks DR, McLennan DA (2002) The nature of diversity: an evolutionary voyage of discovery. University of Chicago Press, Chicago, IL

Brooks DR, Hoberg EP, Gardner SL, Boeger W, Galbreath KE, Herczeg D, Mejía-Madrid HH, Racz E, Tsogtsaikhan Dursahinhan A (2014) Finding them before they find us: informatics, parasites and environments in accelerating climate change. Comp Parasitol 81:155–164

Brooks DR, Hoberg EP, Boeger WA (2015) In the eye of the cyclops- re-examining the classic case of cospeciation: why paradigms are important. Comp Parasitol 82:1–8

Brooks DR, Hoberg EP, Boeger WA (2019) The Stockholm paradigm: climate change and emerging disease. University of Chicago Press, Chicago, IL, p 423

Brooks DR, Hoberg EP, Boeger WA et al (2020) Before the pandemic ends: making sure this never happens again. WCSAJ 1:7–15

Brooks DR, Hoberg EP, Boeger WA, Trivellone V (2022) Emerging infectious disease: an underappreciated area of strategic concern for food security. Transbound Emerg Dis 69:254–267. https://doi.org/10.1111/tbed.14009

Caffrey CR, Rohwer A, Oellien F, Marhöfer RJ, Braschi S, Oliveira G, McKerrow JH, Selzer PM (2009) A comparative chemogenomics strategy to predict potential drug targets in the metazoan pathogen, *Schistosoma mansoni*. PLoS One 4:e4413

Caira JN, Jensen K (eds) (2017) In: Planetary biodiversity inventory (PBI): tapeworms from vertebrate bowels of the earth (2008–2017), vol 25. Special Publication. Natural History Museum, The University of Kansas, Lawrence, KS, p 463p

Carlson CJ, Dallas TA, Alexander LW, Phelan AL, Phillips AJ (2020) What would it take to describe the global diversity of parasites? Proc R Soc B 287:20201841. https://doi.org/10.1098/rspb.2020.1841

Castagnone-Sereno P, Danchin EGJ, Deleury E, Guillemaud T, Malausa T, Abad P (2010) Genome-wide survey and analysis of microsatellites in nematodes, with a focus on the plant-parasitic species Meloidogyne incognita. BMC Genomics 11:598

Chevalier FD, Valentim CL, LoVerde PT, Anderson TJ (2014) Efficient linkage mapping using exome capture and extreme QTL in schistosome parasites. BMC Genomics 15:617. https://doi.org/10.1186/1471-2164-15-617

Ciccarelli FD, Doerks T, von Mering C, Creevey CJ, Snel B, Bork P (2006) Toward automatic reconstruction of a highly resolved tree of life. Science 311:1283–1287. https://doi.org/10.1126/science.1123061

Cleaveland S, Laurenson MK, Taylor LH (2001) Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Phil Trans R Soc Lond B Biol Sci 356:991–999

Coghlan A, Tyagi R, Cotton JA et al (2019) Comparative genomics of the major parasitic worms. Nat Genet 51:163–174. https://doi.org/10.1038/s41588-018-0262-1

Colella JP, Bates J, Burneo S, Camacho A, Carrion C, Constable I, D'Elia G, Dunnum J, Grieman S, Hoberg E, Lessa E, Liphardt S, Londono M, Losos E, Lutz H, Ordóñez Garcia N, Peterson T, Martin ML, Ribas C, Strumminger B, Thompson C, Weksler M, Cook J (2021) Leveraging natural history collections as a global, decentralized pathogen surveillance network. PLoS Pathog 17(6):e1009583. https://doi.org/10.1371/journal.ppat.1009583

Coles GC (1970) A comparison of some isoenyzmes of Schistosoma mansoni and Schistosoma haematobium. Comp Biochem Physiol 33:549–558

Cook JA, Brochman C, Talbot SL, Fedorov VB, Väinölä R, Hoberg EP, Kholodova M, Magnussun KP, Mustonen T (2013) Genetics. In: Meltofte H (ed) Arctic biodiversity assessment. Arctic Council, Convention for Arctic Flora and Fauna, Kiruna, pp 515–539

Cook JA, Galbreath KE, Bell KC et al (2017) The Beringian coevolution project: holistic collections of mammals and associated parasites reveal novel perspectives on changing environments in the north. Arct Sci 3:585–617

Cook JA, Arai S, Armién B et al (2020) Integrating biodiversity infrastructure into pathogen discovery and mitigation of emerging infectious diseases. Bioscience 70:531–534

Copley RR, Aloy P, Russell RB, Telford MJ (2004) Systematic searches for molecular synapomorphies in model metazoan genomes give some support for Ecdysozoa after accounting for the idiosyncrasies of Caenorhabditis elegans. Evol Dev 6:164–169

Criscione CD, Poulin R, Blouin MS (2005) Molecular ecology of parasites: elucidating ecological and microevolutionary processes. Mol Ecol 14:2247–2257

Criscione CD, Anderson JD, Sudimack D, Peng W, Jha B, Williams-Blangero S, Anderson TJC (2007) Disentangling hybridization and host colonization in parasitic roundworms of humans and pigs. Proc R Soc B-Biol Sci 274:2669–2677

Criscione CD, Valentim CLL, Hirai H, LoVerde PT, Anderson TJC (2009) Genomic linkage map of the human blood fluke Schistosoma mansoni. Genome Biol 10:R71

Dagan T, Martin W (2006) The tree of one percent. Genome Biol 7:118. https://doi.org/10.1186/gb-2006-7-10-118

Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife: threats to biodiversity and human health. Science 287:443–449

Davies K, Pagan C, Nadler SA (2020) Host population expansion and the genetic architecture of the pinniped hookworm Uncinaria lucasi. J Parasitol 106:383–391

Davis GM (1993) Evolution of prosobranch snails transmitting Asian Schistosoma; coevolution with Schistosoma: a review. Prog Clin Parasitol 3:145–204

De Ley P, Blaxter ML (2002) Systematic position and phylogeny. In: Lee DL (ed) The biology of nematodes. Taylor and Francis, London, pp 1–30

De Ley P, Blaxter ML (2004) A new system for Nematoda: combining morphological characters with molecular trees and translating clades into ranks and taxa. In: Cook R, Hunt DJ (eds) Nematology monographs and perspectives, vol 2. EJ Brill, Leiden, pp 633–653

de Menocal PB, Stringer C (2016) Human migration: climate and the peopling of the world. Nature 538:49–50

Dean FB, Hosono S, Fang LH et al (2002) Comprehensive human genome amplification using multiple displacement amplification. Proc Natl Acad Sci U S A 99:5261–5266

Delsuc F, Brinkmann H, Philippe H (2005) Phylogenomics and the reconstruction of the tree of life. Nat Rev Genet 6:361–375

Deplazes P, Eichenberger RM, Grimm F (2019) Wildlife-transmitted Taenia and Versteria cysticercosis and coenurosis in humans and other primates. IJP: Parasite Wildl 9:342–358

Detwiler JT, Criscione CD (2010) An infectious topic in reticulate evolution: Introgression and hybridization in animal parasites. Genes 1:102–123

Devi KR, Narain K, Mahanta J, Nirmolia T, Blair D, Saikia SP, Agatsuma T (2013) Presence of three distinct genotypes within the Paragonimus westermani complex in northeastern India. Parasitology 140:76–86

Dobson A, Lafferty KD, Kuris AM, Hechinge RF, Jetz W (2008) Homage to Linnaeus: how many parasites? How many hosts? Proc Natl Acad Sci 105:11482–11489

Dopazo H, Dopazo J (2005) Genome-scale evidence of the nematode-arthropod clade. Genome Biol 6:R41

Dopazo H, Santoyo J, Dopazo J (2004) Phylogenomics and the number of characters required for obtaining an accurate phylogeny of eukaryote model species. Bioinformatics 20(Suppl. 1):I116–I121

Doyle SR, Cotton JA (2019) Genome-wide approaches to investigate anthelmintic resistance. Trends Parasitol 35:289–301

Doyle SR, Sankaranarayanan G, Allan F, Berger D, Castro PDJ, Collins JB, Crellen T, Duque-Correa MA, Ellis P, Jaleta TG, Laing R, Maitland K, McCarthy C, Moundai T, Softley B, Thiele E, Ouakou PT, Tushabe JV, Webster JP, Weiss AJ, Lok J, Devaney E, Kaplan RM, Cotton JA, Berriman M, Holroyd N (2019) Evaluation of DNA extraction methods on individual helminth egg and larval stages for whole-genome sequencing. Front Genet 10:826

Dunams-Morel DB, Reichard MV, Torretti L, Zarlenga DS, Rosenthal BM (2012) Discernible but limited introgression has occurred where Trichinella nativa and the T6 genotype occur in sympatry. Infect Genet Evol 12:530–538

Dunnum JL, Yanagihara R, Johnson KM, Armien B, Batsaikhan N, Morgan L, Cook JA (2017) Biospecimen repositories and integrated databases as critical infrastructure for pathogen discovery and pathobiology research. PLoS Negl Trop Dis 11:e0005133. https://doi.org/10.1371/journal.pntd.0005133

Edwards SV (2009) Is a new and general theory of molecular systematics emerging? Evolution 63:1–19

Eisen JA (1998) Phylogenomics: improving functional predictions for uncharacterized genes by evolutionary analysis. Genome Res 8:163–167

Eisen JA, Fraser CM (2003) Phylogenomics: intersection of evolution and genomics. Science 300:1706–1707

Erwin TL (1985) The taxon pulse: a general pattern of lineage radiation and extinction among carabid beetles. In: Ball GE (ed) Taxonomy, phylogeny, and zoogeography of beetles and ants. Dr. W. Junk, Dordrecht, pp 437–472

Excoffier L, Heckel G (2006) Computer programs for population genetics data analysis: a survival guide. Nat Rev Genet 7:745–758

Flockhart HA, Harrison SE, Dobinson AR, James ER (1982) Enzyme polymorphism in Trichinella. Trans R Soc Trop Med Hyg 76:541–545

Folinsbee KE, Brooks DR (2007) Early hominid biogeography: pulses of dispersal and differentiation. J Biogeogr 43:383–397

Foxman B, Riley L (2001) Molecular epidemiology: focus on infection. Am J Epidemiol 153:1135–1141

Galbreath KE, Hoberg EP (2015) Host responses to historical climate change shape parasite communities in North America's intermountain west. Folia Zool Brno 64:218–232

Ghiselin MT (1974) A radical solution to the species problem. Syst Zool 23:536–544
Gorton MJ, Kasl EL, Detwiler JT, Criscione CD (2012) Testing local-scale panmixia provides insights into the cryptic ecology, evolution, and epidemiology of metazoan animal parasites. Parasitology 139:981–997
Groucutt HS, Grün R, Zalmout ISA et al (2018) Homo sapiens in Arabia by 85,000 years ago. Nat Ecol Evol 2:800–809
Guth S, Visher E, Boots M, Brook CE (2019) Host phylogenetic distance drives trends in virus virulence and transmissibility across the animal–human interface. Phil Trans R Soc Lond B 374: 20190296. https://doi.org/10.1098/rstb.2019.0296
Haas GMS, Hoberg EP, Cook JA, Haukisalmi V, Makarikov AA, Gallagher SR, Dokuchaev NE, Galbreath KE (2020) Taxon pulse dynamics, episodic dispersal and host colonization across Beringia drive diversification of a Holarctic tapeworm assemblage. J Biogeogr 00:1–15. https://doi.org/10.1111/jbi.13949
Harcourt AH (2012) Human biogeography. University of California Press, Berkeley, CA
Haukisalmi V, Hardman LM, Hoberg EP, Henttonen H (2014) Phylogenetic relationships and taxonomic revision of Paranoplocephala Lühe, 1910 sensu lato (Cestoda, Cyclophyllidea, Anoplocephalidae). Zootaxa 3873:371–415
Haukisalmi V, Hardman LM, Fedorov V, Hoberg EP, Henttonen H (2016) Molecular systematics and phylogeography of cestodes of the genus Anoplocephaloides Baer, 1923 s.s. (Cyclophyllidea: Anoplocephalidae) in lemmings (Lemmus, Synaptomys). Zool Scr 45: 88–102
Hawdon JM, Johnston SA (1996) Hookworms in the Americas: an alternative to trans-Pacific contact. Parasitol Today 12:72–74
Hedtke SM, Kuesel AC, Crawford KE, Graves PM, Boussinesq M, Lau CL, Boakye DA, Grant WN (2020) Genomic epidemiology in filarial nematodes: transforming the basis for elimination program decision. Front Genet 10:1282
Heizer E, Zarlenga DS, Rosa B, Gao X, Gasser RB, De Graef J, Geldhof P, Mitreva M (2013) Transcriptome analyses reveal protein and domain families that delineate stage-related development in the economically important parasitic nematodes, Ostertagia ostertagi and Cooperia oncophora. BMC Genomics 14:118
Hoberg EP (1997) Phylogeny and historical reconstruction: host parasite systems as keystones in biogeography and ecology. In: Reaka-Kudla M, Wilson EO, Wilson D (eds) Biodiversity II: understanding and protecting our resources. Joseph Henry Press, National Academy of Sciences, Washington, DC, pp 243–261
Hoberg EP (2006) Phylogeny of Taenia: defining species and origins of human parasites. Parasitol Int 50:S23–S30
Hoberg EP (2010) Invasive processes, mosaics and the structure of helminth parasite faunas. Rev Sci Tech 29:255–272
Hoberg EP, Brooks DR (2008) A macroevolutionary mosaic: episodic host-switching, geographic colonization, and diversification in complex host-parasite systems. J Biogeogr 35:1533–1550
Hoberg EP, Brooks DR (2010) Beyond vicariance: integrating taxon pulses, ecological fitting and oscillation in historical biogeography and evolution. In: Morand S, Krasnov B (eds) The geography of host-parasite interactions. Oxford University Press, Oxford, pp 7–20
Hoberg EP, Brooks DR (2013) Episodic processes, invasion, and faunal mosaics in evolutionary and ecological time. In: Rohde K (ed) The balance of nature and human impact. Cambridge University Press, Cambridge, pp 199–213
Hoberg EP, Brooks DR (2015) Evolution in action: climate change, biodiversity dynamics and emerging infectious disease. Phil Trans R Soc Lond B 370(20130553):10.1098/rstb.2013.0553
Hoberg EP, Zarlenga DS (2016) Evolution and biogeography of Haemonchus contortus, linking faunal dynamics in space and time. In: Gasser RB, von Samson-Himmelstjerna G (eds.) Haemonchus contortus and Haemonchosis: past, present and future trends. Adv Parasitol 93: 1–30

Hoberg EP, Alkire NL, de Queiroz A, Jones A (2001) Out of Africa: origins of the Taenia tapeworms in humans. Proc R Soc Lond B Biol Sci 268:781–787
Hoberg EP, Galbreath KE, Cook JA, Kutz SJ, Polley L (2012) Northern host-parasite assemblages: history and biogeography on the borderlands of episodic climate and environmental transition. Adv Parasitol 79:1–97
Hoberg EP, Agosta SJ, Boeger WA, Brooks DR (2015) An integrated parasitology: revealing the elephant through tradition and invention. Trends Parasitol 31:128–133
Hoberg EP, Boeger WA, Brooks DR, Trivellone V, Agosta S (2022) Steppingstones and mediators of pandemic expansion – a context for humans as ecological super-spreaders. Manter: J Parasite Biodiversity. (in press)
Holterman M, van der Wurff A, van den Elsen S, van Megen H, Bongers T, Holovachov O, Bakker J, Helder J (2006) Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown Clades. Mol Biol Evol 23:1792–1800
Holton TA, Pisani D (2010) Deep genomic-scale analyses of the Metazoa reject coelomata: evidence from single- and multigene families analyzed under a supertree and supermatrix paradigm. Genome Biol Evol 2:310–324
Hull D (1988) Science as a process. University of Chicago Press, Chicago, IL, p 586
Huyse T, Poulin R, Théron A (2005) Speciation in parasites: a population genetics approach. Trends Parasitol 21:469–475
Ito A, Nakao M, Lavikainen A, Hoberg EP (2017) Cystic echinococcosis: future perspectives of molecular epidemiology. Acta Trop 165:3–9
Janz N, Nylin S (2008) The oscillation hypothesis of host-plant range and speciation. In: Tilmon KJ (ed) Specialization, speciation, and radiation: the evolutionary biology of herbivorous insects. University of California Press, Berkeley, CA, pp 203–215
Janzen DH (1985) On ecological fitting. Oikos 45:308–310
Jeffroy O, Brinkmann H, Delsuc F, Philippe H (2006) Phylogenomics: the beginning of incongruence? Trends Genet 22:225–231
Jenkins E, Castrodale L, de Rosemond S, Dixon B, Elmore S, Gesy K, Hoberg E, Polley L, Schurer J, Simard M, Thompson RCA (2013) Tradition and transition: parasitic zoonoses of people and animals in Alaska, northern Canada and Greenland. Adv Parasitol 82:33–204
Jex AR, Liu S, Li B et al (2011) Ascaris suum draft genome. Nature 479:529–533
Jones KE, Patel NG, Levy MA, Storeygard A, Bal D, Gittleman JL, Daszak P (2008) Global trends in emerging infectious diseases. Nature 451:990–993
Kamvar ZN, López-Uribe MM, Coughlan S, Grünwald NJ, Lapp H, Manel S (2017) Developing educational resources for population genetics in R: an open and collaborative approach. Mol Ecol Res 17:120–128
Keiser J, Utzinger J (2010) The drugs we have and the drugs we need against major helminth infections. Adv Parasitol 73:197–230
Korbel JO, Snel B, Huynen MA, Bork P (2002) SHOT: a web server for the construction of genome phylogenies. Trends Genet 18:158–162
Korhonen PK, Pozio E, La Rosa G et al (2016) Phylogenomic and biogeographic reconstruction of the Trichinella complex. Nat Commun 7:10513. https://doi.org/10.1038/ncomms10513
Kuris AM (2012) The global burden of human parasites: who and where are they? How are they transmitted? J Parasitol 98:1056–1064
Kuris AM, Hechinger RF, Shaw JC et al (2008) Ecosystem energetic implications of parasite and free-living biomass in three estuaries. Nature 454:515–518
La Rosa G, Marucci G, Zarlenga DS, Casulli A, Zarnke RL, Pozio E (2003) Molecular identification of natural hybrids between Trichinella nativa and Trichinella T6 provides evidence of gene flow and ongoing genetic divergence. Int J Parasitol 33:209–216
Lafferty KD, Allesina S, Arim M et al (2008) Parasites in foodwebs: the ultimate missing links. Ecol Lett 11:533–546

Lavikainen A, Haukisalmi V, Lehtinen MJ, Laaksonen Henttonen H, Oksanen A, Meri S (2008) A phylogeny of members of the family Taeniidae based on mitochondrial cox1 and nad1 gene data. Parasitology 135:1457–1467

Lavikainen A, Iwaki T, Haukisalmi V et al (2016) Reappraisal of Hydatigera taeniaeformis (Batsch, 1786) (Cestoda: Taeniidae) sensu lato with description of Hydatigera kamiyai n. sp. Int J Parasitol 46:361–374

Lawton SP, Hirai H, Ironside JE, Johnston DA, Rollinson D (2011) Genomes and geography: genomic insights into the evolution and phylogeography of the genus Schistosoma. Parasit Vectors 4:131

Lee LM, Wallace RS, Clyde VL et al (2016) Definitive hosts of a fatal Versteria species (Cestoda: Taeniidae) in North America. Emerg Infect Dis 22:707–710

Longhorn SJ, Foster PG, Vogler AP (2007) The nematode–arthropod clade revisited: phylogenomic analyses from ribosomal protein genes misled by shared evolutionary biases. Cladistics 23:130–144

Louca S, Pennell MW (2020) Extant timetrees are consistent with a myriad of diversification histories. Nature 580:502–505

Lu MR, Lai C-K, Liao B-Y, Tsai IJ (2020) Comparative transcriptomics across nematode life cycles reveal gene expression conservation and correlated evolution in adjacent developmental stages. Genome Biol Evol 12:1019–1030

Lustigman S, Geldhof P, Grant WN, Osei-Atweneboana MY, Sripa B, Basanez MG (2012) A research agenda for helminth diseases of humans: basic research and enabling technologies to support control and elimination of helminthiases. Plos Neglect Trop Dis 6:e1582

Marty AM, Andersen EM (2000) Fasciolopsiasis and other intestinal trematodiases. In: Meyers WM, Neafie RC, Marty AM, Wear DJ (eds) Pathology of infectious diseases, helminthiases, vol 1. Armed Forces Institute of Pathology, Washington, DC, pp 93–105

Mattiucci S, Nascetti G (2008) Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes. Adv Parasitol 66:47–148

McBurney Lin S, Khorram D, Gee S, Hoberg EP, Klassen Fischer MK, Neafie RC (2018) A new worm infiltrating the human cornea: a report of 3 cases. Am J Ophthalmol Case Rep 9:124–130. https://doi.org/10.1016/j.ajoc.2018.01.013

McManus DP (2006) Molecular discrimination of taeniid cestodes. Parasitol Int 55:S31–S37

McMichael AJ, Woodward A, Muir C (2017) Climate change and the health of nations: famines, fevers, and the fate of populations. Oxford University Press, New York, p 370

McNulty SN, Weil GJ, Heinz M, Crosby SD, Fischer PU (2008) Brugia malayi: whole genome amplification for genomic characterization of filarial parasites. Exp Parasitol 119:256–263

McNulty SN, Mitreva M, Weil GJ, Fischer PU (2013) Inter and intra-specific diversity of parasites that cause lymphatic filariasis. Infect Genet Evol 14:137–146

Meldal BH, Debenham NJ, De Ley P, De Ley IT, Vanfleteren JR, Vierstraete AR, Bert W, Borgonie G, Moens T, Tyler PA, Austen MC, Blaxter ML, Rogers AD, Lambshead PJ (2007) An improved molecular phylogeny of the Nematoda with special emphasis on marine taxa. Mol Phylogenet Evol 42:622–636

Mitreva M, Jasmer DP, Zarlenga DS et al (2011) The draft genome of the parasitic nematode Trichinella spiralis. Nat Genet 43:228–235

Mollentze N, Streicker DG (2020) Viral zoonotic risk is homogenous among taxonomic orders of mammalian and avian reservoir hosts. Proc Natl Acad Sci 117:9423–9430

Morens DM, Fauci AS (2020) Emerging pandemic diseases: how we got to COVID-19. Cell 182: 1077–1092

Morlon H, Parsons TL, Plotkin JB (2011) Reconciling molecular phylogenies with the fossil record. Proc Natl Acad Sci 108:16327–16332

Murray CJ, Lopez AD (eds) (1996) The global burden of disease, A comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020. Harvard School of Public Health, World Bank, World Health Organization, Geneva

Nadler SA (2002) Species delimitation and nematode biodiversity: phylogenies rule. Nematology 4: 615–625
Nadler SA, Hudspeth DSS (2000) Phylogeny of the ascaridoidea (Nematoda: Ascaridida) based on three genes and morphology: hypotheses of structural and sequence evolution. J Parasitol 86: 380–393
Nadler SA, Pérez-Ponce de León P (2011) Integrating molecular and morphological approaches for characterizing parasite cryptic species: implications for parasitology. Parasitology 138:1688–1709
Nakao M, McManus P, Schantz PM, Craig PS, Ito A (2007) A molecular phylogeny of the genus Echinococcus inferred from complete mitochondrial genomes. Parasitology 134:713–722
Nakao M, Xiao N, Okomoto M, Yanagida T, Sako Y, Ito A (2009) Geographic pattern of genetic variation in the fox tapeworm Echinococcus multilocularis. Parasitol Int 58:384–389
Nakao M, Lavikainen A, Iwaki T, Haukisalmi V, Konyaev S, Oku Y, Okamoto M, Ito A (2013a) Molecular phylogeny of the genus Taenia (Cestoda: Taeniidae): proposals for the resurrection of Hydatigera Lamarck, 1816 and the creation of a new genus Versteria. Int J Parasitol 43:427–437
Nakao M, Lavikainen A, Yanagida T, Ito A (2013b) Phylogenetic systematics of the genus Echinococcus. Int J Parasitol 43:1017–1029
Nguyen TGT, De NV, Vercruysse J, Dorny P, Le TH (2009) Genotypic characterization and species identification of Fasciola spp. with implications regarding the isolates infecting goats in Vietnam. Exp Parasitol 123:354–361
Nieberding CM, Durette-Desset M-C, Vanderpooten A et al (2008) Geography and host biogeography matter in understanding phylogeography of a parasite. Mol Phylogenet Evol 47:538–554
Norton AJ, Gower CM, Lamberton PHL, Webster BL, Lwambo NJS, Blair L, Fenwick A, Webster JP (2010) Genetic consequences of mass human chemotherapy for Schistosoma mansoni: population structure pre- and post-praziquantel treatment in Tanzania. Am J Trop Med Hyg 83:951–957
Nylin S, Agosta S, Bensch S et al (2018) A new paradigm for species association dynamics. Trends Ecol Evol 33:4–14
O'Brien SJ, Stanyon R (1999) Phylogenomics. Ancestral primate viewed. Nature 402:365–366
Olson PD, Cribb TH, Tkach VV, Bray RA, Littlewood DT (2003) Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). Int J Parasitol 33:733–755
O'Malley MA, Koonin EV (2011) How stands the tree of life a century and a half after the origin? Biol Direct 6:32. https://doi.org/10.1186/1745-6150-6-32
Otranto D, Deplazes P (2019) Zoonotic nematodes of wild carnivores. IJP: Parasite Wildl 9:370–383
Palumbi S (2001) Humans as the world's greatest evolutionary force. Science 293:1786–1790
Pareek CS, Smoczynski R, Tretyn A (2011) Sequencing technologies and genome sequencing. J Appl Genet 52:413–435
Patz JA, Graczyk T, Geller N, Vittor AY (2000) Effects of environmental change on emerging parasitic diseases. Int J Parasitol 30:1395–1405
Patz JA, Gibbs HK, Foley JA, Roger JA, Smith KR (2007) Climate change and global health: quantifying a growing ethical crisis. EcoHealth 4:397–405
Patz JA, Olson SH, Uejio CK, Gibbs HK (2008) Disease emergence from global climate and land use change. Med Clin North Am 92:1473–1491
Pecl GT, Araújo MB, Bell JD et al (2017) Biodiversity redistribution under climate change: impacts on ecosystems and human well-being. Science 355(6332):eaai9214. https://doi.org/10.1126/science.aai9214
Peng W, Criscione CD (2012) Ascariasis in people and pigs: new inferences from DNA analysis of worm populations. Infect Genet Evol 12:227–235
Peng WD, Yuan K, Hu M, Zhou XM, Gasser RB (2005) Mutation scanning-coupled analysis of haplotypic variability in mitochondrial DNA regions reveals low gene flow between human and porcine Ascaris in endemic regions of China. Electrophoresis 26:4317–4326

Pérez-Ponce de Léon G, Nadler SA (2010) What we don't recognize can hurt us: a plea for awareness about cryptic species. J Parasitol 96:453–464
Peterson AT (2011) Ecological niche conservatism: a time-structured view of evidence. J Biogeogr 28:817–827
Philippe H, Snell EA, Bapteste E, Lopez P, Holland PW, Casane D (2004) Phylogenomics of eukaryotes: impact of missing data on large alignments. Mol Biol Evol 21:1740–1752
Philippe H, Delsuc F, Brinkmann H, Lartillot N (2005a) Phylogenomics. Ann Rev Ecol Evol Syst 36:541–562
Philippe H, Lartillot N, Brinkmann H (2005b) Multigene analyses of bilaterian animals corroborate the monophyly of Ecdysozoa, Lophotrochozoa, and Protostomia. Mol Biol Evol 22:1246–1253
Phillips MJ, Delsuc F, Penny D (2004) Genome-scale phylogeny and the detection of systematic biases. Mol Biol Evol 21:1455–1458
Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol Econ 52:273–288
Polley L (2005) Navigating parasite webs and parasite flow: emerging and re-emerging parasitic zoonoses of wildlife origin. Int J Parasitol 35:1279–1294
Polley L, Thompson RCA (2009) Parasite zoonoses and climate change: molecular tools for tracking shifting boundaries. Trends Parasitol 25:285–291
Pozio E, Hoberg EP, La Rosa G, Zarlenga DS (2009) Molecular taxonomy and phylogeny of nematodes of the genus Trichinella. Infect Genet Evol 9:606–616
Quental TB, Marshall CR (2010) Diversity dynamics: molecular phylogenies need the fossil record. Trends Ecol Evol 25:434–441
Rabosky DL, Lovette IJ (2008) Explosive evolutionary radiations: decreasing speciation or increasing extinction through time? Evolution 62:1866–1875
Riccardi A (2007) Are modern biological invasions an unprecedented form of global change? Conserv Biol 21:239–336
Robertson LJ, van der Giessen JWB, Batz MB, Kojima M, Cahill S (2013) Have foodborne parasites finally become a global concern? Trends Parasitol 29:101–103
Robertson LJ, Sprong H, Ortega YR, van der Giessen JWB, Fayer R (2014) Impacts of globalisation on foodborne parasites. Trends Parasitol 30:37–52
Rokas A, Williams BL, King N, Carroll SB (2003) Genome-scale approaches to resolving incongruence in molecular phylogenies. Nature 425:798–804
Rollinson D, Kaukas A, Johnston DA, Simpson AJ, Tanaka M (1997) Some molecular insights into schistosome evolution. Int J Parasitol 27:11–28
Rosenthal B (2008) How has agriculture influenced the geography and genetics of animal parasites? Trends Parasitol 25:67–70
Rosenthal BM, La Rosa G, Zarlenga D, Dunams D, Chunyu Y, Mingyuan L, Pozio E (2008) Human dispersal of Trichinella spiralis in domesticated pigs. Infect Genet Evol 8:799–805
Roy SW, Gilbert W (2005) Resolution of a deep animal divergence by the pattern of intron conservation. Proc Natl Acad Sci U S A 102:4403–4408
Rufener L, Keiser J, Kaminsky R, Mäser P, Nilsson D (2010) Phylogenomics of ligand-gated ion channels predicts monepantel effect. PLoS Patho 6:e1001091
Sapp SGH, Bradbury RS (2020) The forgotten exotic tapeworms: a review of uncommon zoonotic Cyclophyllidea. Parasitology 147:533–558. https://doi.org/10.1017/S003118202000013X
Sharma R, Thompson PC, Hoberg EP et al (2020) Hiding in plain sight: discovery and phylogeography of a cryptic species of Trichinella (Nematoda: Trichinellidae) in wolverine (Gulo gulo). Int J Parasitol 50:277–287
Shinya R, Morisaka H, Kikuchi T, Takeuchi Y, Ueda M, Futai K (2013) Secretome analysis of the pine wood nematode Bursaphelenchus xylophilus reveals the tangled roots of parasitism and its potential for molecular mimicry. PLoS One 8:e67377
Silva L, Liu S, Blanton RE (2006) Microsatellite analysis of pooled Schistosoma mansoni DNA: an approach for studies of parasite populations. Parasitology 132:331–338

Small ST, Labbé F, Coulibaly YI, Nutman TB, King CL, Serre D, Zimmerman PA (2019) Human migration and the spread of the nematode parasite Wuchereria bancrofti. Mol Biol Evol 36: 1931–1941

Smart JJC (1959) Can biology be an exact science? Synthese 11:359–368

Smythe AB, Holovachov O, Kocot KM (2019) Improved phylogenomic sampling of free-living nematodes enhances resolution of higher-level nematode phylogeny. BMC Evol Biol 19:121. https://doi.org/10.1186/s12862-019-1444-x

Snyder SD, Loker ES (2000) Evolutionary relationships among the Schistosomatidae (Platyhelminthes: Digenea) and an Asian origin for Schistosoma. J Parasitol 86:283–288

Steinauer ML (2009) The sex lives of parasites: investigating the mating system and mechanisms of sexual selection of the human pathogen Schistosoma mansoni. Int J Parasit 39:1157–1163

Steinauer ML, Hanelt B, Mwangi IN, Maina GM, Agola LE, Kinuthia JM, Mutuku MW, Mungai BN, Wilson WD, Mkoji GM, Loker ES (2008) Introgressive hybridization of human and rodent schistosome parasites in western Kenya. Mol Ecol 17:5062–5074

Steinauer ML, Blouin MS, Criscione CD (2010) Applying evolutionary genetics to schistosome epidemiology. Infect Genet Evol 10:433–443

Strube C, Buschbaum S, Schnieder T (2012) Genes of the bovine lungworm Dictyocaulus viviparus associated with transition from pasture to parasitism. Infect Genet Evol 12:1178–1188

Swain MT, Larkin DM, Caffrey CR, Davies SJ, Loukas A, Skelly PJ, Hoffmann KF (2011) Schistosoma comparative genomics: integrating genome structure, parasite biology and anthelmintic discovery. Trends Parasitol 27:555–564

Taylor SH, Latham SM, Woolhouse MEJ (2001) Risk factors for human disease emergence. Phil Trans R Soc Lond B Biol Sci 356:983–989

Taylor CM, Wang Q, Rosa BA, Huang SC, Powell K, Schedl T, Pearce EJ, Abubucker S, Mitreva M (2013) Discovery of anthelmintic drug targets and drugs using chokepoints in nematode metabolic pathways. PLoS Pathog 9:e1003505

Terefe Y, Hailemariam Z, Menkir S et al (2014) Phylogenetic characterisation of Taenia tapeworms in spotted hyenas and reconsideration of the 'out of Africa' hypothesis of Taenia in humans. Int J Parasitol 44:533–541

Thompson JN (2005) The geographic mosaic of coevolution. University of Chicago Press, Chicago, IL

Thompson PC, Zarlenga DS, Liu MY, Rosenthal BM (2017) Long-read sequencing improves assembly of Trichinella genomes 10-fold, revealing substantial synteny between lineages diverged over 7 million years. Parasitology 144:1302–1315

Trivellone V, Hoberg EP, Boeger WA, Brooks DR (2022) Food security and emerging infectious disease: risk assessment and risk management. R Soc Open Sci:211687. https://doi.org/10.1098/rsos.211687

Trtanj J, Jantarasami L, Brunkard J et al (2016) Chapter 6: climate impacts on water-related illness. In: The impacts of climate change on human health in the United States: a scientific assessment. U.S. Global Change Research Program, Washington, DC, pp 157–188

Tsai IJ, Zarowiecki M, Holroyd N et al (2013) The genomes of four tapeworm species reveal adaptations to parasitism. Nature 496:57–63

Underwood AP, Supali T, Wu Y, Bianco AE (2000) Two microsatellite loci from Brugia malayi show polymorphisms among isolates from Indonesia and Malaysia. Mol Biochem Parasitol 106: 299–302

Valentim CLL, LoVerde PT, Anderson TJC, Criscione CD (2009) Efficient genotyping of Schistosoma mansoni miracidia following whole genome amplification. Mol Biochem Parasitol 166:81–84

van Megen H, Van den Elsen S, Holterman M, Karssen G, Mooyman P, Bongers T, Holovachov O, Bakker J, Helder J (2009) A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences. Nematology 11:927–950

Waeschenbach A, Littlewood DTJ (2017) Molecular framework for the Cestoda. In: Caira JN, Jensen K (eds) Planetary biodiversity inventory (PBI): tapeworms from vertebrate bowels of the

earth (2008–2017), Special Publication, vol 25. Natural History Museum, The University of Kansas, Lawrence, KS, pp 431–451
Watts N, Adger WN, Ayeb-Karlsson S et al (2017a) The lancet countdown: tracking progress on health and climate change. Lancet 389:1151–1164
Watts N, Amann M, Ayeb-Karlsson S et al (2017b) The lancet countdown on health and climate change: from 25 years of inaction to a global transformation for public health. Lancet 391:1–50
Weaver HJ, Hawdon JM, Hoberg EP (2010) Soil-transmitted helminthiases: implications of climate change and human behavior. Trends Parasitol 26:574–581
Whitmee S, Haines A, Beyrer C et al (2015) Safeguarding human health in the Anthropocene epoch: report of the Rockefeller Foundation–lancet commission on planetary health. Lancet 386:1973–2028
Wiley EO, Lieberman BS (2011) Phylogenetics: the theory and practice of phylogenetic systematics, 2nd edn. Wiley-Blackwell, Hoboken
Wolf YI, Rogozin IB, Koonin EV (2004) Coelomata and not Ecdysozoa: evidence from genome-wide phylogenetic analysis. Genome Res 14:29–36
Wolfe N, Panosian Dunavan C, Diamond J (2007) Origins of major human infectious diseases. Nature 447:279–283
Xu L, Xu M, Sun X et al (2019) The genetic basis of adaptive evolution in parasitic environment from the Angiostrongylus cantonensis genome. PLoS Negl Trop Dis 13:e0007846. https://doi.org/10.1371/journal.pntd.0007846
Yamane K, Suzuki Y, Tachi E et al (2012) Recent hybridization between Taenia asiatica and Taenia saginata. Parasitol Int 61:351–355
Yanagida T, Carod J-F, Mirua T, Nakayama T, Sako Y, Nakao M, Hoberg EP, Ito A (2014) Genetics of the pig tapeworm in Madagascar reveal a history of human dispersal and colonization. PLoS One 9(10):e109003. https://doi.org/10.1371/journal.pone.0109002
Yuan H, Jiang J, Jiménez FA, Hoberg EP, Cook JA, Galbreath KE, Li C (2016) Target gene enrichment in the cyclophyllidean cestodes, the most diverse group of tapeworms. Mol Ecol Res 16:1095–1106
Zarlenga DS, Rosenthal BM, La Rosa G, Pozio E, Hoberg EP (2006) Post Miocene expansion, colonization, and host switching drove speciation among extant nematodes of the archaic genus Trichinella. Proc Natl Acad Sci U S A 103:7354–7359
Zarlenga DS, Wang Z, Mitreva M (2016) Trichinella Spiralis: adaptation and parasitism. Vet Parasitol 231:8–21
Zhu Q, Mai U, Pfeiffer W et al (2019) Phylogenomics of 10,575 genomes reveals evolutionary proximity between domains bacteria and archaea. Nat Commun 10:5477. https://doi.org/10.1038/s41467-019-13443-4

Chapter 3
Paleoparasitology of Helminths

Raffaele Gaeta and Gino Fornaciari

Abstract The paleoparasitology is an important branch of the paleopathology, which is the discipline that studies past diseases from ancient human remains, both skeletal and mummified. This topic is crucial for understanding the lifestyle of the past in terms of hygiene conditions, sanitary measures, and nutrition.

A central role is certainly played by the study of the helminths; in fact, evidences of human ancient parasitism are largely recorded around the world, from prehistory to present age, through the analysis of the coprolites, latrine soils, and mummified remains.

The purpose of the chapter is to illustrate the most significant paleoparasitological findings in the four continents and in various periods, thus showing the wide spread of the whole classes of the helminths (trematodes, cestodes, nematodes).

3.1 Introduction

Paleopathology is the discipline that investigates the diseases of the past through the study of skeletons and mummified human remains (Buikstra 2019). For this reason, it differs from the history of medicine, which debates on past medical theories, therapies, and diseases from historical and literary sources. Paleopathology is therefore closely related to the methods of conventional medicine but enriched and supported by subjects such as history, anthropology, and archaeology. An important branch of this discipline is the paleoparasitology, a term coined in 1978 in the Oswaldo Cruz Foundation (Brazil) (Ferreira et al. 1979), which refers to the study of parasites found in archaeological or paleontological material. This subject is

R. Gaeta (✉)
Division of Paleopathology, Department of Translational Research and of New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy
e-mail: raffaele.gaeta@med.unipi.it

G. Fornaciari
School of Specialization in Archaeological Heritage, Department of Civilisations and Forms of Knowledge, University of Pisa, Pisa, Italy

F. Bruschi (ed.), *Helminth Infections and their Impact on Global Public Health*,
https://doi.org/10.1007/978-3-031-00303-5_3

Fig. 3.1 Geographic localization of the main cases of helminth infection in the world

crucial for understanding the paleoclimate and the lifestyle of the past populations in terms of hygiene conditions, sanitary measures, and nutrition (Bouchet et al. 2003a). However, it also has crucial implications for current medicine and possibly for predicting the evolution of a certain disease. Paleoparasitology has a centennial history since in 1910 Sir Armand Ruffer was the first scientist to identify a parasite (*Schistosoma haematobium*) in Egyptian mummies (Ruffer 1910). Over the years, thanks to technological improvements, the discoveries have multiplied, and many parasites have been identified in ancient human remains in all continents (Fig. 3.1).

Within this discipline, a central role is certainly played by the study of the helminths, since evidences of this type of human ancient parasitism are largely recorded around the world, from prehistory to present age (Ewald 1996).

Paleoparasitology relies on numerous biological sources and adopts a great variety of techniques. One of the major sources are certainly coprolites, i.e., desiccated or mineralized feces, and the latrine soils, which can be recovered from archaeological layers or directly from mummified bodies; this encourages an increasing cooperation among archaeologists, paleopathologists, and paleoparasitologists (Fig. 3.1).

3.2 Materials

Conditions of preservation of parasites in ancient human remains vary from region to region and strictly depend on the rapid interruption of the decay. The optimal climatic conditions are those in which the aridity rate is high or the temperatures are very low. For these reasons, tropical regions are the least suitable due to their high rainfall, acidic soil, and large populations of insects.

Paleofeces can be in organic or mineralized (i.e., coprolites) state and represent the major source for investigating the diet and "also insights into sanitation practices, changing perceptions of cleanliness, and social organization in the past, as well as information on the local ecology and environment" (Shillito et al. 2020). They have been found worldwide in archaeological contexts and may range in size from intact fecal pieces to millimeter-sized sediment inclusions (Borry et al. 2020; Hugot et al. 2014).

Latrine sediments can be assimilated to paleofeces as they are characterized by layers of organic material in latrine pits. However, they differ from feces as they do not have any identifiable shape, a part of the archaeological context, and are usually analyzed as the soil sediments. Latrine sediments may contain eggs, larvae, or parasite DNA.

Mummies represent a rare and precious material and are found all over the world, at every latitude and in every climate zone. Some mummies are artificially prepared, while others are naturally preserved by environmental conditions such as freezing climate or hot, dry, windy climate that result in rapid dehydration of the body. Artificial mummification often occurs through evisceration, where the internal organs are removed. Therefore, natural mummification is the best condition for paleoparasitological studies. In fact, in mummified intestine, parasite eggs and *larvae* can be more easily found than in coprolites or sediments (Bouchet et al. 2003).

Aside from paleofeces, latrine sediments, and mummified tissues, soil samples from the pelvic region of skeletons may also be submitted for analysis.

3.3 Methods

The techniques used for the identification of parasites are essentially morphological and involve genetic analysis using ancient DNA (Fig. 3.2).

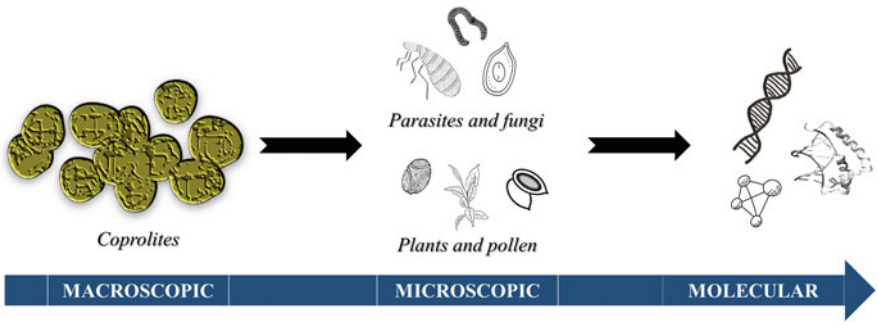

Fig. 3.2 Steps in paleoparasitology analysis, from morphology to aDNA

3.3.1 Morphological Examination

The approach to paleoparasitological study is basically morphological, both macro- and microscopic. The first fundamental step in the study of desiccated samples (like unlithified coprolites or other materials that potentially contain parasites) is the rehydration, which requires the use of trisodium phosphate aqueous solution (Na_3PO_4), introduced by Callen and Cameron (1960). After this step, any parasitological technique for microscopic examination can be applied (Araújo et al. 2015). In many coprolites detected in mummies, Araujo and colleagues discovered that a 4.0% solution of KOH "is effective in dispersing the breakdown products allowing for the disaggregation of the sample" (2015). This is not an uncommon occurrence, since almost half of the mummies contain coprolites.

The most typical finding observed under the microscope are eggs, which can be preserved in coprolites, organic remains, and soil sediments contaminated by the feces. They represent the major residues that can be extracted from archaeological and paleopathological samples. The size of the eggs may vary from 30 to 150 μm, and they are produced by adult worms of helminths parasitizing human or animal gastrointestinal tracts. The different size, shape, color, and other patterns of each parasite egg can be evaluated by using a light microscope to identify specific parasite genera or species.

3.3.2 Ancient DNA

The nuclear and mitochondrial DNA recovered from archaeological and paleontological remains is called ancient DNA (aDNA) and can be extracted from a large variety of biological materials, of different origin, state of preservation, and age. However, the ancient remains always contain only small fragments of aDNA, generally in a poor state of preservation. Postmortem instability of the nucleic acids is responsible for the degradation of DNA. The most serious criticism in the paleogenetic field is the potential contamination of samples by contemporary DNA. Since modern DNA consists of intact template molecules, it will be amplified with much higher efficiency during the PCR process compared to the fragmented and damaged aDNA templates. For this reason, it is fundamental to conduct paleogenetic researches in a laboratory devoted exclusively to ancient DNA, where no investigations with modern DNA are conducted. Moreover, specimen collection and sampling must be performed by taking severe precautions to avoid contaminations (Gaeta 2021). For these reasons, the use of molecular biology in the study of ancient parasite DNA is currently a challenge for paleopathologist and paleoparasitologists. However, it is extremely important, since the determination of aDNA can prove the family, genus, and species of the parasitic elements and can be used in the comparative study of parasites. Moreover, molecular biology provides new possibilities for

comparing ancient and modern parasite genetic profile. Hence, it is almost becoming routine practice in the field of paleoparasitology.

3.4 Review of the Literature

3.4.1 Africa

Several studies demonstrated that many of the most common helminths (e.g., *Ascaris* sp., *Trichuris* sp., *Enterobius vermicularis*, *Taenia* sp., *Diphyllobothrium* sp., *Hymenolepis* sp., *Schistosoma* sp.) were already present 6000 years ago in the populations of the ancient Nile Valley (Harter 2003). This suggests investigating the eating habits that may have facilitated the transmission of infection by the ingestion of specific foods and the climatic and environmental conditions of northern ancient Africa (Fig. 3.3).

Fig. 3.3 African countries with paleoparasitological findings

3.4.1.1 Trematodes

The main range of parasite species has been recovered in Egypt. East Africa seems to be the center of dispersion of schistosomiasis: from there, the infection would have dispersed to other parts of the world, most likely due to the trade routes (Chamot and Amat-roze 1993; Nozais 1987).

At the beginning of the twentieth century, the development of a technique of rehydration of desiccated tissues allowed the finding of calcified ova of *S. haematobium* in the kidneys of two Egyptian mummies from the 20th dynasty (ca 1184–ca 1087 BC) (Ruffer 1910). This was the earliest demonstration of a parasitic infection in ancient human tissues.

Instead, the oldest known case of infection by *Schistosoma* in mummies was identified using immunodiagnostics (enzyme-linked immunosorbent assay, ELISA), in an Egyptian adolescent of 5000 years ago (Deelder et al. 1990). The ELISA also revealed *S. haematobium* in other two mummies of 3000 and 4000 years ago (Contis and David 1996).

Calcified *Schistosoma* sp. ova were identified radiologically in several mummies from later periods by the Manchester Mummy Project that developed a program to study the paleoepidemiology of schistosomiasis in ancient Egypt using computed tomography (CT), scanned electron microscopy (SEM), ELISA, and immunocytochemistry (David 1997).

PCR primers suitable for the fragmented ancient DNA detected *Schistosoma mansoni* and *S. haematobium* in samples of the liver from the mummy of Nekht-Ankh (ca 1900 BC) and in intestinal samples from his brother Khnum-Nakht (Matheson et al. 2014), both located in the Manchester Museum.

It is no by chance that there are several reported cases of infection (up to 65%) among mummies between 350 and 550 CE from the Sudan-Egyptian border (Miller et al. 1992). In fact, the development of irrigation in Egypt, characterized by Nile's annual flooding of cultivated fields, provided the contact of the aquatic snail intermediate hosts with humans (definitive host) in surface waters. This condition is extremely conducive to the spread of schistosomiasis, especially *S. haematobium* (Kloos and David 2001).

3.4.1.2 Cestodes

It was possible to identify ova of *Taenia saginata* in a mummified intestine placed in canopic jars dated to the 25th dynasty (715–656 BC) (Harter et al. 2003).

The presence of *Taenia* sp. eggs was already revealed by SEM in histological sections of the intestine of the Egyptian mummy ROM I (Royal Ontario Museum I), dated at 1198 years BC (Horne and Lewin 1977).

Diagnosis of cysticercosis, dating back to the late Ptolemaic period (first–second century BC), confirms the large diffusion in Egypt of the farming of pigs,

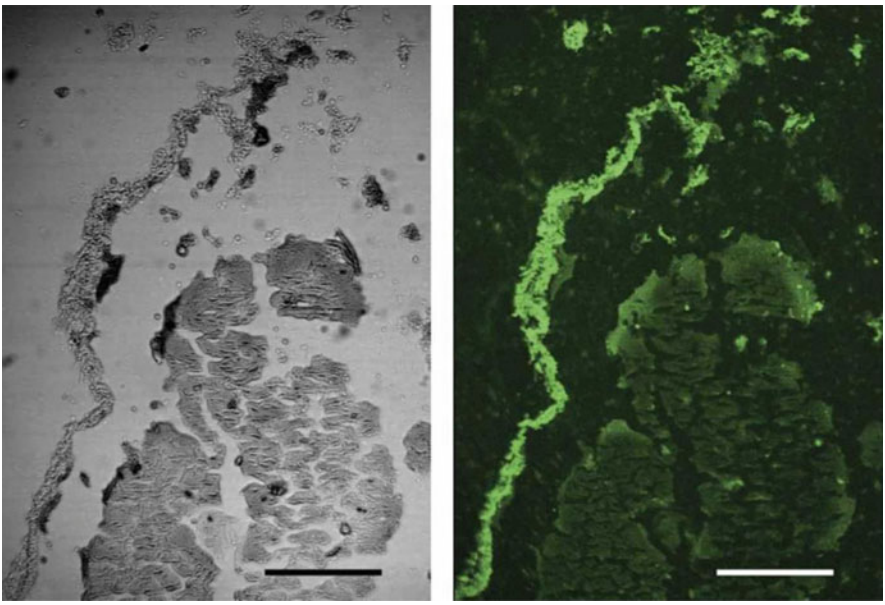

Fig. 3.4 Cysticercus cellulosae and cyst wall observed by laser confocal microscopy after incubation of sections with Taenia solium-positive control serum. Left: transmission light microscope view. Right: corresponding fluorescent image. The bladder wall is strongly stained (from Bruschi et al. 2006)

representing the most common intermediate hosts of *Taenia solium* (Bruschi et al. 2006) (Fig. 3.4).

3.4.1.3 Nematodes

Cockburn et al. (1975) found eggs of *Ascaris lumbricoides* in an Egyptian mummy dated to 170 BC (Ptolemaic period) of an unknown man (called PUM II – Pennsylvania University Museum), approximately of 35–40 years of age. The eggs were located in the intestinal mucosa, thus demonstrating the antiquity of *Ascaris* infection.

Evans et al. (1996) examined coprolites dated from 12,000 to 9000 BP, recovered in the Kruger Cave (South Africa), probably inhabited by ancestors of the modern San (also known as Bushmen), hunter-gatherer people who live in the southern areas of Africa. Surprisingly, the presence of both *Ascaris* sp. and *Trichuris* sp. eggs was detected, demonstrating "the presence of these human parasites in the sub-Saharan region and the antiquity of the *Ascaris-Trichuris* association" (Araújo and Ferreira 2014; p. 408).

This condition was confirmed by Harter et al. (2003), who reported the presence of nematodes in samples from Sai (Sudan) dated from 2700 BC up to 1500 AD, consisting in eggs of *A. lumbricoides*, *Trichuris* sp., and *E. vermicularis.*

In 2002, Horne described for the first time embryonated eggs of *E. vermicularis* in fecal samples of two mummies from Kellis site, in the Dakhleh Oasis (Egypt), dated to 30 BC–395 AD (Horne 2002).

3.4.2 Middle East

In the Middle East, parasites have been identified in ancient human remains mainly in Israel, Cyprus, Turkey, Syria, and Iran (Fig. 3.5).

In particular, some of the most important works in paleoparasitology of the Middle East derive from the studies of medieval latrine sediments, useful to determine the frequency of helminth infections in the crusader population.

In order to help pilgrims in the Holy Land, many hospital organizations were founded in the twelfth and thirteenth century, like the Knights Hospitallers, which set up a network of hospitals with thousands of beds. The several paleoparasitological findings confirm a significant rate of parasite infections, because of war, plagues, and poor hygienic conditions as described by the Muslim traveler Ibn Jubayr who visited crusader Acre in the twelfth century and commented how the city was "full of refuse and excrement" (Ibn Jubayr 1952, p. 318).

Fig. 3.5 Middle Eastern countries with paleoparasitological findings

In Israel, on the other hand, the paleoparasitological research has been carried out so far on samples before the crusader period. Zias and Mumcuoglu presented two calcified cysts of the abdominal cavity of an individual buried in a tomb in Jerusalem. The analyses revealed that these cysts, dated back to the Herodian period (first century AD), were probably hydatid cysts caused by *Echinococcus granulosus* (Zias and Mumcuoglu 1991). In the first-century BC to first-century AD organic sediment samples from Qumran, eggs of *Ascaris* sp., *Trichuris* sp., and *Taenia* sp. were observed (Harter et al. 2004). Finally, in Nahal-Mishmar Valley, eggs of *T. trichiura* dated back to the second century AD have been described (Witenberg 1961).

3.4.2.1 Trematodes

In Mesopotamia, agricultural workers were repeatedly exposed to infection by *Schistosoma* from snail-infested irrigation channels. The priestly caste was also at risk due to the use of freshwater for religious ceremonies in temples. The situation was probably further complicated by the capture of slaves, who may therefore have assisted in spreading the disease and introducing new strains of parasites (Adamson 1976).

Cyprus is a land of millennial history for its role of crossroads between East and West. Indeed, helminth eggs of *Fasciola* sp. discovered in Shillourokambos (Cyprus) from the Neolithic periods (8500–7500 BC) are the most ancient findings in the Middle East region (Harter 2003). Moreover, by the analysis of five sediment samples collected from the area of the hip bone of human skeletons, the authors found eggs of *A. lumbricoides*, *Trichuris* sp., *Taenia* sp., and *Diphyllobothrium* sp. (Harter 2003; Harter-Lailheugue et al. 2005).

Analysis of sediment from the pelvic region of human skeletons dated 4500–4000 BC, recovered in Tell Zeidan (Syria), revealed the presence of *Schistosoma* eggs (Anastasiou et al. 2014), suggesting the presence of trematodes 6000 years ago in this part of the Middle East.

3.4.2.2 Cestodes

Eggs of *Diphyllobothrium latum* were identified in the thirteenth-century latrine sediments of the crusader Hospital of St. John of Acre (Israel), representing the most ancient sample of this parasite in archeological findings of Middle East. This may be the proof that tapeworm must have been introduced in that area by the crusaders (Mitchell and Stern 2001), even though dog tapeworm (*E. granulosus*) and beef/pork tapeworm (*Taenia* sp.) have been found in pre-medieval Israel.

The most ancient presence of parasitism in Iran was detected from soil samples recovered in the salt mine of Chehrabad (northwestern Iran), dated back to 2500 and 1500 years BP. Samples were collected from different archeological levels, which contained coprolites of human and animal origin. Parasite extraction led to the

recovery of a wide variety of human and animal parasites, in particular tapeworm (*Taenia* sp. and *Echinococcus* sp.), detected in 22.6% of the samples (Nezamabadi et al. 2013a, b).

3.4.2.3 Nematodes

The first report of ancient parasite from Israel was published by Witenberg in 1961, who observed, at light microscopy, eggs of *T. trichiura* in two coprolite samples dated to the Bar Kochba period (132–135 AD) from Judean Desert cave in Nahal-Mishmar Valley (Witenberg 1961).

The most common ova in the latrine sediments of Acre, as well as in pre-crusader Israel sites, are certainly of *T. trichiura*, followed by *A. lumbricoides* (Mitchell and Stern 2001). Referring to the crusader period, there are two cases found in latrine sediments inside the castle of Saranda Kolones at Paphos, Cyprus, built after the conquest of the island by King Richard I of England in 1191 AD, during the third Crusade. The microscopic examination demonstrated the presence of eggs of *A. lumbricoides* and *T. trichiura* (Anastasiou and Mitchell 2013).

In Turkey, the famous Neolithic site of Çatalhöyük (7100–6150 BC) provided the most ancient evidences of whipworm (*T. trichiura*) eggs in two coprolites from human burials (Ledger et al. 2019). This suggests that the well-organized settlement of Çatalhöyük, i.e., housing, infrastructure, sociocultural practices, and subsistence strategies, resulted in "crowded living conditions, with middens containing human excrement located directly adjacent to houses," that "probably played a role in the transmission of whipworm, as it is spread by the faecal-oral route" (Ledger et al. 2019; p. 577).

The city of Sagalassos (Turkey) was a major urban settlement in Pisidia region during the Roman imperial age (second–fifth century AD). Five latrine sediment samples from the Roman bath resulted positive for *Ascaris* sp. eggs (Williams et al. 2017).

Whipworm, roundworm (*Ascaris* sp.), and pinworm (*E. vermicularis*) have been identified in the abovementioned sites of Chehrabad (Iran), dating back to 2500 and 1500 years BP (Nezamabadi et al. 2013a, b). From the same site, Nezamabadi et al. (2013a, b) identified tapeworm eggs from the genus *Taenia* sp. in human mummified remains dated to 2286 ± 28 BP.

3.4.3 Europe

Analyses of European latrines have been the source of many paleoepidemiological studies, comparing disease of different settlements and villages.

Parasitic infections were a common occurrence in medieval life, as emerges from numerous archaeological sites. In particular, the *Ascaris/Trichuris* pair is known for its almost systematic presence from Roman times until the Renaissance.

Fig. 3.6 European countries with paleoparasitological findings

Given the high number of findings (Fig. 3.6), only a few papers will be mentioned, while Tables 3.1 and 3.2 refer for a more complete summary.

3.4.3.1 Trematodes

Trematode infection was a well-known problem in ancient times in the Old World, especially the lancet fluke, *Dicrocoelium* sp., that is attested in Western Europe from middle Pleistocene to the sixteenth century AD (Le Bailly and Bouchet 2010).

Hoeppli (1959) published a dissertation about diseases in ancient populations based on old documents, such as Hippocratic texts and the *Ebers Papyrus*, and identified the parasitic infections, characterized by hydropsy and anemia, in ancient Rome and Greece. In fact, during the Roman period and the Middle Ages, *Dicrocoelium* sp. and *Fasciola* sp. infections were frequent and thus well documented in samples collected from several excavations in archaeological sites such as Paris, Montbeliard, Reims, and Bordeaux (Bouchet et al. 2003b). Another demonstration of the high incidence of *Dicrocoelium dendriticum* in Roman

Table 3.1 Some intestinal parasites reported in European countries in the Roman period (first century BC to fifth century AD) (modified from Ledger et al. 2019)

Country	Site	Date	Parasites	Sample Type
Austria	Carnuntum	101–300 CE	*Ascaris lumbricoides* *Taenia* sp. *Trichuris trichiura*	sewer and latrine
Belgium	Arlon	1–300 CE	*Ascaris* sp. *Trichuris* sp.	vats or pits
	Mageroy	Roman	*Entamoeba histolytica*	latrine
Britain	Leicester	1–200 CE	*Ascaris* sp. *Fasciola* sp. *Trichuris* sp.	cesspit
	London, Hibernia Wharf	1–200 CE	*Ascaris lumbricoides* *Diphyllobothrium latum* *Taenia* sp. *Trichuris trichiura*	well
	Carlisle	1–300 CE	*Ascaris lumbricoides* *Fasciola* sp. *Trichuris trichiura*	occupation sediment
	Ambleside	1–400 CE	*Ascaris* sp. *Trichuris trichiura*	pit
	Church Street Sewer, York	1–500 CE	*Ascaris* sp. *Trichuris* sp.	sewer
	London, 15–35 Copthall Ave.	101–400 CE	*Dicrocoelium dendriticum* *Trichuris trichiura*	occupation sediment
	Bearsden	142–158 CE	*Ascaris* sp. *Trichuris* sp.	sewer
	Lincoln, Waterside NW	301–400 CE	*Trichuris* sp.	occupation sediment
	Owslebury, Winchester	Roman	*Ascaris* sp. *Dicrocoelium* sp. *Trichuris*/*Capillaria* sp.	pit
France	Bobigny Hospital	201 BCE–100 CE	*Ascaris lumbricoides* *Trichuris trichiura*	pelvic soil
	Marseille	14 BCE–27 CE	*Diphyllobothrium* sp. *Fasciola* sp. *Trichuris* sp.	occupation sediment
	Lattes	1–200 CE	*Entamoeba histolytica*	cesspit
	Bordeaux	40–51 CE	*Ascaris* sp. *Taenia* sp. *Trichuris* sp.	sewer or latrine
	Reims	101–200 CE	*Ascaris* sp. *Fasciola* sp. *Trichuris* sp.	pits, wells, occupation sediment
	Jaunay-Clan	201–300 CE	*Trichuris trichiura*	coffin sediment
	La Gramiere, Castillon du Gard	201–300 CE	*Entamoeba histolytica*	cesspit
	Amiens	201–400 CE	*Capillaria hepatica*	cyst with skeletal remains
	Lisses	Roman	*Entamoeba histolytica*	pit
	Mikelauen-Zilo	Roman	*Dicrocoelium* sp. *Diphyllobothrium* sp.	occupation sediment
	Reims, Rue Carnot	Roman	*Dicrocoelium* sp.	cesspit
	Troyes, Place de la Liberation	Roman	*Dicrocoelium* sp. *Entamoeba histolytica*	cesspit
Germany	Ladenburg	1–200 CE	*Ascaris* sp. *Trichuris* sp.	latrine
	Belginum	101–300 CE	*Ascaris* sp. *Capillaria* sp. *Taenia* sp.	pit
	Künzing	140–250 CE	*Trichuris trichiura*	pit
Italy	Pompeii	100 BCE–79 CE	*Trichuris trichiura*	sewer
	Uffizi Gallery Burials	301–500 CE	*Ascaris* sp.	pelvic soil
	Roma	Roman	*Entamoeba histolytica*	–
Netherlands	Utigeest	101 BCE–300 CE	*Trichuris* sp.	well
	Alphen on the Rhine	1–100 CE	*Ascaris* sp. *Taenia*/*Echinococcus* sp. *Trichuris* sp.	latrine
	Valkenburg Army Camp	1–100 CE	*Ascaris lumbricoides* *Trichuris trichiura*	occupation sediment
Switzerland	Augst	1–100 CE	*Ascaris* sp. *Trichuris* sp.	latrine
	Eschenz	1–200 CE	*Ascaris lumbricoides* *Trichuris trichiura*	latrine
	Vindonissa	30–45 CE	Ascarididae *Trichuris* sp.	latrines

age derives from the study of the Zweeloo Woman, a bog mummy from the Netherlands dated back to the fifth century. This finding is extremely important since it is the oldest known patent infection of *D. dendriticum* in humans (Searcey et al. 2013).

Regarding *Schistosoma* sp., some samples were found in a pit adjacent to a fifteenth–sixteenth-century house in France (Bouchet and Paicheler 1995). This is an interesting case given that both urogenital and intestinal schistosomiases are considered to be of African origin, so it is possibly a diffusion by infected individuals from this continent (Bouchet et al. 2002a, 2002b).

3.4.3.2 Cestodes

Taenia and *Diphyllobothrium* eggs are a frequent finding in archaeological remains, especially in France (Bouchet et al. 2001) and Germany (Jansen and Over 1962; Herrmann 1987), mainly in the houses owned by the élite, since only wealthy people could afford to eat meat. However, it was often undercooked or nearly raw: all processes insufficient to kill the cysticerci in the muscles (in the case of *Taenia*).

A case of *cysticercosis* was found in an anatomic specimen, an encephalon, belonging to the collection of the University of Turin (Italy), dating back to 1911 AD (Ferrari and Micalizio 2001).

The presence of *Echinococcus* sp. in Europe has been reported in some studies. Calcified hydatid cysts have been rarely found in archaeological human remains due to their fragility and the difficulty of recognizing and recovering them. In Europe, there are about 25 cases from 18 different sites described in the literature, from the Hellenistic to the postmedieval periods, both from Southern and Northern Europe (Fornaciari et al. 2020).

Eight individuals with calcified hydatid cysts preserved in the thorax and abdomen were recovered during excavations in Skriðuklaustur, a medieval monastic site in eastern Iceland, which also functioned as hospital from 1493 to 1554 AD (Kristjansdottir and Collins 2010).

Two cases of cyst of tapeworm (*E. granulosus*) were found in the fourteenth-century cemetery of the hospital of S. Maria della Scala in Siena (Tuscany, Central Italy) (Fornaciari et al. 1991) and in an early twentieth-century ossuary of Saint Maddalena church in Castel di Sangro (L'Aquila, Abruzzo, central Italy) (D'Anastasio et al. 2008).

Recently, a calcified hydatid cyst has been detected in the medieval site of Badia Pozzeveri, Lucca (Tuscany, Italy), where several burials, dated back to the eleventh–fourteenth centuries, have been investigated. One skeleton, belonging to a female who died between 35 and 45 years of age, showed a calcified formation in the thoracoabdominal region, adjacent to the ninth and tenth right ribs, where the liver is located (Fornaciari et al. 2020).

Table 3.2 Some intestinal parasites reported in European countries in the medieval period (fifth–fifteenth century AD) (modified from Knorr et al. 2019, and Slepchenko and Reinhard 2018)

Country	Site Name	Sample Type	Species
Austria	Am Hof 1	Sewer	*Ascaris* sp.
Belgium	Namur	Latrine	*Ancylostoma* sp. *Ascaris* sp. *Diphyllobothrium* sp. *Entamoeba histolytica* *Fasciola hepatica* *Taenia* sp. *Trichuris* sp.
	Unknown	Latrine	*Giardia duodenalis*
Britain	Winchester	Pit	*Dicrocoelium dendriticum* *Trichuris* sp.
	Hibernia Wharf	Pit	*Ascaris* sp. *Diphyllobothrium latum* *Fasciola hepatica* *Taenia* sp. *Trichuris trichiura*
Czech Republic	Breclav-Pohansko	Pelvic soil	*Ascaris* sp. *Trichuris trichiura*
	Hradební Street	Cesspits	*Ascaris* sp. *Diphyllobothrium latum* *Fasciola hepatica* *Giardia duodenalis* *Trichuris trichiura*
Denmark	Viborg	Latrine	*Ascaris* sp. *Diphyllobothrium latum* *Enterobius vermicularis* *Fasciola hepatica* *Trichuris trichiura*
	Odense	Latrine	*Ascaris* sp. *Dicrocoelium dendriticum* *Taenia hydatigena* *Trichuris trichiura*
France	Ile de la Cité	Cesspit	*Dicrocoelium dendriticum* *Fasciola hepatica* *Taenia* sp. *Trichuris* sp.
	Pineuilh	Sediment	*Entamoeba histolytica* *Giardia duodenalis*
	Montbéliard	Latrine	*Ascaris* sp. *Dicrocoelium* sp. *Diphyllobothrium* sp. *Schistosoma haematobium* *Schistosoma mansoni* *Trichuris trichiura*
Germany	Lübeck	Latrine	*Ascaris* sp. *Diphyllobothrium latum* *Fasciola hepatica* *Taenia saginata* *Trichuris trichiura*
	Göttingen	Unknown	*Enterobius vermicularis*
Latvia	Riga	Latrine	*Ascaris* sp. *Diphyllobothrium* sp. *Trichuris trichiura*
Netherlands	Utrecht	Cesspit	*Ascaris* sp. *Trichuris trichiura*
	Kampen	Latrine	*Ascaris* sp. *Diphyllobothrium* sp. *Enterobius vermicularis* *Taenia* sp. *Toxocara* sp.
Norway	Oslo	Cesspit	*Ascaris* sp. *Diphyllobothrium* sp. *Trichuris trichiura*
Switzerland	Chevenez	Pelvic soil	*Entamoeba histolytica* *Giardia duodenalis*
Italy	Piazza Garibaldi, Parma	Rubbish pits	*Ascaris* sp. *Taenia/Echinococcus* *Diphyllobothrium* sp. *Trichuris trichiura*
Portugal	Sartilhos Grandes Santarém	Pelvic soil Pelvic soil	*Ascaris* sp. *Ascaris* sp. *Trichuris trichiura*
Spain	Collegiate-Basilica of St. Isodore	Mummified remains	*Ascaris* sp. *Trichuris trichiura*

3.4.3.3 Nematodes

As previously mentioned, ascaridiosis and trichuriasis are two common parasitic diseases infecting human populations since the antiquity. In fact, there are numerous cases described in the scientific literature.

Trichuris infection is a longtime companion of humans; in fact, *Trichuris* eggs were found in the intestine of a glacier mummy from the Copper Age (3400–3100 BCE), known as Ötzi, who was recovered on the Alps at the border between Italy and Austria (Aspöck et al. 1996; Dickson et al. 2000).

The oldest record of *Ascaris*, dated between 800 and 350 BC (Iron Age), was found in human coprolites from Hallstatt salt mines (Austria) (Aspöck et al. 1973) and from bodies of a girl and a man recovered in a bog in East Prussia dated back to the Iron Age (600 BC) (Szidat 1944).

Roundworm (*A. lumbricoides*) and whipworm (along with the fish tapeworm, genus *Diphyllobothrium*) are reported on four Merovingian skeletons, dated from the late fifth to the late ninth centuries, recovered in the church of Saint-Martin-au-Val in Chartres (Center Region, France) (Dufour et al. 2019).

In the medieval site of Chevenez, France (Le Bailly and Bouchet 2012), the paleopathologists performed parasitological study of the soil located in the pelvis and abdomen of the skeletons. The analyses highlighted two intestinal parasites, *A. lumbricoides* and *T. trichiura*.

In the medieval site of "Place d'Armes" in Namur (Belgium), several coprolites mixed with soil organic matter were recovered from a fourteenth-century latrine (Plumier et al. 1997). The coprolites revealed a very high concentration of parasite eggs, and some of those eggs still contained embryo remains. After rehydration, DNA from 104 eggs was collected and extracted with an ultrasound and phenol-chloroform-based method. The analysis of the sequences confirmed the origin from *Ascaris* sp. (Loreille et al. 2001), one of the most abundant parasites found in archeological sites.

Sediments from Islamic Spain sites, four cesspits from the tenth–eleventh-century AD Cordoba (Spain) and two from the twelfth–thirteenth-century Mertola (Portugal), were analyzed using light microscopy and ELISA. The authors revealed eggs of *A. lumbricoides* in every cesspit analyzed, affirming that Islamic Al-Andalus was less hygienic than historically depicted, since no differences between parasites found in Muslim and Christian regions of Iberia have been noted (Knorr et al. 2019).

A. lumbricoides eggs were found in the mummified bodies of Infanta María (thirteenth century AD) and (along with *T. trichiura* eggs) Infante Fernando (twelfth century AD) from the Collegiate Basilica of St. Isidoro, León (Spain) (Hidalgo-Argüello et al. 2003).

Finally, *T. trichiura* eggs were also identified by immunohistochemistry and laser confocal microscopy in the colon of a sixteenth-century natural Italian mummy from Naples (Masetti et al. 2008) (Fig. 3.7).

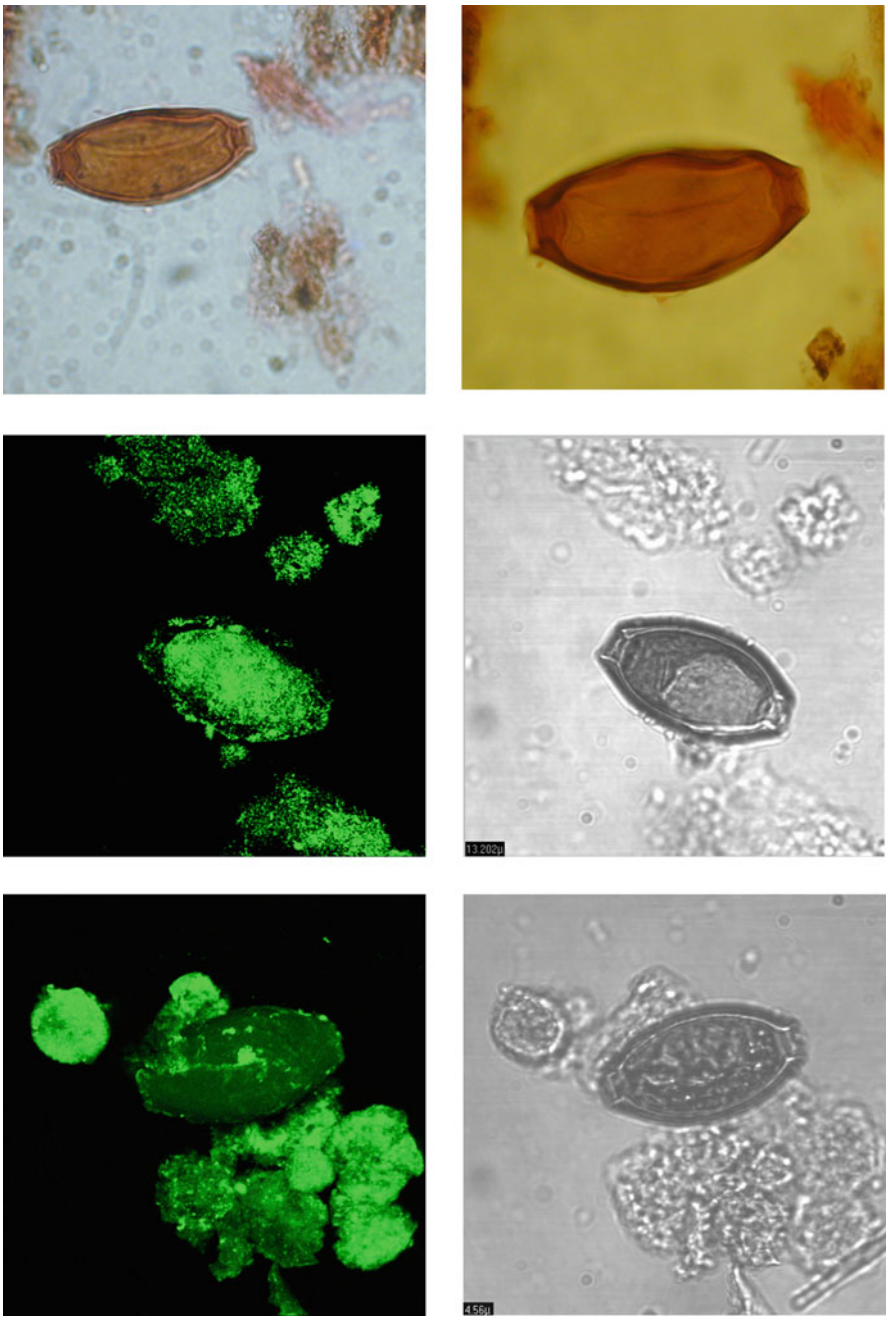

Fig. 3.7 Upper left, appearance of the organisms retrieved in the cecum of mummy NASD29 (1000x); upper right (1200x), H&E stain. Middle left, immunohistochemistry and CSLM. positive reaction, both on the surface and on the internal structure of the egg, against a specific hyperimmune serum for Trichuris muris; middle right, corresponding light microscope view (1000x). Bottom left, immunohistochemistry and CLSM with negative reaction, both on the surface and on the interior part of the egg, after exposition with pre-immune mouse serum; bottom right, corresponding light microscope view (1000x)

3.4.4 Asia

The finding of eggs of parasites from archaeological materials has been reported in several sites, mainly from South Korea, China (Chen 1956; Wei 1973; Chen and Hung 1981; Wei et al. 1981; Yang et al. 1984; Su 1987), and Japan (Matsui et al. 2003) (Fig. 3.8).

3.4.4.1 Trematodes

Korean mummies are among the most studied findings in Asia, discovered in medieval Joseon tombs (1392–1910 AD). *Clonorchis sinensis* infection (Fig. 3.9) remained one of the most common parasite infections among Koreans even up to 1970, when 12.1% of the population resulted affected (Kim et al. 1971). Thus, given the extensive historical literature regarding ancient cuisine based on raw fish consumption in the Joseon culture, it is almost certain that ancient Korean society was also infected with the parasite by this kind of food (Seo et al. 2008).

Another trematode frequently found in Joseon mummy coprolites is *Paragonimus westermani*, so much so that infection rate during the Joseon period reached 33.3% (Seo et al. 2020).

In China, paleopathologists found, in 1956, the eggs of *C. sinensis* from coprolites of a Ming Dynasty mummy (1513 AD) in the suburbs of Guangzhou (Chen 1956). Then, in 1975, *C. sinensis* eggs were found from a mummy of the West Han Dynasty in Jiangling (202 BC–220 AD) (Chen and Hung 1981; Wei et al. 1981) and from a tomb of the Chu Dynasty (170 BC–70 AD) (Hu 1984; Yang et al. 1984; Su 1987).

In Japan, *C. sinensis* was found in an early medieval cesspit of a palace of Kashihara City (Matsui et al. 2003). These findings suggest that *clonorchiasis* has been prevalent over the last 2300 years in South Korea, China, and Japan.

3.4.4.2 Cestodes

In Japan, *Taenia* sp. eggs were found in a cesspit of the Fujiwara Palace Site at Kashihara City, the capital of Japan from 694 to 710 AD (Matsui et al. 2003).

There are only two paleoparasitological reports of tapeworms in China, both from the Han Dynasty, dated back to 202 BC–220 AD (Zhan et al. 2020).

3.4.4.3 Nematodes

Several paleoparasitological findings of *A. lumbricoides* and *T. trichiura* in China, from archaeological contexts dating from the Neolithic period to the Ming Dynasty (1368–1644 AD), have been reported (Zhan et al. 2020).

Fig. 3.8 Asian countries with paleoparasitological findings

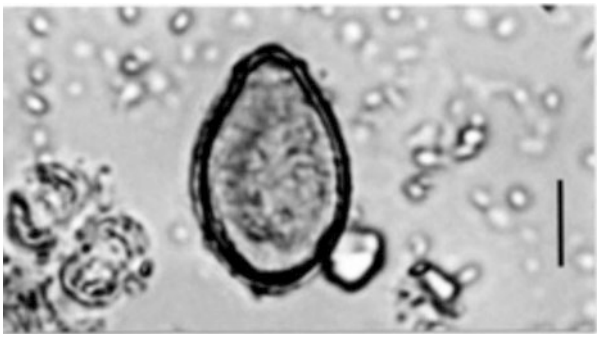

Fig. 3.9 Clonorchis sinensis (bar = 10 μm) (from Eun-Taek et al. 2003)

Eggs of *C. sinensis*, *A. lumbricoides*, and *T. trichiura* were found in the feces of a fifteenth-century child mummy from Yangju, Korea. *T. trichiura* eggs were detected in far greater numbers than other parasite eggs, with intact bipolar plugs and the *larvae* still visible in some eggs. The eggs of *C. sinensis* and *A. lumbricoides* were also well preserved. A SEM study revealed *Metagonimus yokogawai*, *P. westermani*, and *Gymnophalloides seoi* eggs recovered from Korean mummies of the Joseon Dynasty, dated back from the fifteenth to the seventeenth century (Shin et al. 2009). Moreover, Korean paleopathologists phylogenetically analyzed multiple aDNA regions of *T. trichiura* and *A. lumbricoides*, discovering the genetic features of the parasites prevalent in ancient South Korea (Seo et al. 2020).

In Japan, *A. lumbricoides*, *T. trichiura*, *C. sinensis*, and *M. yokogawai* eggs were found in a cesspit of the Fujiwara Palace Site at Kashihara City, which was the capital of Japan from 694 to 710 AD (Matsui et al. 2003).

3.4.5 America

The most debated question in paleoparasitology of America is how much the contact with the Old World influenced the population of helminths and when the different species of helminths were introduced into the New World (Reinhard 1998) (Fig. 3.10). There are three theories about this dilemma:

1. During prehistoric migrations across the Bering land bridge (10,000 BP), several Old World human parasites reached America despite polar temperatures. However, Arctic conditions played the role of a "filter" for many parasitic infections, since many of them need peculiar conditions of humidity, pH, and temperature to keep eggs or larvae alive (Araújo et al. 1988).
2. The climatic conditions of Bering region encountered by migrating people have been considered too extreme for tropical hookworms to survive, suggesting that hookworms must have been introduced to South America by "storm-tossed" fisherman or transpacific explorers or by coastal navigation from Asia.
3. Based on the analysis of the life cycle and morphology of hookworm, some paleopathologists stated that the presence of hookworm in the Americas prior to 1492 is doubtful, so helminths are considered some of many pathogens brought to the Americas after contact in 1492 (Fuller 1997).

Analyses of coprolites and intestinal contents of mummies are most frequently performed on remains discovered in the desert of the Southwestern United States (Moore et al. 1974; Fry 1980), central and northeastern Brazil (Araújo et al. 1981; Confalonieri et al. 1981; Ferreira et al. 1983), and Peru (Patrucco et al. 1983). Cold and dry environments in fact result in the preservation of helminth remains in mummies.

Fig. 3.10 American countries with paleoparasitological findings

3.4.5.1 Trematodes

Fluke eggs, which could not be specifically identified, were reported from Nevada and Utah. A single case of *P. westermani* (Chile) and *Cryptocotyle lingua* belonging to the family Heterophyidae (Alaska) have been described (Zimmerman 1998).

Echinostoma sp. eggs, most likely *E. paraensei*, are described in an adult male mummy from the Peruaçu Valley, Brazil, dated by ^{14}C method to 560 ± 40 years ago (Reinhard and Araùjo 2016).

3.4.5.2 Cestodes

Clinical data show a clear relationship between the presence of *Diphyllobothrium pacificum* and the El Niño-Southern Oscillation (ENSO) phenomenon along the Chilean coast, especially the warmer waters in the northern coast. The ENSO phenomenon causes a drastic inversion in the seawater temperatures in this region as well as dramatic environmental changes. The abundance of human mummies and archaeological coastal sites in the Atacama Desert (Chile) provides an excellent model to test the impact of ENSO in antiquity (Arriaza et al. 2010).

This arid region was the place where Chinchorro culture emerged about 9000 years ago. This population used to mummify their deceased since approximately 7000 BC, so they are considered the oldest examples of artificial mummies. The diet of the Chinchorro, reconstructed using bone chemistry (Aufderheide and Allison 1992) and coprolite samples, revealed the consumption of a variety of marine foods (Reinhard and Aufderheide 1990). Paleoparasitology shows that this population was affected by *D. pacificum*, suggesting the presence of warmer water associated with the cyclic ENSO phenomenon. The tapeworm lives in the lower intestines and competes with the host for nutrients, particularly B12 vitamin, so the cases of anemia (porotic hyperostosis) reported in ancient Chilean and Peruvian skulls may be related, in some cases, to intestinal parasite infection (Arriaza and Standen 2009).

Two parasite species were found in the desert of Atacama, in mummies of the Inca period: fish tapeworm (*D. pacificum*) and hymenolepidid tapeworm (*Hymenolepis nana*) (Santoro et al. 2003).

Tapeworms of the genus *Diphyllobothrium* have been reported from Chile and Peru with a possible finding in Michigan (McClary 1972).

Various reports of *taeniid* eggs come from studies conducted in Utah, Michigan, and Arizona (Reinhard et al. 1987).

3.4.5.3 Nematodes

Hookworm appears to have been a common disease in ancient South America, as it has been found in many archaeological sites.

The oldest case of hookworm eggs in human coprolites comes from the archaeological site of Boqueirão do Sítio da Pedra Furada, Piauí, Brazil, which is 7230 ± 80 years old (Ferreira et al. 1987). Allison et al. (1974) detected *Ancylostoma duodenale* in the intestinal mucosa of a Peruvian mummy from 900 BC. *Trichuris trichiura* and hookworm eggs and *larvae* were found in human coprolites recovered in sediment layers of an archaeological site in Minas Gerais, Brazil, dated from 3490 ± 120 to 430 ± 70 years BP (Ferreira et al. 1983). Later, the same parasites were also found in coprolites collected from a natural mummy of a child from the same site and period (Ferreira et al. 1987). Araújo (1987) also detected hookworm eggs in human coprolites from Minas Gerais, Brazil, dated from 4905 ± 90 to 1325 ± 60 years. In a Tiahuanaco mummy, dating from around 900 AD, was identified another case of hookworm and *A. duodenale* in the small intestine (Gerszten et al. 2012).

DNA results indirectly contributed to design a new panorama of *Ascaris* paleodistribution, showing that the parasite has been present in South America since 6800 years BC in the prehistoric populations from Brazil and Chile (Martinez Machado et al. 2003; Leles et al. 2008).

Several cases of pinworm (*Enterobius vermicularis*) has been reported from North America (Arizona, Colorado, Kentucky, New Mexico, Oregon, Utah) to South America (Chile, Peru, and Brazil) (Reinhard et al. 2016). Ancient DNA of *E. vermicularis* was extracted from 27 coprolites of archaeological sites in Chile and western Utah, USA, dated 7837 BC, which represents one of the oldest human parasite (Fry and Moore 1969). Enzymatic amplification of human mtDNA sequences confirmed the presence of the parasite in ancient American population (Iñiguez et al. 2003).

Whipworm (*Trichuris trichiura*) has been described from Brazil, Chile, and Peru. Of the two hookworms that infect man, *Ancylostoma duodenale* has been reported in Peru and *Necator americanus* in Brazil. Eggs of the thorny-headed worm have been reported from four sites in Utah and one in Oregon (Horne 1985).

Finally, several cases of *Anisakis* infection were detected in the aforementioned Chinchorro mummies.

Two parasite species were found in mummies of the Inca period in the desert of Atacama: pinworm (*E. vermicularis*) and whipworm (*T. trichiura*) (Santoro et al. 2003).

Recently, Valverde and colleagues described the first report of *E. vermicularis* in pre-Columbian mummies from Bolivia belonging to the post-Tiwanaku era or the Late Intermediate period (1150–1450 AD) (2020).

3.5 Conclusions

A review on the origin of human parasites (Araújo et al. 2013) demonstrates that paleoparasitology has totally changed the common knowledge based on reports and interpretations of some nineteenth-century theories. For example, all the intestinal

helminth infections, once considered introduced to America by African slaves, already existed in prehistoric American populations.

Records of ancient human tapeworms are scarce. There is a unique case of cysticercosis in an Egyptian mummy of Ptolemaic age (200–100 years BC) and other findings of ova in ancient Egypt of the Christian period, but it was impossible to determine the species of tapeworm only by the eggs. Recent phylogenetic analysis showed that human parasitism by *T. solium* and *T. saginata* predates the domestication of pigs and cattle.

A. lumbricoides is another intestinal parasite that has a long history of association with humans. There was a debate about the origin of the *ascarid*, that is, whether the human species, *A. lumbricoides*, originated from the parasite of pigs, *Ascaris suum*, or vice versa. This should have occurred 10,000 years ago, after the domestication of the pig. Molecular biology techniques have shown that infection of *A. lumbricoides* was common in prehistoric groups, in North America as well as South America, despite the rare findings in coprolites from New World archaeological sites. Since wild pigs in the New World are not parasitized by roundworms, it is very probable that prehistoric migrants brought the parasite from the Old World to the Americas. When comparing the morphological and genetic characteristics of both species of roundworm, there are few differences between them, found indistinctly in humans and pigs. Therefore, it was assumed that *A. lumbricoides* is the oldest species that was transferred to pigs after domestication and thus would be only one species of parasite, of both humans and pigs.

Many nematodes that infect the human intestinal tract have been found in prehistoric populations of the Americas. The first migrants that arrived on the continent most probably introduced them. However, not all intestinal helminths were introduced by the same prehistoric route, and the conditions of transmission of these helminths in the Americas were completely different from those in Europe. For example, in the Americas, the horse pinworm (*Oxyuris equi*) has not been detected before between 150 and 50 years before present. The absence of the parasite is explained by the fact that in the American continent in antiquity, there were no horses until the arrival of the conquistadors about 500 years ago (Dufour et al. 2015). *E. vermicularis* has no environmental restrictions on the transmission from one host to another individual, but other intestinal helminths could not survive in the cold conditions of the Siberian-Arctic regions of the Bering land bridge. However, hookworms, *T. trichiura* and *A. lumbricoides*, found in archaeological sites in both North and South America, date back 9000 years ago. Thus, alternative routes, such as transpacific or coastal navigation, introduced these parasites. Paleoparasitological data have shown that they were common among prehistoric populations in the Americas. However, the eggs found in coprolites were always in few cases, indicating a low prevalence. This is not surprising, as most prehistoric populations in the Americas were nomad and had hunter-gathering subsistence to survive. However, when Europeans arrived, they established new conditions as colonizers, building villages, enslaving Native Americans, and later deporting Africans, thus totally changing the lifestyle in the Americas.

Table 3.3 Paleoparasitological finds from human remains, in the New and Old World, in pre and post-Columbian Era (from Gonçalves et al. 2003)

	New World		Old World	
Human paleoparasitological finds	Pre-Columbian parasite finds	Post-Columbian parasite finds	Pre-Columbian parasite finds	Post-Columbian parasite finds
Ancylostomids	Y	Y	Y	Y
Ascaris lumbricoides	Y	Y	Y	Y
Trichuris trichiura	Y	Y	Y	Y
Enterobius vermicularis	Y	NF	Y	NF
Strongyloides stercoralis	?	NF	Y	NF
Trichostrongylus spp.	Y	Y	NF	NF
Fasciola spp.	?	NF	Y	?
Schistosoma spp.	NF	NF	Y	Y
Dicrocoelium spp.	NF	?	Y	NF
Opisthorchiformes	Y	NF	Y	NF
Paragonimus spp.	Y	NF	NF	NF
Taenia spp.	NF	Y	Y	Y
Diphyllobothrium spp.	Y	Y	Y	Y
Hymenolepis spp.	Y	NF	NF	NF
Acanthocephala	Y	NF	NF	NF
Entamoeba spp.	Y	NF	Y	NF
Giardia duodenalis	Y	NF	Y	Y
Chilomastix mesnili	NF	NF	Y	NF
Cryptosporidium parvum	Y	NF	NF	NF
Cyclospora cayetanensis	Y	NF	NF	NF
Isospora belli	Y	NF	NF	NF
Sarcocystis hominis	Y	NF	NF	NF
Echinoccus granulosus	Y	Y	Y	?
Trichinella spiralis	NF	Y	Y	Y
Dracunculus medinensis	NF	NF	Y	NF
Filarial worm	NF	NF	Y	NF

Y: yes; NF: not found

It is interesting to compare paleoparasitological data from Europe in the Middle Ages and the Americas, before and after the arrival of Europeans. While latrines and mummies in Europe are full of intestinal worms, in the Americas, the intestinal helminth eggs are very rare, although positive for the same helminth species. This is supposed to be a consequence of differences in the population density, but also as a result of different occupational patterns. When the first villages were founded in America, the environment changed as well as the lifestyle of many people (Hugot et al. 1999). There was a real explosion of soil-transmitted helminths caused by population growth and very poor hygienic conditions.

Below (Table 3.3), it is possible to see a summary list of the paleoparasitological findings from human remains, in the New and Old World, in pre- and post-Columbian era.

In conclusion, paleoparasitology is a multidisciplinary subject that stimulates international cooperation with the aim of formulating hypotheses and verifying results. Therefore, those involved are not only parasitologists, paleopathologists, and archaeologists but also many other specialists who can contribute to the understanding of the origin and evolution of parasitic diseases.

References

Adamson PB (1976) Schistosomiasis in antiquity. Med Hist 20:176–188
Allison MJ, Pezzia A, Hasegawa I, Gerszten E (1974) A case of hookworm infestation in a precolumbian American. Am J Phys Anthropol 41:103–106
Anastasiou E, Mitchell PD (2013) Human intestinal parasites from a latrine in the twelfth century Frankish castle of Saranda Kolones in Cyprus. Int J Paleopathol 3:218–223
Anastasiou E, Lorentz KO, Stein GJ, Mitchell PD (2014) Prehistoric schistosomiasis parasite found in the Middle East. Lancet Infect Dis 14:553–554
Araújo A (1987) Paleoepidemiologia da Ancilostomose. Tese de Doutorado, Escola Nacional de Saúde Pública, Fiocruz, Rio de Janeiro, 118 pp
Araújo A, Ferreira LF (2014) The findings in Africa. In: Ferreira LF, Reinhard KJ, Araújo A (eds) Foundations of paleoparasitology [online]. Editora FIOCRUZ, Rio de Janeiro, pp 403–416
Araújo A, Ferreira LF, Confalonieri U (1981) A contribution to the study of helminth findings in archaeological material in Brazil. Rev Bras Biol 41:873–881
Araújo A, Ferreira LF, Confalonieri U, Chame M (1988) Hookworms and the peopling of America. Cad Saúde Pública 4:226–233
Araújo A, Reinhard K, Ferreira LF, Pucu E, Chieffi PP (2013) Paleoparasitology: the origin of human parasites. Arq Neuropsiquiatr 71:722–726
Araújo A, Reinhard K, Ferreira LF (2015) Paleoparasitology—human parasites in ancient material. Adv Parasitol 90:349–387
Arriaza B, Standen V (2009) Bioarqueología. Historia biocultural de los antiguos pobladores del extremo norte de Chile. Editorial Universitaria, Santiago, p 148
Arriaza BT, Reinhard KJ, Araujo AG, Orellana NC, Standen VG (2010) Possible influence of the ENSO phenomenon on the pathoecology of diphyllobothriasis and anisakiasis in ancient Chinchorro populations. Mem Inst Oswaldo Cruz 105:66–72
Aspöck H, Flamm H, Picher O (1973) Darmparasiten in menschlichen Exkrementen aus prähistorischen Salzbergwerken der Hallstatt-Kultur (800-350 v. Chr.). Zlb Bakt Hyg I Ab Orig 223:549–558
Aspöck H, Aver H, Picher O (1996) Trichuris trichiura eggs in the neolithic glacier mummy from the alps. Parasitol Today 12:255–256
Aufderheide A, Allison M (1992) Chemical dietary reconstruction of North Chile prehistoric populations by trace mineral analysis. Department of Pathology, University of Minnesota, Duluth. Proceedings of the I World Congress on Mummy Studies 2:451–461
Borry M, Cordova B, Perri A, Wibowo M, Prasad Honap T, Ko J et al (2020) CoproID predicts the source of coprolites and paleofeces using microbiome composition and host DNA content. PeerJ 8:e9001
Bouchet F, Paicheler JC (1995) Presumption of Bilharziose on an archaeological site from XV° century in Montbéliard (Doubs, France). CR Acad Sci Sér III 318:811–814
Bouchet F, Bentrad S, Martin C (2001) Analyse paleoparasitologique. In: Le quartier Gallo-Romain de la rue de Venise et sa réoccupation à l'époque Moderne. Archéologie Urbaine. Bull Soc Arch Champenoise 2–3:148–150
Bouchet F, Harter M, Le Bailly M (2002a) Apport de la Paléoparasitologie à la connaissance des pathologies infectieuses dans les sites médiévaux de Belgique et de France. In: Au-delà de l'écrit. Les homes et leurs vécus matériels au Moyen Age à la lumière des sciences et des techniques, Proceedings of Marche-en-Famenne, 16–20 October 2002. Brepols, Turnhout, pp 99–108
Bouchet F, Harter S, Paicheler JC, Aráujo A, Ferreira LF (2002b) The first recovery of Schistosoma mansoni eggs from a latrine in Europe (15th -sixteenth century). J Parasitol 88:404–405
Bouchet F, Harter M, Le Bailly M (2003) The state of the art of paleoparasitological research in the Old World. Mem Inst Oswaldo Cruz 98(suppl. 1):95–101

Bouchet F, Guidon N, Dittmar K, Harter S, Ferreira LF, Chaves SM, Reinhard K, Araújo A (2003a) Parasite remains in archaeological sites. Mem Inst Oswaldo Cruz 98(Suppl. 1):47–52
Bouchet F, Guidon N, Dittmar K, Harter S, Ferreira LF, Chaves SM, Reinhard K, Araujo A (2003b) Parasite remains in archaeological sites. Mem Inst Oswaldo Cruz 98(suppl 1):47–52
Bruschi F, Masetti M, Locci MT, Ciranni R, Fornaciari G (2006) Cysticercosis in an Egyptian mummy of the late Ptolemaic period. Am J Trop Med Hyg 74:598–599
Buikstra J (ed) (2019) Ortner's identification of pathological conditions in human skeletal remains, 3rd edn. Academic Press, London
Callen EO, Cameron TWM (1960) A prehistoric diet revealed in coprolites. New Scientist 8:35–40
Chamot G, Amat-roze JM (1993) Les Bilharzioses Revue du Praticien 43:401–404
Chen XT (1956) Medical Parasitology. Public Health Publication, Beijing, pp 155–156
Chen LB, Hung T (1981) Scanning electron microscopic view of parasitic worm ova in an ancient corpse. Acta Chin Acad Med Sci 3:64–65
Cockburn A, Barraco RA, Reyman TA, Peck WH (1975) Autopsy of an Egyptian mummy. Science 187(4182):1155–1160
Confalonieri UE, De Araujo AJG, Ferreira LF (1981) Trichuris trichiura infection in colonial Brazil. Paleopathol Newsletter 35:13–14
Contis G, David AR (1996) The epidemiology of bilharzia in ancient Egypt: 5000 years of schistosomiasis. Parasitol Today 12:253–255
D'Anastasio R, Vitullo G, Paolucci A, Michetti E (2008) A paleopathological case of Echinococcus cyst. J Paleopathol 20:67–73
David AR (1997) Disease in Egyptian mummies: the contribution of new technologies. Lancet 349(9067):1760–1763
Deelder AM, Miller RL, De Jonge N, Krijker FW (1990) Detection of schistosome antigen in mummies. Lancet 335:724–725
Dickson JH, Oeggl K, Holden TG, Handley LL, O'Connell TC, Preston T (2000) The omnivorous Tyrolean iceman: colon contents (meat, cereals, pollen, moss and whipworm) and stable isotope analyses. Phil Trans R Soc Lond B Biol Sci 355(1404):1843–1849
Dufour B, Hugot JP, Lepetz S, Le Bailly M (2015) The horse pinworm (Oxyuris equi) in archaeology during the Holocene: review of past records and new data. Infect genet Evol 33: 77–83
Dufour B, Portat E, Bazin B, Le Bailly M (2019) Paleoparasitology of Merovingian corpses buried in stone sarcophagi in the saint-Martin-au-Val church (Chartres, France). Korean J Parasitol Dec 57(6):613–619
Eun-Taek H, Sang-Mee G, Jae-Lip K, Hoon-Jin J, Soo-Nam K, Jong-Yil C (2003) Detection of parasite eggs from archaeological excavations in the Republic of Korea. Mem Inst Oswaldo Cruz 98(suppl.1):123–126
Evans AC, Markus MB, Mason RJ, Steel R (1996) Late stone-age coprolite reveals evidence of prehistoric parasitism. S Afr Med J. mar 86(3):274–275
Ewald PW (1996) Evolution of infectious disease. Oxford University Press, Oxford, pp 3–13
Ferrari L, Micalizio S (2001) A case of cerebral cysticercosis in an anatomical collection of the last century. In: La Verghetta M, Capasso L (eds) Proceedings of the XIII European Meeting of the Paleopathology Association, pp 103–104
Ferreira LF, Araújo A, Confalonieri U (1979) Subsidios para a Paleoparasitologia do Brasil I—Parasitos encontrados em coprólitos no município de Unai, Minas Gerais. In: Congr. Bras. Parasitol., Abstr.,Campinas, SP, p. 56
Ferreira LF, Araujo A, Confalonieri U (1983) The finding of helminth eggs in a Brazilian mummy. Trans R Soc Trop Med Hyg 77:65–67
Ferreira LF, Araújo A, Chame M, Ribeiro BM (1987) Encontro de ovos de ancilostomídeos em coprólitos humanos datados de 7230 ± 80 anos, Piauí, Brasil. An Acad Bras Cienc 59:280–281
Fornaciari G, Tornaboni D, Pollina L, Tognetti A (1991) Nota paleopatologica: un caso di cisti da echinococco. In: Boldrini E, Parenti R (eds) Santa Maria della Scala. Archeologia e edilizia sulla piazza dello Spedale. Edizioni "All'Insegna del Giglio", Firenze, pp 443–445

Fornaciari A, Gaeta R, Cavallini L, Aringhieri G, Ishak R, Bruschi F, Giuffra V (2020) A thirteenth-century cystic echinococcosis from the cemetery of the monastery of Badia Pozzeveri (Lucca, Italy). Int J Paleopathol Dec 31:79–88

Fry GF (1980) Prehistoric diet and parasites in the desert west of North America. In: Browman DL (ed) Early native Americans. Mouton, The Hague, pp 325–339

Fry GF, Moore JG (1969) Enterobius vermicularis: 10,000-year-old human infection. Science 166: 1620

Fuller K (1997) Hookworm: not a pre-Columbian pathogen. Med Anthropol 17:297–308

Gaeta R (2021) Ancient DNA and paleogenetics: risks and potentiality. Pathologica 113:141–146

Gerszten E, Allison MJ, Maguire B (2012) Paleopathology in south American mummies: a review and new findings. Pathobiology 79(5):247–256

Gonçalves MLC, Araujo A, Ferreira LF (2003) Human intestinal parasites in the past: new findings and a review. Mem Inst Oswaldo Cruz 98(suppl. 1):103–118

Harter S (2003) Implication de la Paléoparasitologie dans l'Étude des Populations Anciennes de la Vallée du Nil et du Proche-Orient: études de cas. Thèse, Reims: UFR de Pharmacie, Université de Reims Champagne-Ardènne

Harter S, Le Bailly M, Janot F, Bouchet F (2003) First paleoparasitological study of an embalming rejects jar found in Saqqara, Egypt. Memórias do Instituto Oswaldo Cruz 98(suppl. 1):119–121

Harter S, Bouchet F, Mumcuoglu KY, Zias JE (2004) Toilet practices among members of the dead sea scrolls sect at Qumran (100 BCE-68 CE). Rev Qumran 20:579–584

Harter-Lailheugue S, Le Mort F, Vigne JD, Guilaine J, Le Brun A, Bouchet F (2005) Premiéres Données Parasitologiques sur les populations humaines précéramiques chypriotes (VIII e et VII e millénaires av. J-C). Paléorient 31(2):43–54

Herrmann B (1987) Parasitologische Untersuchung mittelalterlicher Kloaken. Mensch und Umwelt im Mittelalter 3:160–169

Hidalgo-Argüello MR, Díez Baños N, Fregeneda Grandes J, Prada Marcos E (2003) Parasitological analysis of Leonese royalty from collegiate-basilica of St. Isidoro, León (Spain): helminths, protozoa, and mites. J Parasitol 89(4):738–743

Hoeppli R (1959) Parasites and parasitic infection in early medicine and science. University of Malaya Press, Singapore

Horne PD (1985) A review of the evidence of human Endoparasitism in the pre-Columbian New World through the study of coprolites. J ArchaeoI Science 12:299–310

Horne PD (2002) First evidence of enterobiasis in ancient Egypt. J Parasitol 88:1019–1021

Horne PD, Lewin PK (1977) Electron microscopy of mummified tissue: autopsy of an Egyptian mummy. Can Med Ass J 117:472–473

Hu SY (1984) Study on the parasite eggs in an ancient corpse from Zhangguo Chu Tomb No. 1 in Mashan brickfield of Jiangling County, Hubei. Chin J Parasitol Parasit Dis 2:8

Hugot JP, Reinhard KJ, Gardner SL, Morand S (1999) Human enterobiasis in evolution: origin, specificity and transmission. Parasite 6(3):201–208

Hugot JP, Gardner SL, Borba V, Araujo P, Leles D, Stock Da-Rosa ÁA, Dutra J, Ferreira LF, Araújo A (2014) Discovery of a 240 million year old nematode parasite egg in a cynodont coprolite sheds light on the early origin of pinworms in vertebrates. Parasit Vect 7:486

Iñiguez AM, Reinhard KJ, Araujo A, Ferreira LF, Vicente ACP (2003) Enterobius vermicularis: ancient DNA from north and south American human coprolites. Mem Inst Oswaldo Cruz 98 (suppl. 1):67–69

Jansen JJR, Over HJ (1962) The occurrence of parasites in protohistorical material from north-West Germany. Tijdschr Diergeneesk 87:1377–1379

Ibn Jubayr (1952) The travels of Ibn Jubayr, transl. R.J.C. Broadhurst. Jonathan Cape, London

Kim CH, Park CH, Kim HJ, Chun HB, Min HK, Koh TY, Soh CT (1971) Prevalence of intestinal parasites in Korea. Korean J Parasitol 9:25–38

Kloos H, David R (2001) The Paleoepidemiology of schistosomiasis in ancient Egypt. Hum Ecol 9: 14–25

Knorr DA, Smith WPW, Ledger ML, Peña-Chocarro L, Pérez-Jordà G, Clapés R, de Fátima PM, Mitchell PD (2019) Intestinal parasites in six Islamic medieval period latrines from tenth-eleventh century Córdoba (Spain) and 12th-thirteenth century Mértola (Portugal). Int J Paleopathol Sep 26:75–83

Kristjansdottir S, Collins C (2010) Cases of hydatid disease in medieval Iceland. Int J Osteoarchaeol 21:479–486

Le Bailly M, Bouchet F (2010) Ancient dicrocoeliosis: occurrence, distribution and migration. Acta Trop 115:175–180

Le Bailly M, Bouchet F (2012) Paléoparasitologie. In: Occupations du Haut Moyen Age à Chevenez: inhumations et atelier métallurgique. Cahier d'archéologie jurassienne, Porrentruy

Ledger M, Anastasiou E, Shillito L-M, Mackay H, Bull I, Haddow S, Knüsel C, Mitchell P (2019) Parasite infection at the early farming community of Çatalhöyük. Antiquity 93(369):573–587

Leles D, Araujo A, Ferreira LF, Paulo Vicente AC, Mayo Iñiguez A (2008) Molecular paleoparasitological diagnosis of Ascaris sp. from coprolites: new scenery of ascariasis in pre-Columbian South America times. Mem Inst Oswaldo Cruz 103:106–108

Loreille O, Roumat E, Verneau O, Bouchet F, Hänni C (2001) Ancient DNA from Ascaris: extraction amplification and sequences from eggs collected in coprolites. Int J Parasitol 31: 1101–1106

Martinez Machado E, Correia Santos JA, Villela Verissimo E, Duarte Nascimento A, Ferreira LF, Bello Ribeiro A (2003) Random amplified polymorphic DNA analysis of DNA extracted from Trichuris trichiura (Linnaeus, 1771) eggs and its prospective. In: application to paleoparasitological Studies. Memórias do Instituto Oswaldo Cruz 98(suppl. I):59–62

Masetti M, Bruschi F, Locci MT, Johnson K, Pangoli D, Fornaciari G (2008) Identification of Trichuris Trichiura eggs in a sixteenth century Italian mummy. In: Atoche PP, Martin CR, Rodriguez Ramirez A (eds) Proceedings of the VI World congress on mummy studies. Academia Canaria de la Historia, Santa Cruz de Tenerife, pp 673–676

Matheson CD, David R, Spigelman M, Donoghue HD (2014) Molecular confirmation of Schistosoma and family relationship in two ancient Egyptian mummies. In: Gill-Frerking H, Rosendahl W, Zink A, Alterauge A (eds) Yearbook of mummy studies, vol 2. Verlag Dr. Friedrich Pfeil, Munich, pp 39–47

Matsui A, Kanehara M, Kanehara M (2003) Palaeoparasitology in Japan—discovery of toilet features. Mem Inst Oswaldo Cruz 98(suppl.1):127–136

McClary A (1972) Notes on some late middle woodland coprolites. In: Fitting J (ed) The Schultz site at Green point, a stratified occupation area in the Saginaw Valley of Michigan, memoirs of the Museum of Anthropology, number 4. University of Michigan, Ann Arbor, pp 131–136

Miller RL, Armelagos GJ, Ikram S, De Jonge N, Krijger FW, Deelder AM (1992) Palaeoepidemiology of Schistosoma infection in mummies. BMJ 304:555–556

Mitchell PD, Stern E (2001) Parasitic intestinal helminth ova from the latrines of the thirteenth century crusader Hospital of St. John in acre, Israel. In: La Verghetta M, Capasso L (eds) Proceedings of the XIII European meeting of the paleopathology association, pp 207–213

Moore JG, Grundmann AW, Hall HJ, Fry GF (1974) Human fluke infection in Glen canyon at AD 1250. Am J Phys Anthropol 41:115–117

Nezamabadi M, Aali A, Stöllner T, Mashkour M, Le Bailly M (2013a) Paleoparasitological analysis of samples from the Chehrabad salt mine (northwestern Iran). Int J Paleopathol. Sep 3(3): 229–233

Nezamabadi M, Mashkour M, Aali A, Stöllner T, Le Bailly M (2013b) Identification of Taenia sp. in a natural human mummy (third century BC) from the Chehrabad salt mine in Iran. J Parasitol Jun 99(3):570–572

Nozais JP (1987) Hypothèses sur le rôle du Sahara préhistorique dans la répartition de certaines affections parasitaires et hématologiques. Bull Soc Path Ex 80:121–131

Patrucco R, Tello R, Bonavia D (1983) Parasitological studies of coprolites of pre-hispanic Peruvian populations. Curr Anthrop 24:393–394

Plumier J, Vanmechelen R, Dupont C (1997) Namur Place d'armes Archeologia Mediaevalis 21: 51–52
Reinhard KJ (1998) Mummy studies and archaeoparasitology. In: Cockburn A, Cockburn E, Reyman TA (eds) Mummies, diseases & ancient cultures. Cambridge University Press, Cambridge
Reinhard KJ, Araùjo A (2016) Prehistoric pathoecology as represented by parasites of a mummy from the Peruaçu Valley. Brazil Korean J Parasitol 54(5):585–590
Reinhard KJ, Aufderheide AC (1990) Diphyllobothriasis in prehistoric Chile and Peru: adaptive radiation of a helminth species to native American populations. Paleopathol Newsletter 72: 18–19
Reinhard KJ, Hevly RH, Anderson GA (1987) Helminth remains from prehistoric Indian coprolites on the Colorado plateau. J Parasitol. Jun 73(3):630–639
Reinhard KJ, Araùjo A, Morrow JJ (2016) Temporal and spatial distribution of Enterobius vermicularis (Nematoda: Oxyuridae) in the prehistoric Americas. Korean J Parasitol 54(5): 591–603
Ruffer MA (1910) Note on the presence of bilharzia haematobia in Egyptian mummies of the XXth dynasty (1250-1000 B.C.). Br Med J 1:16
Santoro C, Vinton Dorsey S, Reinhard KJ (2003) Inca expansion and parasitism in the Lluta Valley: preliminary data. Mem Inst Oswaldo Cruz 98:161–163
Searcey N, Reinhard KJ, Egarter-Vigl E, Maixner F, Piombino-Mascali D, Zink AR, Van der Sanden W, Gardner SL, Bianucci R (2013) Parasitism of the Zweeloo woman: dicrocoeliasis evidenced in a Roman period bog mummy. Int J Paleopathol 3:224–228
Seo M, Shin DH, Guk SM, Oh CS, Lee EJ, Shin MH, Kim MJ, Lee SD, Kim YS, Yi YS, Spigelman M, Chai JY (2008) Gymnophalloides seoi eggs from the stool of a seventeenth century female mummy found in Hadong, Republic of Korea. J Parasitol 94:467–472
Seo M, Hong JH, Reinhard K, Shin DH (2020) Archaeoparasitology of Korean mummies. In: Shin DH, Bianucci R (eds) The handbook of mummy studies. Springer, Singapore
Shillito LM, Blong JC, Green EJ, van Asperen EN (2020) The what, how and why of archaeological coprolite analysis. Earth Sci Rev 207:103196
Shin DH, Lim DS, Choi KJ, Oh CS, Kim M, Lee IS, Kim Bae S, Shin J, Bok G, Chai YJ, Min S (2009) Scanning electron microscope study of ancient parasite eggs. J Parasitol 95:137–145
Slepchenko S, Reinhard K (2018) Paleoparasitology and pathoecology in Russia: investigations and perspectives. Int J Paleopathol. Sep 22:39–44
Su TC (1987) A scanning electron microscopic study on the parasite eggs in an ancient corpse from a tomb of Chu dynasty, the warring state, in Jiangling County, Hubei Province. J Tongji Med Univ 7:63–64
Szidat L (1944) Uber die Erhaltungsfähigkeit von Helmintheneiern in Vor- und Frühgeschichtlichen Moorleichen. Z Parasitenk 13:265
Valverde G, Ali V, Durán P, Castedo L, Paz JL, Martínez E (2020) First report in pre-Columbian mummies from Bolivia of Enterobius vermicularis infection and capillariid eggs: a contribution to paleoparasitology studies. Int J Paleopathol. Dec 31:34–37
Wei O (1973) Internal organs of a 2100-year-old female corpse. Lancet 24:1198
Wei DX, Yang WY, Huang SQ, Lu YF, Su TC, Ma JH, Hu WX, Xie NF (1981) Parasitological investigation on the ancient corpse of the Western Han dynasty unearthed from tomb no. 168 on Phoenix Hill in Jiangling County. Acta Acad Med Wuhan 1:16–23
Williams FS, Arnold-Foster T, Yeh HY, Ledger ML, Baeten J, Poblome J, Mitchell PD (2017) Intestinal parasites from the second-fifth century AD latrine in the Roman baths at Sagalassos (Turkey). Int J Paleopathol 19:37–42
Witenberg G (1961) Human parasites in archaeological findings. Bull Israel Expl Soc 25:86

Yang WY, Wei DX, Song GF, Wu ZB, Teng RS (1984) Parasitologic investigations on the ancient corpse of Chu dynasty the warring states unearthed from the Ma-zhuan tomb no. 1. Jiangling County Acta Acad Med Wuhan 14:43–45

Zhan X, Qi W, Yeh HY (2020) Ancient parasites seen in the archaeology and medical contexts in the Han dynasty, China. In: Shin DH, Bianucci R (eds) The handbook of mummy studies. Springer, Singapore

Zias J, Mumcuoglu KY (1991) Case reports of paleopathology: calcified hydatid cysts. Paleopathol Newsletter 73:7–8

Zimmerman MR (1998) Aleutian and Alaskan mummies. In: Cockburn A, Cockburn E, Reyman TA (eds) Mummies, disease, and ancient cultures. Cambridge Univ. Press, Cambridge, pp 138–153

Chapter 4
Under the Influence: The Systemic Consequences of Helminth Infection

Rick M. Maizels

Abstract It is well established that the survival and transmission of parasitic helminths (worms) in their mammalian hosts is dependent on down-regulation of the immune system; less well-characterised are the ramifications of helminth infection on other physiological functions. Immune modulation by helminth parasites has, for example, been linked to reduced susceptibility to inflammatory disorders, as well as poorer vaccine responses, and altered resistance to microbial pathogens. More broadly, systemic metabolism can also be affected, while in some instances the propensity for cancer is increased. In this chapter, I briefly review the pathways of immunomodulation and then consider the broader physiological and medical impact of helminths on the body.

4.1 Introduction

Helminth parasites are extraordinary in their ubiquity, adaptation, specialisation and diversification. They continue to impose an enormous disease burden across the world today, with new drugs and vaccines stubbornly slow to be developed (Keiser and Utzinger 2010; Diemert et al. 2018). Their ability to divert and evade the host immune response is well-established (Maizels and McSorley 2016), but in parallel, it is clear they exert wider-ranging effects with systemic ramifications (Mishra et al. 2014).

Helminth worms evoke a characteristic mode of the immune response, the type 2, involving a specific suite of cytokines (such as IL-4, IL-5, IL-9 and IL-13) and driving differentiation of specialised cell subsets (including eosinophils, M2 macrophages and Th2 helper T cells) while promoting specific isotypes of antibody

R. M. Maizels (✉)
Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK
e-mail: Rick.Maizels@glasgow.ac.uk

F. Bruschi (ed.), *Helminth Infections and their Impact on Global Public Health*,
https://doi.org/10.1007/978-3-031-00303-5_4

(Harris and Loke 2017; El-Naccache et al. 2020). When well-orchestrated, the type 2 response can successfully eliminate helminths and is most likely to be the primary evolutionary function of this arm of the immune system (Allen and Maizels 2011). However, the helminth parasites found today are able to subvert and evade every phase of the type 2 response from initial antigen recognition and immune cell activation through to final effector mechanisms, allowing them to establish chronic infections which persist for many months or years.

4.2 Immunomodulation

Seminal studies in helminth-endemic areas provided evidence that infection was negatively associated with allergic reactivity (van den Biggelaar et al. 2000; Araujo et al. 2000), and longitudinal data from children treated with anthelmintic drugs established a causal relationship between helminth infection and reduced allergy (van den Biggelaar et al. 2004). Similarly, levels of autoimmune anti-nuclear antibodies were found to be lower in helminth-infected subjects and to increase following chemotherapeutic clearance (Mutapi et al. 2011). A striking study in multiple sclerosis patients in Argentina found a cohort who unintentionally acquired gastrointestinal helminth parasites entered long-term remission, which was broken in individuals subsequently given anthelmintic treatment (Correale and Farez 2007; Correale and Farez 2011). Mechanistically, these instances of dampened inflammatory responses correlated with immune suppressive parameters such as the cytokine IL-10 and regulatory T cells as discussed below (Weinstock and Elliott 2014; Wammes et al. 2014).

These down-modulatory factors not only preserve the tenure of helminth parasites but may help explain the wider effects in dampening immunity and inflammation even in tissues distal to the site of infection (McSorley and Maizels 2012; Maizels 2020; Logan et al. 2018). As discussed in recent reviews, multiple species are effective at abating inflammatory colitis (Varyani et al. 2017; Maruszewska-Cheruiyot et al. 2018), allergic inflammation (Cruz et al. 2017; Schwartz et al. 2018) and autoimmune disorders (Smallwood et al. 2017). To account for these effects, two distinct mechanistic models are loosely grouped together under the term "hygiene hypothesis" (Maizels et al. 2014). One posits that early-life infection imprints a more moderated tone on the immune system so that it does not overreact to harmless environmental antigens (Cooper et al. 2018); the other suggests that infectious agents such as helminths exert ongoing regulatory effects even in the mature immune system of individuals who have not previously been infected (Bach 2018). There is good evidence for both scenarios, although the latter is the setting for helminth therapy of inflammatory diseases and is more readily testable by experimental laboratory work.

Key concepts which have emerged from the many reports of helminths suppressing inflammatory disorders have been modulation of dendritic cell responses required for immune activation (Carvalho et al. 2009; Motran et al.

2017) and expansion of the regulatory T cell compartment that normally controls responses to self-antigen and innocuous exogenous specificities such as allergens and food antigens (Taylor et al. 2012; White et al. 2020). Regulatory B cells are also implicated in helminth-mediated protection from inflammatory diseases (Correale et al. 2008; Wilson et al. 2010; Hussaarts et al. 2011). In addition, critical innate pathways such as the production of alarmins at barrier surfaces undergoing helminth invasion (Osbourn et al. 2017) and the ability of granulocytes to trap helminth larvae (Bouchery et al. 2020) can be compromised by helminth parasites. Importantly, these and other immunomodulatory activities are replicated by molecular products released by parasites (Maizels et al. 2018), reflecting a well-tuned evolutionary adaptation of helminths towards the immune system of their host.

It is important to note, however, that each helminth species has adapted in its own way to modulate different elements of the immune system, even if the physiological outcome of infection may be similar. Thus, a meta-analysis found that each of the major human parasites (*Ascaris lumbricoides*, hookworms, *Schistosoma* and the whipworm *Trichuris trichiura*) was protective against allergic reactivity (Feary et al. 2011) although it is unlikely they do so through identical mechanisms. In any event, the magnitude of immune regulatory effects differs significantly between helminth species, and even in the setting of one species (*S. mansoni*), immunomodulation depends on sufficient infection intensity and chronicity to reach maximal effect (Smits et al. 2007).

4.3 Therapies with, and from, Helminths

The wealth of evidence from both human and animal studies that helminth infections can be anti-inflammatory has fuelled interest in the possibility of therapeutic infection with live helminths (Helmby 2015; Fleming and Weinstock 2015; Sobotkova et al. 2019). Two strategies have been pursued through to clinical trials. In the first approach, porcine parasite *Trichuris suis* ova (TSO) have been administered to patients, as this organism is minimally pathogenic in humans and remains in the large bowel for only a few weeks (Summers et al. 2003). Despite early promising results with individuals and small cohorts (Weinstock and Elliott 2013), however, larger trials of TSO in multiple sclerosis (Voldsgaard et al. 2015), allergic rhinitis (Bager et al. 2010) and inflammatory bowel disease (Garg et al. 2014) patients did not find significant benefit. In the second approach, the human hookworm *Necator americanus* is administered, in doses low enough to avoid pathogenicity either during its lung migratory phase or during residence in the small intestine. Hookworm-infected coeliac disease patients were able to tolerate higher gluten diets (Croese et al. 2015), while in asthma, no significant benefit was recorded (Feary et al. 2010). A number of commentators have discussed the

underperformance of live helminth therapy compared to evident protection in endemic populations, highlighting factors such as dose, intensity and duration of infection, choice of helminth species best suited to the disease in question and clinical or genetic heterogeneity of the treatment cohort (Wammes et al. 2014; Sobotkova et al. 2019; Evans and Mitre 2015), all of which may mitigate against achieving a statistically robust outcome from the trials so far performed.

With the understanding that released products can protect as well as live infections, there is now an increasing emphasis on identifying helminth molecules with protective effects, which could offer future defined therapeutics for treatment of human disorders (Harnett and Harnett 2010; Shepherd et al. 2015). Strategically, this approach would disentangle the pathogenic consequences of parasite infection from whatever immunomodulatory benefit they may confer and would provide new drugs with clear modes of action, based on natural products which are known to be well-tolerated by the human host. The emerging spectrum of parasite molecules with immunomodulatory properties and therapeutic potential has recently been reviewed in detail elsewhere (Maizels et al. 2018).

4.4 Vaccine Unresponsiveness

A major public health question is the degree to which helminth-infected individuals, particularly children, show impaired responses to vaccination. For example, *Onchocerca volvulus* infection has been reported to compromise the efficacy of tetanus vaccine, with infected subjects showing higher IL-10 levels (Cooper et al. 1998), while the BCG vaccine was found to be less immunogenic in helminth-infected individuals, in this case associated with raised production of the immunosuppressive cytokine TGF-β (Elias et al. 2008). Following tetanus toxoid (TT) immunisation, *S. mansoni* cases showed diminished IFNγ responses to TT with a switch towards type 2 cytokines (Sabin et al. 1996), while children immunised with a candidate malaria vaccine showed a threefold reduction in antibody levels if infected with *Trichuris trichiura* (Esen et al. 2012). Conversely, responses to cholera vaccination were improved by albendazole anthelmintic treatment of children with *Ascaris* infection (Cooper et al. 2000). Similar findings have been reported in mouse models, in which *H. polygyrus* infection depressed responses to a malaria vaccine (Su et al. 2006) and *S. mansoni* impeded BCG vaccination against mycobacterial infection (Elias et al. 2005).

Most recently, *Schistosoma* infections have been found to result in shorter-lived antibody responses to hepatitis B, tetanus and measles vaccinations (Riner et al. 2016; Nono et al. 2018), indicating a subtle effect that may nevertheless have major epidemiological consequences. Anti-schistosome treatment with praziquantel has recently been shown to improve responses to the measles vaccine, suggesting an

important public health strategy for optimal childhood immunisation in endemic areas (Tweyongyere et al. 2019).

4.5 Co-Infection: Ups and Downs

Helminth-infected hosts can show dramatic shifts in susceptibility or resistance to other infectious agents, as a consequence of both immunological and physiological changes (Graham 2008; Bullington et al. 2021). In examples of great significance in poorly resourced countries, the intestinal helminth *H. polygyrus* renders animals susceptible to enteric *Salmonella* infection (Reynolds et al. 2017) and dampens the host response to *Mycobacterium bovis (*Obieglo et al. 2016*)*. The same parasite also enhances systemic susceptibility to neurotropic West Nile virus, by deviating the host immune response towards an IL-4/Th2-dominated mode, rather than protective Th1/CD8 response (Desai et al. 2021). More subtle interactions may also occur, as with herpes virus in which a major transcription promoter is bound by STAT6, downstream of IL-4 signalling, resulting in viral activation during *H. polygyrus* infection (Reese et al. 2014). A similar shift, but involving IL-5 and IL-33, is indirectly responsible for increased vaginal viral susceptibility in mice infected with *Nippostrongylus brasiliensis* through epithelial lesions driven by activated eosinophils (Chetty et al. 2021).

Data for virus susceptibility in helminth-infected humans is more scarce, but importantly, cases of *Wuchereria bancrofti* filarial infection in Tanzania were associated with a twofold higher acquisition of HIV infection (Kroidl et al. 2016), and a number of studies have found positive associations between *Schistosoma* spp. and HIV infections (reviewed by (Furch et al. 2020)). Hence, even subtle changes in immune status caused by helminths may read out into life-threatening consequences. Indeed, in a study of African buffalo naturally exposed to *Mycobacterium tuberculosis*, anthelmintic treatment was found to reduce mortality ninefold while not affecting the baseline prevalence of infection (Ezenwa and Jolles 2015).

Interactions between different pathogens are inevitably complex, and so it is not surprising that in some cases, helminth infection proves protective against other organisms. Helminths which deplete host erythrocytes through blood feeding are associated with reduced levels of protozoal parasites that invade red blood cells (Graham 2008). In the case of respiratory syncytial virus, *H. polygyrus*-infected mice stimulate an innate type 1 interferon response at other mucosal tissues such as the lung, limiting the infectivity of virus in the airway (McFarlane et al. 2017). In the course of *S. mansoni* infection of mice, the replication of a modified mouse hepatitis B virus is changed in a phase-dependent manner; during prepatency, IFNγ responses are elevated and the virus is suppressed; however, once egg production commences and a switch to Th2 occurs, there is no longer any inhibition (Loffredo-Verde et al. 2020).

4.6 Microbiome Interactions

A fascinating triangular dynamic exists between the host, the helminth parasites and the intestinal microbiome, operating at the physiological, metabolic and immunological levels (Reynolds et al. 2015; Zaiss and Harris 2016; Rapin and Harris 2018). In mouse models, helminth infection modifies the composition of the microbiota, in some instances indirectly altering the host immune response (Zaiss et al. 2015; Brosschot and Reynolds 2018). In one instance, there is also reciprocity, in which *H. polygyrus* infection of the small intestine promotes expansion of a *Lactobacillus* species, while mice pre-seeded with this bacteria are more susceptible to infection with the parasite (Reynolds et al. 2014). Notably, outgrowth of lactobacilli has been reported in no fewer than ten different helminth studies (reviewed by (Cortes et al. 2019)). In a different environment, *Trichuris muris* infection of the caecum enhances clostridial species at the expense of *Bacteroides* and thereby alleviates intestinal inflammation in a model of Crohn's disease (Ramanan et al. 2016).

While both helminths and intestinal bacteria exert multiple effects on the host immune response, studies so far have focussed more on macromolecules (proteins and glycans) of parasites, compared to metabolites and other small molecules elaborated from bacteria. For example, regulatory T cells can be induced by species that produce short-chain fatty acids (SCFAs, acetate, butyrate and propionate) that activate the host Ffar2/GPR41 receptor (Smith et al. 2013). Notably, SCFA levels rise during *H. polygyrus* infection, attributable to a modified microbiota which on transfer to an uninfected host can reduce allergic inflammation (Zaiss et al. 2015). Hence, both helminths and microbes can exert similar anti-inflammatory effects and appear to act in concert at least in this mouse model.

A more challenging question is whether such interactions are seen, and are physiologically significant, in real-world populations. The *Bacteroides*-to-*Clostridiales* switch seen in *T. muris*-infected mice, remarkably, was reversed in a Malaysian cohort in whom anthelmintic treatment resulted in lower *Clostridiales* and expanded *Bacteroides* colonisation (Ramanan et al. 2016). Beyond the analysis of specific bacterial taxa, an important parameter is the overall diversity of the intestinal microbiota, which is associated with better health. In another Malaysian study, helminth-infected subjects showed a broader range of bacterial species than uninfected controls (Lee et al. 2014), and the richness of the intestinal flora was found to increase following deliberate hookworm infection of coeliac patients (Giacomin et al. 2015). However, across a range of human studies, quite disparate outcomes have been reported, in terms of both key species representation and overall diversity, probably reflecting the strength of confounding genetic, environmental and dietary variables in determining the makeup of the microbiota. Although this might suggest that helminths can establish infection in a microbial-independent manner, this is not the case for *T. muris* at least, which requires cues from the intestinal microbiota to induce egg hatching (Hayes et al. 2010).

4.7 Metabolism: Inflammation and Helminth Infection

Increasing attention has recently been focused on the consequences of helminth infection on the metabolic status of the host (Shea-Donohue et al. 2017; Rajamanickam et al. 2020b; Wiria et al. 2014; Guigas and Molofsky 2015). Investigations have been conducted at two levels, the systemic impact of infection and the changes in individual cell types (such as macrophages) or niches (such as adipose tissue) when exposed to helminth products (Wiria et al. 2014; Guigas and Molofsky 2015). Most notably, helminths may mitigate metabolic disorders across the broad range of the metabolic syndrome, type 2 diabetes, obesity and cardiovascular diseases, each linked to heightened inflammatory responses (Chawla et al. 2011; Gregor and Hotamisligil 2011) and increasing in prevalence in areas where helminths are less common (Guigas and Molofsky 2015). Although intestinal helminths can significantly diminish the host's nutritional intake and may affect appetite, the protective properties of infection are more consistent with the anti-inflammatory effects of these parasites (Wiria et al. 2012).

In human studies in India, patients with type 2 diabetes showed lower levels of lymphatic filariasis infection than the general population and higher inflammatory cytokine production (Aravindhan et al. 2010), while in an Australian aboriginal community, *Strongyloides stercoralis* infection was associated with a lower T2D risk (Hays et al. 2015). Indian patients with the same infection had significantly lower inflammatory cytokine and chemokine levels and lower insulin, which increased following anthelminthic therapy (Rajamanickam et al. 2019; Rajamanickam et al. 2020a). Turning to cardiovascular disease, which often develops in tandem with metabolic disorder, previous schistosome infection in a region of China that historically was endemic for *S. japonicum* was found to reduce the incidence of measures such as dyslipidaemia (Shen et al. 2014) and general markers of the metabolic syndrome (Shen et al. 2015). Such effects may not be purely immune-mediated, as helminths have also been suggested to directly alter blood glucose and lipid levels (reviewed by (Gurven et al. 2016)).

In animal models, obesity resulting from feeding mice high-fat diet (HFD) is attenuated by helminths such as *H. polygyrus* (Su et al. 2018; Shimokawa et al. 2019), *N. brasiliensis (*Yang et al. 2013*)*, *S. mansoni (*Hussaarts et al. 2015*)* or *Trichinella spiralis* (Kang et al. 2021). In these models, a conversion of macrophages from pro-inflammatory M1 to a counter-inflammatory M2 phenotype appears to be the critical feature, reinforcing a general conclusion that the macrophages are the determining immune population in metabolic homeostasis and disease (Red Eagle and Chawla 2010; Cortes-Selva and Fairfax 2021). M2 macrophages are induced by the type 2 cytokines IL-4 and IL-13, acting through the STAT6 transcription factor, and most critically active in adipose tissue that regulates metabolic activity. Thus, *N. brasiliensis* infection stimulated eosinophil release of IL-4, driving M2 differentiation that protected mice from the obesity-inducing effects of HFD (Wu et al. 2011).

4.8 Transgenerational Effects

Where infants are exposed to the influence of helminths in utero, it may be expected that their subsequent immune response to the same parasites will be altered, for example, skewing towards type 2 responses (Malhotra et al. 1999; Wright et al. 2009), influencing antigen-specific immune memory or generating immune tolerance (Dauby et al. 2012; Lacorcia and Prazeres da Costa 2018). Prenatal exposure to helminth antigens is also evident from IgE (which does not cross the placenta) and memory-like antibody responses in neonates from infected mothers (King et al. 1998; Seydel et al. 2012). A remarkable example of immune tolerisation was shown in studies spanning 20 years in the Cook Islands, endemic for lymphatic filariasis; young adults infected with *Wuchereria bancrofti* showed a dramatic impairment of anti-filarial immune responses if they were born to mothers infected with the same parasite at the time of pregnancy (Steel et al. 1994).

In mouse models, offspring of *S. mansoni*-infected mothers were found to be hypo-responsive when the latter were infected as young adults; as well as lower antibody titres, they suffered less granulomatous pathology, although they also sustained lower worm numbers (Attallah et al. 2006; Lenzi et al. 1987). Notably, both gestational and nursing effects were found when offspring of uninfected mothers were suckled on infected dams and vice versa (Santos et al. 2016). A further layer of complexity is added by studies demonstrating that the impact of *S. mansoni* on neonates depends on the stage of infection in the mothers; only during the early (Th1-dominated) phase prior to egg production, does maternal IFNγ cross the placenta and imprint the developing immune system, rendering it less prone to develop Th2 disorders such as airway allergy (Straubinger et al. 2014).

This implication that maternal helminth infection can have more extensive impact on the general immune status of the offspring is well supported by human studies (Lacorcia and Prazeres da Costa 2018; Mpairwe et al. 2014). For example, in a Ugandan study, maternal anthelmintic treatment during pregnancy significantly increased the incidence of infant eczema (Mpairwe et al. 2011). However, in Ecuador, the protective effect of maternal infection was limited to skin allergic sensitivity rather than patent eczema (Cooper et al. 2016). Children of helminth-infected mothers developed poorer IgG antibody levels to the major microbial vaccines (Malhotra et al. 2015), but anthelminthic administration during pregnancy did not consistently raise responses, suggesting that treatment at this time did not confer an overall benefit (Ndibazza et al. 2012).

Finally, there is even a suggestion that helminth infection can modulate pregnancy itself. In a unique study over 9 years in Bolivia, it was found that while *Ascaris lumbricoides* infection was associated with increased fecundity (including earlier first pregnancy), hookworm infection had the opposite effect, delaying primiparous age and reducing birth frequency (Blackwell et al. 2015).

4.9 Pathology, Neurotropism and Cancer

The primary pathologies of helminth infection are well known, for example, hepatosplenomegaly and fibrosis in schistosomiasis (Wynn et al. 2004), lymphatic disease and elephantiasis in filariasis (Babu and Nutman 2012) and dermatitis and ocular damage in onchocerciasis (Hoerauf et al. 2003; Murdoch 2010). In general, these pathologies have an immune aetiology, being associated with localised inflammatory reactions to parasite eggs, microfilariae or adult worms which give rise through various pathways to the specific consequences of each infection. However, as the degree of immunological responses varies across endemic populations, a wide spectrum of diseases is observed ranging from asymptomatic to life-shortening fibrosis.

A less appreciated aspect is neurological damage from helminths; the most well-recognised example is neurocysticercosis caused by the porcine tapeworm *Taenia solium* (Garcia 2018), but *Taenia* larvae are not the only parasites that can invade the brain; *Toxocara canis* is known to do so in mice (Kolbekova et al. 2011; Strube et al. 2012), and human cases have also been reported (Hill et al. 1985). It is now recognised that *Onchocerca volvulus* may also do so, being implicated in a severe form of epilepsy, nodding syndrome (Colebunders et al. 2021).

Soil-transmitted helminths are not known to be neurotropic but nevertheless have been associated in some studies with subtle cognitive deficits in school-aged children (Nokes et al. 1992; Jardim-Botelho et al. 2008; Pabalan et al. 2018). Aggregating the numerous reports now in the public domain, no cognitive benefit could be found at the population level from anthelmintic treatment of children (Taylor-Robinson et al. 2015), although the possibility remains that polyparasitism is more detrimental and/or that the most susceptible children are most rapidly re-infected following treatment.

Although helminths and cancer are not often linked, there are in fact a subset of specific parasites associated with increased risk of cancer (Fried et al. 2011; Scholte et al. 2018). *S. haematobium* is a well-known causative agent of bladder cancer and, in highly endemic settings such as Egypt during the 1970s, was responsible for >25% of all cancer cases (Mostafa et al. 1999). In Thailand, bile duct cancer due to *Opisthorchis viverrini* remains a major problem, with transmission due to consumption of undercooked fish, leading to larval flukes parasitising the hepatic ducts (Sripa et al. 2012). Disease develops in as many as one-sixth of infections, and as a consequence, cholangiocarcinoma in the endemic area is >40 times more prevalent than elsewhere. In nonhuman hosts, *Spirocerca lupi* is associated with oesophageal sarcomas of dogs and other canids following long-term nodule formation at this site of infection (Rojas et al. 2019).

Mechanistically, helminth-induced cancers are thought to primarily arise from chronic inflammation and tissue damage, as, for example, incurred by the bladder wall in *S. haematobium* infection. In the case of *O. viverrini*, however, a more precise molecular interaction has been uncovered, with the parasite producing a granulin-like factor that drives proliferation of mammalian cells (Smout et al. 2009).

Possibly, this serves a wound-healing function to optimise the parasite environment which, over time, advances the carcinogenic pathway in the host (Botelho et al. 2016).

4.10 Conclusions

The systemic impact of helminths is increasingly recognised as pervading every compartment of the body, influencing physiological systems and the response to multiple environmental stimuli (Mishra et al. 2014; Wammes et al. 2014). As more of the observations discussed are subjected to mechanistic analysis, it will be interesting to follow some key threads that run across multiple systems. For example, the primary interface between helminths and the host is found at barrier surfaces, such as epithelial cells (Coakley and Harris 2020); how these respond to infection and correspond with other populations across the body will have great implications for the outcome of infection (Saenz et al. 2008). Much more is now understood about how the innate immune network retains "memory", allowing generalised changes in host immune status to spread systemically (Netea et al. 2016) and imprinting critical populations such as the macrophage during helminth infection (Cortes-Selva et al. 2021). In combination with our existing appreciation of how the adaptive immune system of B and T lymphocytes follows helminth-specific differentiation programmes in infection (Harris and Loke 2017), we are increasingly able to understand, and soon to modify, the systemic effects of helminth infection.

4.11 Acknowledgements

The author gratefully acknowledges funding from the Wellcome Trust through Investigator Awards (ref. 106122 and 219530) and the core-funded Wellcome Centre for Integrative Parasitology (ref. 104111).

References

Allen JE, Maizels RM (2011) Diversity and dialogue in immunity to helminths. Nat Rev Immunol 11:375–388

Araujo MI, Lopes AA, Medeiros M, Cruz AA, Sousa-Atta L, Solé D, Carvalho EM (2000) Inverse association between skin response to aeroallergen and *Schistosoma mansoni* infection. Int Arch Allergy Immunol 123:145–148

Aravindhan V, Mohan V, Surendar J, Muralidhara Rao M, Pavankumar N, Deepa M, Rajagopalan R, Kumaraswami V, Nutman TB, Babu S (2010) Decreased prevalence of lymphatic filariasis among diabetic subjects associated with a diminished pro-inflammatory cytokine response (CURES 83). PLoS Negl Trop Dis 4:e707

Attallah AM, Abbas AT, Dessouky MI, El-emshaty HM, Elsheikha HM (2006) Susceptibility of neonate mice born to Schistosoma mansoni-infected and noninfected mothers to subsequent S. mansoni infection. Parasitol Res 99:137–145

Babu S, Nutman TB (2012) Immunopathogenesis of lymphatic filarial disease. Semin Immunopathol 34:847–861

Bach JF (2018) The hygiene hypothesis in autoimmunity: the role of pathogens and commensals. Nat Rev Immunol 18:105–120

Bager P, Arnved J, Rønborg S, Wohlfahrt J, Poulsen LK, Westergaard T, Petersen HW, Kristensen B, Thamsborg S, Roepstorff A, Kapel C, Melbye M (2010) *Trichuris suis* ova therapy for allergic rhinitis: a randomized, double-blind, placebo-controlled clinical trial. J Allergy Clin Immunol 125:123–130

Blackwell AD, Tamayo MA, Beheim B, Trumble BC, Stieglitz J, Hooper PL, Martin M, Kaplan H, Gurven M (2015) Helminth infection, fecundity, and age of first pregnancy in women. Science 350:970–972

Botelho MC, Alves H, Richter J (2016) Wound healing and cancer progression in Opisthorchis viverrini associated cholangiocarcinoma. Parasitol Res 115:2913–2914

Bouchery T, Moyat M, Sotillo J, Silverstein S, Volpe B, Coakley G, Tsourouktsoglou TD, Becker L, Shah K, Kulagin M, Guiet R, Camberis M, Schmidt A, Seitz A, Giacomin P, Le Gros G, Papayannopoulos V, Loukas A, Harris NL (2020) Hookworms evade host immunity by secreting a deoxyribonuclease to degrade neutrophil extracellular traps. Cell Host Microbe 27(277–289):e6

Brosschot TP, Reynolds LA (2018) The impact of a helminth-modified microbiome on host immunity. Mucosal Immunol 11:1039–1046

Bullington BW, Klemperer K, Mages K, Chalem A, Mazigo HD, Changalucha J, Kapiga S, Wright PF, Yazdanbakhsh MM, Downs JA (2021) Effects of schistosomes on host anti-viral immune response and the acquisition, virulence, and prevention of viral infections: a systematic review. PLoS Pathog 17:e1009555

Carvalho L, Sun J, Kane C, Marshall F, Krawczyk C, Pearce EJ (2009) Mechanisms underlying helminth modulation of dendritic cell function. Immunology 126:28–34

Chawla A, Nguyen KD, Goh YP (2011) Macrophage-mediated inflammation in metabolic disease. Nat Rev Immunol 11:738–749

Chetty A, Darby MG, Vornewald PM, Martin-Alonso M, Filz A, Ritter M, McSorley HJ, Masson L, Smith K, Brombacher F, O'Shea MK, Cunningham AF, Ryffel B, Oudhoff MJ, Dewals BG, Layland LE, Horsnell WGC (2021) *Il4ra*-independent vaginal eosinophil accumulation following helminth infection exacerbates epithelial ulcerative pathology of HSV-2 infection. Cell Host Microbe 29(579–593):e5

Coakley G, Harris NL (2020) The intestinal epithelium at the forefront of host-helminth interactions. Trends Parasitol 36(9):761–772. https://doi.org/10.1016/j.pt.2020.07.002

Colebunders R, Njamnshi AK, Menon S, Newton CR, Hotterbeekx A, Preux PM, Hopkins A, Vaillant M, Siewe Fodjo JN (2021) Onchocerca volvulus and epilepsy: a comprehensive review using the Bradford Hill criteria for causation. PLoS Negl Trop Dis 15:e0008965

Cooper PJ, Espinel I, Paredes W, Guderian RH, Nutman TB (1998) Impaired tetanus-specific cellular and humoral responses following tetanus vaccination in human onchocerciasis: a possible role for interleukin-10. J Infect Dis 178:1133–1138

Cooper PJ, Chico ME, Losonsky G, Sandoval C, Espinel I, Sridhara R, Aguilar M, Guevara A, Guderian RH, Levine MM, Griffin GE, Nutman TB (2000) Albendazole treatment of children with ascariasis enhances the vibriocidal antibody response to the live attenuated oral cholera vaccine CVD 103-HgR. J Infect Dis 182:1199–1206

Cooper PJ, Chico ME, Amorim LD, Sandoval C, Vaca M, Strina A, Campos AC, Rodrigues LC, Barreto ML, Strachan DP (2016) Effects of maternal geohelminth infections on allergy in early childhood. J Allergy Clin Immunol 137:899–906e2

Cooper PJ, Chico ME, Vaca MG, Sandoval CA, Loor S, Amorim LD, Rodrigues LC, Barreto ML, Strachan DP (2018) Effect of early-life geohelminth infections on the development of wheezing at 5 years of age. Am J Respir Crit Care Med 197:364–372

Correale J, Farez M (2007) Association between parasite infection and immune responses in multiple sclerosis. Ann Neurol 61:97–108

Correale J, Farez MF (2011) The impact of parasite infections on the course of multiple sclerosis. J Neuroimmunol 233:6–11

Correale J, Farez M, Razzitte G (2008) Helminth infections associated with multiple sclerosis induce regulatory B cells. Ann Neurol 64:187–199

Cortes A, Peachey L, Scotti R, Jenkins TP, Cantacessi C (2019) Helminth-microbiota cross-talk—a journey through the vertebrate digestive system. Mol Biochem Parasitol 233:111222

Cortes-Selva D, Fairfax K (2021) Schistosome and intestinal helminth modulation of macrophage immunometabolism. Immunology 162:123–134

Cortes-Selva D, Gibbs L, Maschek JA, Nascimento M, Van Ry T, Cox JE, Amiel E, Fairfax KC (2021) Metabolic reprogramming of the myeloid lineage by Schistosoma mansoni infection persists independently of antigen exposure. PLoS Pathog 17:e1009198

Croese J, Giacomin P, Navarro S, Clouston A, McCann L, Dougall A, Ferreira I, Susianto A, O'Rourke P, Howlett M, McCarthy J, Engwerda C, Jones D, Loukas A (2015) Experimental hookworm infection and gluten microchallenge promote tolerance in celiac disease. J Allergy Clin Immunol 135:508–516

Cruz AA, Cooper PJ, Figueiredo CA, Alcantara-Neves NM, Rodrigues LC, Barreto ML (2017) Global issues in allergy and immunology: parasitic infections and allergy. J Allergy Clin Immunol 140:1217–1228

Dauby N, Goetghebuer T, Kollmann TR, Levy J, Marchant A (2012) Uninfected but not unaffected: chronic maternal infections during pregnancy, fetal immunity, and susceptibility to postnatal infections. Lancet Infect Dis 12:330–340

Desai P, Janova H, White JP, Reynoso GV, Hickman HD, Baldridge MT, Urban JF Jr, Stappenbeck TS, Thackray LB, Diamond MS (2021) Enteric helminth coinfection enhances host susceptibility to neurotropic flaviviruses via a tuft cell-IL-4 receptor signaling axis. Cell 184(1214–1231):e16

Diemert DJ, Bottazzi ME, Plieskatt J, Hotez PJ, Bethony JM (2018) Lessons along the critical path: developing vaccines against human helminths. Trends Parasitol 34:747–758

Elias D, Akuffo H, Pawlowski A, Haile M, Schon T, Britton S (2005) *Schistosoma mansoni* infection reduces the protective efficacy of BCG vaccination against virulent *mycobacterium tuberculosis*. Vaccine 23:1326–1334

Elias D, Britton S, Aseffa A, Engers H, Akuffo H (2008) Poor immunogenicity of BCG in helminth infected population is associated with increased *in vitro* TGF-β production. Vaccine 26:3897–3902

El-Naccache DW, Hasko G, Gause WC (2020) Early events triggering the initiation of a type 2 immune response. Trends Immunol 42(2):151–164. https://doi.org/10.1016/j.it.2020.11.006

Esen M, Mordmuller B, de Salazar PM, Adegnika AA, Agnandji ST, Schaumburg F, Hounkpatin AB, Bruckner S, Theisen M, Belard S, Ngoa UA, Issifou S, Yazdanbakhsh M, Kremsner PG (2012) Reduced antibody responses against plasmodium falciparum vaccine candidate antigens in the presence of Trichuris trichiura. Vaccine 30:7621–7624

Evans H, Mitre E (2015) Worms as therapeutics for allergy and asthma: understanding why benefits in animal studies have not translated into clinical success. J Allergy Clin Immunol 135:343–353

Ezenwa VO, Jolles AE (2015) Epidemiology. Opposite effects of anthelmintic treatment on microbial infection at individual versus population scales. Science 347:175–177

Feary JR, Venn AJ, Mortimer K, Brown AP, Hooi D, Falcone FH, Pritchard DI, Britton JR (2010) Experimental hookworm infection: a randomized placebo-controlled trial in asthma. Clin Exp Allergy 40:299–306

Feary J, Britton J, Leonardi-Bee J (2011) Atopy and current intestinal parasite infection: a systematic review and meta-analysis. Allergy 66:569–578

Fleming JO, Weinstock JV (2015) Clinical trials of helminth therapy in autoimmune diseases: rationale and findings. Parasite Immunol 37:277–292
Fried B, Reddy A, Mayer D (2011) Helminths in human carcinogenesis. Cancer Lett 305:239–249
Furch BD, Koethe JR, Kayamba V, Heimburger DC, Kelly P (2020) Interactions of Schistosoma and HIV in sub-Saharan Africa: a systematic review. Am J Trop Med Hyg 102:711–718
Garcia HH (2018) Neurocysticercosis. Neurol Clin 36:851–864
Garg SK, Croft AM, Bager P (2014) Helminth therapy (worms) for induction of remission in inflammatory bowel disease. Cochrane Database Syst Rev 1:CD009400
Giacomin P, Zakrzewski M, Croese J, Su X, Sotillo J, McCann L, Navarro S, Mitreva M, Krause L, Loukas A, Cantacessi C (2015) Experimental hookworm infection and escalating gluten challenges are associated with increased microbial richness in celiac subjects. Sci Rep 5:13797
Graham AL (2008) Ecological rules governing helminth-microparasite coinfection. Proc Natl Acad Sci USA 105:566–570
Gregor MF, Hotamisligil GS (2011) Inflammatory mechanisms in obesity. Annu Rev Immunol 29: 415–445
Guigas B, Molofsky AB (2015) A worm of one's own: how helminths modulate host adipose tissue function and metabolism. Trends Parasitol 31:435–441
Gurven MD, Trumble BC, Stieglitz J, Blackwell AD, Michalik DE, Finch CE, Kaplan HS (2016) Cardiovascular disease and type 2 diabetes in evolutionary perspective: a critical role for helminths? Evol Med Public Health 2016(1):338–357. https://doi.org/10.1093/emph/eow028
Harnett W, Harnett MM (2010) Helminth-derived immunomodulators: can understanding the worm produce the pill? Nat Rev Immunol 10:278–284
Harris NL, Loke P (2017) Recent advances in Type-2-cell-mediated immunity: insights from helminth infection. Immunity 47:1024–1036
Hayes KS, Bancroft AJ, Goldrick M, Portsmouth C, Roberts IS, Grencis RK (2010) Exploitation of the intestinal microflora by the parasitic nematode *Trichuris muris*. Science 328:1391–1394
Hays R, Esterman A, Giacomin P, Loukas A, McDermott R (2015) Does Strongyloides stercoralis infection protect against type 2 diabetes in humans? Evidence from Australian aboriginal adults. Diabetes Res Clin Pract 107:355–361
Helmby H (2015) Human helminth therapy to treat inflammatory disorders—where do we stand? BMC Immunol 16:12
Hill IR, Denham DA, Scholtz CL (1985) *Toxocara canis* larvae in the brain of a British child. Trans R Soc Trop Med Hyg 79:351–354
Hoerauf A, Buttner DW, Adjei O, Pearlman E (2003) Onchocerciasis. BMJ 326:207–210
Hussaarts L, van der Vlugt LE, Yazdanbakhsh M, Smits HH (2011) Regulatory B-cell induction by helminths: implications for allergic disease. J Allergy Clin Immunol 128:733–739
Hussaarts L, Garcia-Tardon N, van Beek L, Heemskerk MM, Haeberlein S, van der Zon GC, Ozir-Fazalalikhan A, Berbee JF, Willems van Dijk K, van Harmelen V, Yazdanbakhsh M, Guigas B (2015) Chronic helminth infection and helminth-derived egg antigens promote adipose tissue M2 macrophages and improve insulin sensitivity in obese mice. FASEB J 29:3027–3039
Jardim-Botelho A, Raff S, Rodrigues Rde A, Hoffman HJ, Diemert DJ, Correa-Oliveira R, Bethony JM, Gazzinelli MF (2008) Hookworm, Ascaris lumbricoides infection and polyparasitism associated with poor cognitive performance in Brazilian schoolchildren. Tropical Med Int Health 13:994–1004
Kang SA, Choi JH, Baek KW, Lee DI, Jeong MJ, Yu HS (2021) Trichinella spiralis infection ameliorated diet-induced obesity model in mice. Int J Parasitol 51:63–71
Keiser J, Utzinger J (2010) The drugs we have and the drugs we need against major helminth infections. Adv Parasitol 73:197–230
King CL, Malhotra I, Mungai P, Wamachi A, Kioko J, Ouma JH, Kazura JW (1998) B cell sensitization to helminthic infection develops in utero in humans. J Immunol 160:3578–3584
Kolbekova P, Vetvicka D, Svoboda J, Skirnisson K, Leissova M, Syrucek M, Mareckova H, Kolarova L (2011) *Toxocara canis* larvae reinfecting BALB/c mice exhibit accelerated speed of migration to the host CNS. Parasitol Res 109:1267–1278

Kroidl I, Saathoff E, Maganga L, Makunde WH, Hoerauf A, Geldmacher C, Clowes P, Maboko L, Hoelscher M (2016) Effect of *Wuchereria bancrofti* infection on HIV incidence in Southwest Tanzania: a prospective cohort study. Lancet 388:1912–1920

Lacorcia M, Prazeres da Costa CU (2018) Maternal schistosomiasis: immunomodulatory effects with lasting impact on allergy and vaccine responses. Front Immunol 9:2960

Lee SC, Tang MS, Lim YA, Choy SH, Kurtz ZD, Cox LM, Gundra UM, Cho I, Bonneau R, Blaser MJ, Chua KH, Loke P (2014) Helminth colonization is associated with increased diversity of the gut microbiota. PLoS Negl Trop Dis 8:e2880

Lenzi JA, Sobral AC, Araripe JR, Grimaldi Filho G, Lenzi HL (1987) Congenital and nursing effects on the evolution of Schistosoma mansoni infection in mice. Mem Inst Oswaldo Cruz 82 (Suppl 4):257–267

Loffredo-Verde E, Bhattacharjee S, Malo A, Festag J, Kosinska AD, Ringelhan M, Rim Sarkar S, Steiger K, Heikenwaelder M, Protzer U, Prazeres da Costa CU (2020) Dynamic, helminth-induced immune modulation influences the outcome of acute and chronic hepatitis B virus infection. J Infect Dis 221:1448–1461

Logan J, Navarro S, Loukas A, Giacomin P (2018) Helminth-induced regulatory T cells and suppression of allergic responses. Curr Opin Immunol 54:1–6

Maizels RM (2020) Regulation of immunity and allergy by helminth parasites. Allergy 75:524–534

Maizels RM, McSorley HJ (2016) Regulation of the host immune system by helminth parasites. J Allergy Clin Immunol 138:666–675

Maizels RM, McSorley HJ, Smyth DJ (2014) Helminths in the hygiene hypothesis—sooner or later? Clin Exp Immunol 177:38–46

Maizels RM, Smits HH, McSorley HJ (2018) Modulation of host immunity by helminths: the expanding repertoire of parasite effector molecules. Immunity 49:801–818

Malhotra I, Mungai P, Wamachi A, Kioko J, Ouma JH, Kazura JW, King CL (1999) Helminth- and bacillus Calmette-Guerin-induced immunity in children sensitized in utero to filariasis and schistosomiasis. J Immunol 162:6843–6848

Malhotra I, McKibben M, Mungai P, McKibben E, Wang X, Sutherland LJ, Muchiri EM, King CH, King CL, LaBeaud AD (2015) Effect of antenatal parasitic infections on anti-vaccine IgG levels in children: a prospective birth cohort study in Kenya. PLoS Negl Trop Dis 9:e0003466

Maruszewska-Cheruiyot M, Donskow-Lysoniewska K, Doligalska M (2018) Helminth therapy: advances in the use of parasitic worms against inflammatory bowel diseases and its challenges. Helminthologia 55:1–11

McFarlane AJ, McSorley HJ, Davidson DJ, Fitch PM, Errington C, Mackenzie KJ, Gollwitzer ES, Johnston CJ, MacDonald AS, Edwards MR, Harris NL, Marsland BJ, Maizels RM, Schwarze J (2017) Enteric helminth-induced type I interferon signaling protects against pulmonary virus infection through interaction with the microbiota. J Allergy Clin Immunol 140(4):1068–1078. e6. https://doi.org/10.1016/j.jaci.2017.01.016

McSorley HJ, Maizels RM (2012) Helminth infections and host immune regulation. Clin Microbiol Rev 25:585–608

Mishra PK, Palma M, Bleich D, Loke P, Gause WC (2014) Systemic impact of intestinal helminth infections. Mucosal Immunol 7:753–762

Mostafa MH, Sheweita SA, O'Connor PJ (1999) Relationship between schistosomiasis and bladder cancer. Clin Microbiol Rev 12:97–111

Motran CC, Ambrosio LF, Volpini X, Celias DP, Cervi L (2017) Dendritic cells and parasites: from recognition and activation to immune response instruction. Semin Immunopathol 39:199–213

Mpairwe H, Webb EL, Muhangi L, Ndibazza J, Akishule D, Nampijja M, Ngom-Wegi S, Tumusime J, Jones FM, Fitzsimmons C, Dunne DW, Muwanga M, Rodrigues LC, Elliott AM (2011) Anthelminthic treatment during pregnancy is associated with increased risk of infantile eczema: randomised-controlled trial results. Pediatr Allergy Immunol 22(3):5–31

Mpairwe H, Tweyongyere R, Elliott A (2014) Pregnancy and helminth infections. Parasite Immunol 36:328–337

Murdoch ME (2010) Onchodermatitis. Curr Opin Infect Dis 23:124–131

Mutapi F, Imai N, Nausch N, Bourke CD, Rujeni N, Mitchell KM, Midzi N, Woolhouse ME, Maizels RM, Mduluza T (2011) Schistosome infection intensity is inversely related to autoreactive antibody levels. PLoS One 6:e19149

Ndibazza J, Mpairwe H, Webb EL, Mawa PA, Nampijja M, Muhangi L, Kihembo M, Lule SA, Rutebarika D, Apule B, Akello F, Akurut H, Oduru G, Naniima P, Kizito D, Kizza M, Kizindo R, Tweyongere R, Alcock KJ, Muwanga M, Elliott AM (2012) Impact of anthelminthic treatment in pregnancy and childhood on immunisations, infections and eczema in childhood: a randomised controlled trial. PLoS One 7:e50325

Netea MG, Joosten LA, Latz E, Mills KH, Natoli G, Stunnenberg HG, O'Neill LA, Xavier RJ (2016) Trained immunity: a program of innate immune memory in health and disease. Science 352:aaf1098

Nokes C, Grantham-McGregor SM, Sawyer AW, Cooper ES, Robinson BA, Bundy DAP (1992) Moderate to heavy infections of *Trichuris trichiura* affect cognitive function in Jamaican school children. Parasitology 104:539–547

Nono JK, Kamdem SD, Netongo PM, Dabee S, Schomaker M, Oumarou A, Brombacher F, Moyou-Somo R (2018) Schistosomiasis burden and its association with lower measles vaccine responses in school children from rural Cameroon. Front Immunol 9:2295

Obieglo K, Feng X, Bollampalli VP, Dellacasa-Lindberg I, Classon C, Osterblad M, Helmby H, Hewitson JP, Maizels RM, Gigliotti Rothfuchs A, Nylen S (2016) Chronic gastrointestinal nematode infection mutes immune responses to mycobacterial infection distal to the gut. J Immunol 196:2262–2271

Osbourn M, Soares DC, Vacca F, Cohen ES, Scott IC, Gregory WF, Smyth DJ, Toivakka M, Kemter AM, Le Bihan T, Wear M, Hoving D, Filbey KJ, Hewitson JP, Henderson H, Gonzàlez A, Errington C, Vermeren S, Astier A, Wallace WA, Schwarze J, Ivens AC, Maizels RM, McSorley HJ (2017) HpARI protein secreted by a helminth parasite suppresses interleukin-33. Immunity 47:739–751

Pabalan N, Singian E, Tabangay L, Jarjanazi H, Boivin MJ, Ezeamama AE (2018) Soil-transmitted helminth infection, loss of education and cognitive impairment in school-aged children: a systematic review and meta-analysis. PLoS Negl Trop Dis 12:e0005523

Rajamanickam A, Munisankar S, Bhootra Y, Dolla C, Thiruvengadam K, Nutman TB, Babu S (2019) Metabolic consequences of concomitant *Strongyloides stercoralis* infection in patients with type 2 diabetes mellitus. Clin Infect Dis 69:697–704

Rajamanickam A, Munisankar S, Dolla C, Menon PA, Thiruvengadam K, Nutman TB, Babu S (2020a) Helminth infection modulates systemic pro-inflammatory cytokines and chemokines implicated in type 2 diabetes mellitus pathogenesis. PLoS Negl Trop Dis 14:e0008101

Rajamanickam A, Munisankar S, Thiruvengadam K, Menon PA, Dolla C, Nutman TB, Babu S (2020b) Impact of helminth infection on metabolic and immune homeostasis in non-diabetic obesity. Front Immunol 11:2195

Ramanan D, Bowcutt R, Lee SC, Tang MS, Kurtz ZD, Ding Y, Honda K, Gause WC, Blaser MJ, Bonneau RA, Lim YA, Loke P, Cadwell K (2016) Helminth infection promotes colonization resistance via type 2 immunity. Science 352:608–612

Rapin A, Harris NL (2018) Helminth-bacterial interactions: cause and consequence. Trends Immunol 39:724–733

Red Eagle A, Chawla A (2010) In obesity and weight loss, all roads lead to the mighty macrophage. J Clin Invest 120:3437–3440

Reese TA, Wakeman BS, Choi HS, Hufford MM, Huang SC, Zhang X, Buck MD, Jezewski A, Kambal A, Liu CY, Goel G, Murray PJ, Xavier RJ, Kaplan MH, Renne R, Speck SH, Artyomov MN, Pearce EJ, Virgin HW (2014) Helminth infection reactivates latent γ-herpesvirus via cytokine competition at a viral promoter. Science 345:573–577

Reynolds LA, Smith KA, Filbey KJ, Harcus Y, Hewitson JP, Yebra M, Maizels RM (2014) Commensal-pathogen interactions in the intestinal tract: lactobacilli promote infection with, and are promoted by, helminth parasites. Gut Microbes 5:10–19

Reynolds LA, Finlay BB, Maizels RM (2015) Cohabitation in the intestine: interactions among helminth parasites, bacterial microbiota, and host immunity. J Immunol 195:4059–4066

Reynolds LA, Redpath SA, Yurist-Doutsch S, Gill N, Brown EM, van der Heijden J, Brosschot TP, Han J, Marshall NC, Woodward SE, Valdez Y, Borchers CH, Perona-Wright G, Finlay BB (2017) Enteric helminths promote salmonella coinfection by altering the intestinal metabolome. J Infect Dis 215:1245–1254

Riner DK, Ndombi EM, Carter JM, Omondi A, Kittur N, Kavere E, Korir HK, Flaherty B, Karanja D, Colley DG (2016) Schistosoma mansoni infection can jeopardize the duration of protective levels of antibody responses to immunizations against hepatitis B and tetanus toxoid. PLoS Negl Trop Dis 10:e0005180

Rojas A, Dvir E, Baneth G (2019) Insights on Spirocerca lupi, the carcinogenic dog nematode. Trends Parasitol 36(1):52–63. https://doi.org/10.1016/j.pt.2019.10.004

Sabin EA, Araujo MI, Carvalho EM, Pearce EJ (1996) Impairment of tetanus toxoid-specific Th1-like immune responses in humans infected with *Schistosoma mansoni*. J Infect Dis 173: 269–272

Saenz SA, Taylor BC, Artis D (2008) Welcome to the neighborhood: epithelial cell-derived cytokines license innate and adaptive immune responses at mucosal sites. Immunol Rev 226: 172–190

Santos P, Lorena VM, Fernandes Ede S, Sales IR, Nascimento WR, Gomes Yde M, Albuquerque MC, Costa VM, Souza VM (2016) Gestation and breastfeeding in schistosomotic mothers differently modulate the immune response of adult offspring to postnatal Schistosoma mansoni infection. Mem Inst Oswaldo Cruz 111:83–92

Scholte LLS, Pascoal-Xavier MA, Nahum LA (2018) Helminths and cancers from the evolutionary perspective. Front Med (Lausanne) 5:90

Schwartz C, Hams E, Fallon PG (2018) Helminth modulation of lung inflammation. Trends Parasitol 34:388–403

Seydel LS, Petelski A, van Dam GJ, van der Kleij D, Kruize-Hoeksma YC, Luty AJ, Yazdanbakhsh M, Kremsner PG (2012) Association of in utero sensitization to Schistosoma haematobium with enhanced cord blood IgE and increased frequencies of CD5- B cells in African newborns. Am J Trop Med Hyg 86:613–619

Shea-Donohue T, Qin B, Smith A (2017) Parasites, nutrition, immune responses and biology of metabolic tissues. Parasite Immunol 39:5

Shen SW, Lu Y, Li F, Shen ZH, Xu M, Yao WF, Feng YB, Yun JT, Wang YP, Zhou L, Tong DX (2014) The potential long-term effect of previous schistosome infection may reduce the risk factors for cardiovascular diseases. Int J Cardiol 177:566–568

Shen SW, Lu Y, Li F, Shen ZH, Xu M, Yao WF, Feng YB, Yun JT, Wang YP, Ling W, Qi HJ, Tong DX (2015) The potential long-term effect of previous schistosome infection reduces the risk of metabolic syndrome among Chinese men. Parasite Immunol 37:333–339

Shepherd C, Navarro S, Wangchuk P, Wilson D, Daly NL, Loukas A (2015) Identifying the immunomodulatory components of helminths. Parasite Immunol 37:293–303

Shimokawa C, Obi S, Shibata M, Olia A, Imai T, Suzue K, Hisaeda H (2019) Suppression of obesity by an intestinal helminth through interactions with intestinal microbiota. Infect Immun 87:6

Smallwood TB, Giacomin PR, Loukas A, Mulvenna JP, Clark RJ, Miles JJ (2017) Helminth immunomodulation in autoimmune disease. Front Immunol 8:453

Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly YM, Glickman JN, Garrett WS (2013) The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science 341:569–573

Smits HH, Hammad H, van Nimwegen M, Soullie T, Willart MA, Lievers E, Kadouch J, Kool M, Oosterhoud JK, Deelder AM, Lambrecht BN, Yazdanbakhsh M (2007) Protective effect of *Schistosoma mansoni* infection on allergic asthma depends on intensity and chronicity of infection. J Allergy Clin Immunol 120:932–940

Smout MJ, Laha T, Mulvenna J, Sripa B, Suttiprapa S, Jones A, Brindley PJ, Loukas A (2009) A granulin-like growth factor secreted by the carcinogenic liver fluke, *Opisthorchis viverrini*, promotes proliferation of host cells. PLoS Pathog 5:e1000611

Sobotkova K, Parker W, Leva J, Ruzkova J, Lukes J, Jirku PK (2019) Helminth therapy—from the parasite perspective. Trends Parasitol 35:501–515

Sripa B, Brindley PJ, Mulvenna J, Laha T, Smout MJ, Mairiang E, Bethony JM, Loukas A (2012) The tumorigenic liver fluke Opisthorchis viverrini--multiple pathways to cancer. Trends Parasitol 28:395–407

Steel C, Guinea A, McCarthy JS, Ottesen EA (1994) Long-term effect of prenatal exposure to maternal microfilaraemia on immune responsiveness to filarial antigens. Lancet 343:890–893

Straubinger K, Paul S, Prazeres da Costa O, Ritter M, Buch T, Busch DH, Layland LE, Prazeres da Costa CU (2014) Maternal immune response to helminth infection during pregnancy determines offspring susceptibility to allergic airway inflammation. J Allergy Clin Immunol 134(1271–1279):e10

Strube C, Heuer L, Janacek E (2012) *Toxocara* infections in paratenic hosts. Vet Parasitol 193(4): 375–389

Su Z, Segura M, Stevenson MM (2006) Reduced protective efficacy of a blood-stage malaria vaccine by concurrent nematode infection. Infect Immun 74:2138–2144

Su CW, Chen CY, Li Y, Long SR, Massey W, Kumar DV, Walker WA, Shi HN (2018) Helminth infection protects against high fat diet-induced obesity via induction of alternatively activated macrophages. Sci Rep 8:4607

Summers RW, Elliott DE, Qadir K, Urban JF Jr, Thompson R, Weinstock JV (2003) *Trichuris suis* seems to be safe and possibly effective in the treatment of inflammatory bowel disease. Am J Gastroenterol 98:2034–2041

Taylor MD, van der Werf N, Maizels RM (2012) T cells in helminth infection: the regulators and the regulated. Trends Immunol 33:181–189

Taylor-Robinson DC, Maayan N, Soares-Weiser K, Donegan S, Garner P (2015) Deworming drugs for soil-transmitted intestinal worms in children: effects on nutritional indicators, haemoglobin, and school performance. Cochrane Database Syst Rev 7:CD000371. https://doi.org/10.1002/14651858.CD000371.pub6:CD000371

Tweyongyere R, Nassanga BR, Muhwezi A, Odongo M, Lule SA, Nsubuga RN, Webb EL, Cose SC, Elliott AM (2019) Effect of *Schistosoma mansoni* infection and its treatment on antibody responses to measles catch-up immunisation in pre-school children: a randomised trial. PLoS Negl Trop Dis 13:e0007157

van den Biggelaar A, van Ree R, Roderigues LC, Lell B, Deelder AM, Kremsner PG, Yazdanbakhsh M (2000) Decreased atopy in children infected with *Schistosoma haematobium*: a role for parasite-induced interleukin-10. Lancet 356:1723–1727

van den Biggelaar AH, Rodrigues LC, van Ree R, van der Zee JS, Hoeksma-Kruize YC, Souverijn JH, Missinou MA, Borrmann S, Kremsner PG, Yazdanbakhsh M (2004) Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. J Infect Dis 189:892–900

Varyani F, Fleming JO, Maizels RM (2017) Helminths in the gastrointestinal tract as modulators of immunity and pathology. Am J Physiol Gastrointest Liver Physiol 312:G537–G549

Voldsgaard A, Bager P, Garde E, Akeson P, Leffers AM, Madsen CG, Kapel C, Roepstorff A, Thamsborg SM, Melbye M, Siebner H, Sondergaard HB, Sellebjerg F, Sorensen PS (2015) Trichuris suis ova therapy in relapsing multiple sclerosis is safe but without signals of beneficial effect. Mult Scler 21:1723–1729

Wammes LJ, Mpairwe H, Elliott AM, Yazdanbakhsh M (2014) Helminth therapy or elimination: epidemiological, immunological, and clinical considerations. Lancet Infect Dis 14:1150–1162

Weinstock JV, Elliott DE (2013) Translatability of helminth therapy in inflammatory bowel diseases. Int J Parasitol 43:245–251

Weinstock JV, Elliott DE (2014) Helminth infections decrease host susceptibility to immune-mediated diseases. J Immunol 193:3239–3247

White MPJ, McManus CM, Maizels RM (2020) Regulatory T-cells in helminth infection: induction, function and therapeutic potential. Immunology 160:248–260

Wilson MS, Taylor MD, O'Gorman MT, Balic A, Barr TA, Filbey K, Anderton SM, Maizels RM (2010) Helminth-induced $CD19^{+}CD23^{hi}$ B cells modulate experimental allergic and autoimmune inflammation. Eur J Immunol 40:1682–1696

Wiria AE, Djuardi Y, Supali T, Sartono E, Yazdanbakhsh M (2012) Helminth infection in populations undergoing epidemiological transition: a friend or foe? Semin Immunopathol 34: 889–901

Wiria AE, Sartono E, Supali T, Yazdanbakhsh M (2014) Helminth infections, type-2 immune response, and metabolic syndrome. PLoS Pathog 10:e1004140

Wright VJ, Ame SM, Haji HS, Weir RE, Goodman D, Pritchard DI, Mohamed MR, Haji HJ, Tielsch JM, Stoltzfus RJ, Bickle QD (2009) Early exposure of infants to GI nematodes induces Th2 dominant immune responses which are unaffected by periodic anthelminthic treatment. PLoS Negl Trop Dis 3:e433

Wu D, Molofsky AB, Liang HE, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, Chawla A, Locksley RM (2011) Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science 332:243–247

Wynn TA, Thompson RW, Cheever AW, Mentink-Kane MM (2004) Immunopathogenesis of schistosomiasis. Immunol Rev 201:156–167

Yang Z, Grinchuk V, Smith A, Qin B, Bohl JA, Sun R, Notari L, Zhang Z, Sesaki H, Urban JF Jr, Shea-Donohue T, Zhao A (2013) Parasitic nematode-induced modulation of body weight and associated metabolic dysfunction in mouse models of obesity. Infect Immun 81:1905–1914

Zaiss MM, Harris NL (2016) Interactions between the intestinal microbiome and helminth parasites. Parasite Immunol 38:5–11

Zaiss MM, Rapin A, Lebon L, Dubey LK, Mosconi I, Sarter K, Piersigilli A, Menin L, Walker AW, Rougemont J, Paerewijck O, Geldhof P, McCoy KD, Macpherson AJ, Croese J, Giacomin PR, Loukas A, Junt T, Marsland BJ, Harris NL (2015) The intestinal microbiota contributes to the ability of helminths to modulate allergic inflammation. Immunity 43:998–1010

Chapter 5
Schistosomiasis

Ahmad Othman and Rashika El Ridi

Abstract Schistosomiasis is one of the most widespread and debilitating helminthic diseases. The immune system strives to be protective vis-à-vis *Schistosoma* infection, but the inevitable immunopathology may lead to fibrosis and organ dysfunction. Moreover, the skewing of immune response axis to polarized Th2 phenotype can impair resistance to other pathogens and has been associated with neoplasms, further complicating the clinical sequelae of schistosomiasis. Recent research has focused on subtle morbidities of schistosomiasis, including anaemia, malnutrition, and persistent inflammatory state which have significant impact on vulnerable populations such as growing children and pregnant females. Techniques and tools for diagnosing schistosomiasis either are cumbersome or lack sensitivity and specificity. Accordingly, many patients remain undiagnosed and receive no treatment. Currently, the only available drug is praziquantel (PZQ), the use of which in mass treatment raises concerns about development of drug resistance. PZQ also neither affects juvenile parasites nor prevents reinfection. Indeed, *Schistosoma* infection is a fascinating model for gaining insight about the mutual interplay between host and parasite factors, which ultimately determines the net pathology. Despite decades of intensive research on schistosomiasis, unresolved issues are still intriguing scientists; one of which is the development of a vaccine. Fortunately, however, significant progress has been achieved towards the elimination of schistosomiasis. Information provided in this review should help opening avenues for better understanding, management, and control of schistosomiasis.

A. Othman (✉)
Medical Parasitology Department, Faculty of Medicine, Tanta University, Tanta, Egypt
e-mail: ahmed.osman1@med.tanta.edu.eg

R. El Ridi
Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt
e-mail: rashika@sci.cu.edu.eg

F. Bruschi (ed.), *Helminth Infections and their Impact on Global Public Health*,
https://doi.org/10.1007/978-3-031-00303-5_5

Abbreviations

ARA	Arachidonic acid
CAA	Circulating anodic antigen
CCA	Circulating cathodic antigen
ESP	Excretory–secretory products
EV	Excretory vesicles
Ig	Immunoglobulin
Il	Interleukin
IFN-γ	Interferon gamma
HA	Hyaluronic acid
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HSCs	Hepatic stellate cells
MDA	Mass drug administration
miRNA	MicroRNA
MAP	Multiple antigen peptide
PZQ	Praziquantel
SAWA	Soluble adult worm antigen
SEA	Soluble egg antigen
STH	Soil-transmitted helminth
Th	T helper cell
TNF-α	Tumour necrosis factor alpha
Tregs	Regulatory T cells
WHO	World Health Organization

5.1 Introduction

Schistosomiasis, a neglected tropical disease, widespread in 74 tropical and subtropical countries, is caused by blood flukes of the genus *Schistosoma*. Historically, it was in Cairo, Egypt, in 1851 that the adult worms of *Schistosoma haematobium* (*S. haematobium*) were first observed and depicted by the German physician Theodor Bilharz (1825–1852), working at the time in Kasr el-Aini Teaching Hospital. The sinuously moving worms were detected during autopsy in pelvic veins of a patient with antecedents of haematuria (Cox 2002). Bilharz confirmed an etiological relationship between the agent and the mysterious *endemic haematuria* in Egypt. Therefore, the name *bilharzia* has been widely used in the medical literature, and in Egypt as well as many other countries, the disease used to be named bilharziasis (Koraitim 1994). The life cycle of *Schistosoma* was however not clarified until 1915 when the British parasitologist Robert Leiper demonstrated that the aquatic pulmonate snails of the genera *Bulinus* and *Biomphalaria* are the intermediate hosts of *S. haematobium* and *S. mansoni*, respectively (Leiper 1916). Meanwhile, Japanese

scientists worked out the life cycle of *S. japonicum*. In 1910, Marc Ruffer found eggs of *S. haematobium* and urinary bladder calcifications in two mummies dating from the 20th dynasty of ancient Egypt (1250 to 1000 B.C.), thus launching the discipline of paleoparasitology (Ziskind 2009; Cox 2002; Fornaciari and Gaeta 2014).

Currently, over 780 million people, principally children, are at risk of infection, and 250 million people are infected, with 201.5 million of them living in Africa. The infection leads to considerable morbidity and the estimated loss of a minimum of 1.9 million disability-adjusted life years (DALYs) (McManus et al. 2018, 2020). Millions of children suffer anaemia, growth deficiency, abdominal pain, exercise intolerance, poor school performance, cognitive defects, and other sequelae resulting from infection with schistosomes. Millions of adult males and females endure fever, headache, lethargy, and lowered work capacity and quality of life because of severe lesions and damage in the liver, colon, rectum, and/or bladder and lower urinary tract consequent to the infection. At least, 200,000 people die annually of haematemesis, liver failure, or cancer of the urinary bladder. Sound information is required for setting the platform for elimination of this serious affliction.

5.2 The Agent and Life Cycle

Schistosomes are flatworms (kingdom Animalia, phylum Platyhelminthes, class Trematoda), which are exclusively different from other trematodes in having separate sexes; yet, they form pairs, mimicking the hermaphrodite condition. They are endoparasites (subclass Digenea) of an intermediate host snail where they reproduce asexually and a final vertebrate host where copulation of adult male and female leads to daily production of hundreds of eggs. They belong to order Strigeidida, characterized by a fork-tailed cercaria, which infects hosts using enzymes of penetration glands. The suborder is Strigeata, superfamily Schistosomatoidea, which contains three families: Sanguinocolidae (which infect fish), Spirorchidae (parasites of turtles), and Schistosomatidae (parasites of crocodilians, birds, and mammals). They demonstrate unsurpassed precision in laying eggs near the conduit for egg passage to the external environment to complete the life cycle (Platt and Brooks 1997). The family Schistosomatidae comprises approximately 100 species, among which *S. mansoni*, *S. haematobium*, *S. japonicum*, and to a less prevalent extent *S. guineensis*, *S. intercalatum*, and *S. mekongi* cause human schistosomiasis, which leads to considerable morbidity in sub-Saharan Africa, the Middle East, South America, and Southeast Asia (Tamarozzi et al. 2021).

While in the final host, the adult worms mate and produce many eggs that pass to the external milieu via the urine or faeces (depending on the species of schistosome). These eggs hatch in water, and the miracidia locate and penetrate the snail intermediate host where they reproduce as sporocysts, sometimes for several generations. They exit the snail as cercariae, which are the infective stage. These cercariae can penetrate the skin of humans during contact with contaminated water, shed their tails, and enter the circulation. On migration to the portal veins of the liver, they

mature into adult flukes. These migrate again in the bloodstream to the venules of the bladder or bowel where they mate, produce eggs, and complete the life cycle (Timson 2020) (Fig. 5.1).

5.3 Epidemiology of Schistosomiasis

Incidence and prevalence of schistosomiasis reflect the distribution of the freshwater snail the schistosome species use as intermediate host. Thus, the presence of *Oncomelania* snails (*Oncomelania hupensis*) in the marsh and lake regions of southern China (Zhao et al. 2012), the Philippines (Leonardo et al. 2013), and the Central Sulawesi Province of Indonesia (Budiono et al. 2019) allows the spread of *S. japonicum*. In the Middle East and sub-Saharan Africa, the broad geographical range of susceptible snail species of the genera *Biomphalaria* and *Bulinus* coincides with the widespread incidence of schistosomiasis mansoni and schistosomiasis haematobium, respectively (Hailegebriel et al. 2020; Habib et al. 2021). In contrast, schistosomiasis is absent from Cape Verde, Comoros, and Seychelles owing to the absence of permissive snail intermediate hosts (Utzinger et al. 2009). In Brazil, the distribution of *Biomphalaria* spp. (*B. glabrata*, *B. straminea*, and *B. tenagophila*) is closely associated with the occurrence of schistosomiasis mansoni (Scholte et al. 2012). Transmission of the parasite to the human population in Zanzibar is related to the distribution of the intermediate snail host, *Bulinus globosus* (Allan et al. 2013). Abundance of *Bulinus truncatus* is declining in Egypt and so is urinary schistosomiasis (Barakat 2013). On the other hand, occurrence of *B. truncatus* and *B. beccari* in Saudi Arabia might allow spread of *S. haematobium* (Mostafa et al. 2012).

Prevalence of schistosomiasis is inversely associated with adequate financial, social, and political conditions that would permit rise in living standards, and access to clean water, improved sanitary conditions, and health education to residents of rural areas (Utzinger et al. 2011; King 2010; McManus et al. 2018, 2020). Improving socioeconomic conditions has contributed to the interruption of schistosomiasis transmission in Japan, Iran, Jordan, Morocco, and Tunisia (Utzinger et al. 2011; World Health Organization 2011; Sokolow et al. 2018). In contrast, more than 90% of all estimated cases of hepato-intestinal and urinary schistosomiasis reside in poorly developed regions of sub-Saharan Africa (Stothard et al. 2017) with Nigeria and Tanzania having the highest burden. Of note, prevalence of infection increased with rise in the population in Tanzania from 19% in 1977 to 51.5% in 2012 (Mazigo et al. 2012). Implementation of mass drug administration (MDA) programmes, improvement in sanitation, provision of clean water, and behaviour changes through public health education have led to substantial decline in schistosomiasis prevalence among Tanzanian school-aged children and young women (Rite et al. 2020; Mazigo et al. 2021).

As importantly, epidemiology is dependent on the level of accuracy of diagnostic methods. The World Health Organization (WHO) in 2009 estimates 235 million cases of schistosomiasis worldwide, with 732 million people at risk for infection in

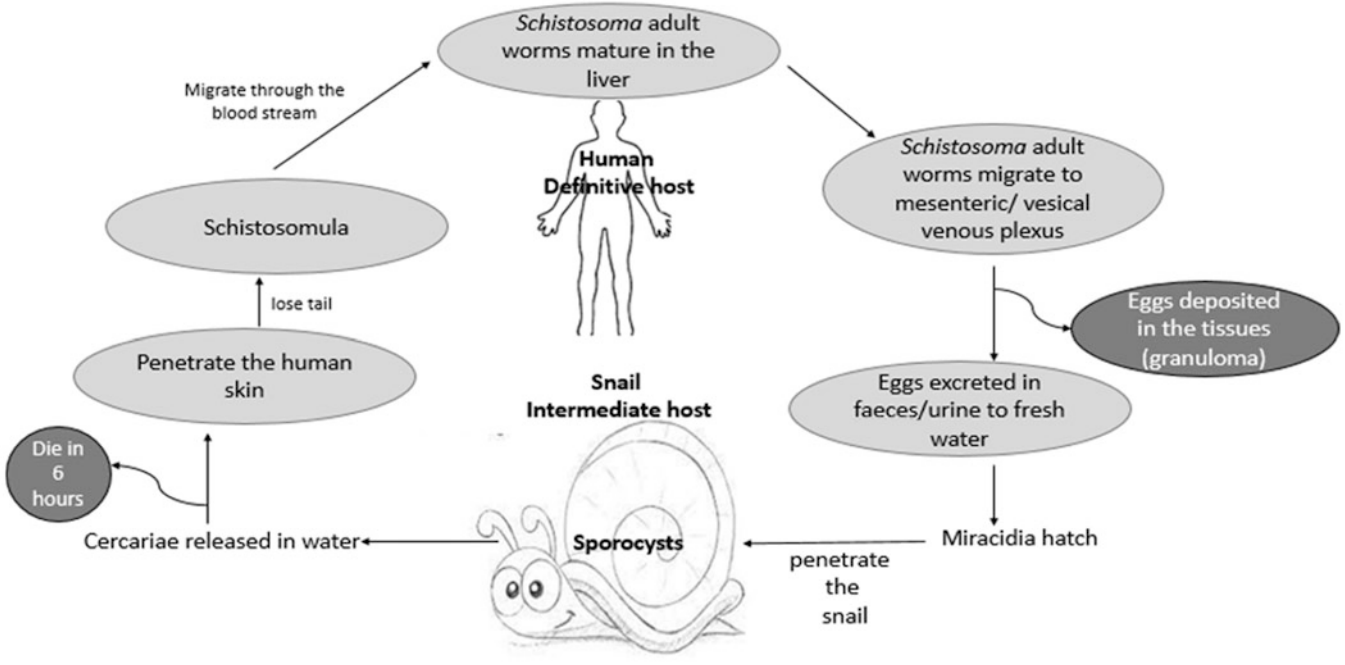

Fig. 5.1 Life cycle of *Schistosoma* spp. (Picture designed and kindly provided by prof. Dalia S. Ashour, Faculty of Medicine, Tanta University, Egypt)

known transmission areas (WHO 2009). Since standard methods of field testing are admittedly insensitive (Gryseels 1996; Gad et al. 2011), true prevalence and worm loads in endemic communities may be considerably underestimated, and the WHO statistics may underestimate active and potential cases (Enk et al. 2008; Coulibaly et al. 2012). King (2010) argued that probably 40–60% of patients are likely misdiagnosed and suggested that the WHO values should be adjusted to between 391 and 587 million people worldwide. In support, WHO estimates show that still 290.8 million people required preventive treatment for schistosomiasis in 2018 (WHO 2020).

S. mansoni is endemic in a few countries in South America, principally Brazil, where prevalence rates are low, and in around 50 countries in the Middle East and sub-Saharan Africa where prevalence rates can exceed 50% in some parts of Nigeria, Ghana, Mozambique, Burkina Faso, Mali, Sierra Leone, Madagascar, and Tanzania (Utzinger et al. 2011; WHO 2009; Elmorshedy et al. 2020). Schistosomiasis haematobium is found only in the Middle East and in sub-Saharan African countries, causing two thirds of schistosomiasis cases (King et al. 2020; Adam et al. 2021). The third schistosome species parasitizing humans in Africa, *S. intercalatum* (4.8%), was only reported in surveys carried out in Cameroon and Nigeria, confirming that this species is restricted to some parts of West and Central Africa. Co-occurrence of *S. mansoni* and *S. haematobium* was reported in 20% of the survey locations. Based on the WHO prevalence cut-offs of $<10\%$, 10–50%, and $>50\%$ as low, moderate, and high endemicity areas, respectively, *S. haematobium* is highly prevalent in Western and Southern sub-Saharan African countries, while *S. mansoni* predominates in East Africa (King et al. 2020; Adam et al. 2021).

S. japonicum affects humans and more than 40 mammalian host species, all of which can act as reservoirs of infection, in the Philippines, China, and Indonesia. However, only 7 out of 12 previously endemic provinces in China reported schistosome infections with prevalence rates of 6–7% (Zhou et al. 2011a, b, 2013). In 2017, only 38,000 schistosomiasis cases were reported in China justifying excellent prospects for elimination of human infections in the near future (Chen et al. 2021). In the Philippines, there are currently only 560,000 cases of schistosomiasis (Olveda and Gray 2019). Schistosomiasis transmission is also under control in Indonesia in the two previously endemic areas of Lindu Valley and Napu Valley, both located in the Province of Central Sulawesi where prevalence rates range between 0 and 13% (Garjito et al. 2008). Interruption of *S. japonicum* transmission is hindered due to the prevalence of schistosomiasis in bovines, goats, and sheep, important reservoir hosts (Jumawan and Estaño 2021).

Beside the numerous cases of imported schistosomiasis in Europe (Leblanc et al. 2021), in the summer of 2013, an unexpected outbreak of urogenital schistosomiasis occurred in Corsica, France, with more than 120 local people or tourists infected. The investigations showed that *B. truncatus* snails in Cavu river in the village of Sainte-Lucie-de-Porto-Vecchio, Southern Corsica, were infected with *S. haematobium* (Boissier et al. 2015, 2016) and that the transmission appears to be ongoing (Rothe et al. 2021).

5.4 Immunopathogenesis of *Schistosoma* Infection

Barsoum et al. (2013) stated that "almost all the clinical features of schistosomiasis are caused, directly or indirectly, by the host's immune response to different stages of the parasite in the body". Most of our concepts of the immunopathological processes during *Schistosoma* infection are derived from animal studies especially on *S. mansoni* and *S. japonicum*, and much less frequently on *S. haematobium*. Human studies are relatively few and seem difficult to be controlled and interpreted. Most authorities, however, believe that the immunopathology is rather similar in human and animal hosts.

5.4.1 Acute Schistosomiasis

Cercarial dermatitis is a local IgE-mediated hypersensitivity response directed against penetrating cercariae. It occurs infrequently among endemic populations but is common among visitors and migrants and after primary infections (Gryseels et al. 2006). Upon adherence to host skin, *S. mansoni* cercariae exposed to linoleic acid produce prostaglandin E2 (PGE2) and induce mouse and human keratinocytes to produce PGE2 and immunosuppressive IL-10 (Ramaswamy et al. 2000). After invasion of host skin, cercariae transform into schistosomula and release large amounts of proteins that can activate lymph node cells of irradiated cercariae-vaccinated mice to release copious amounts of interferon (IFN)-γ (Harrop et al. 2000). In vivo, cercariae-derived proteins likely interact with the Langerhans cells and keratinocytes' surface membrane toll-like receptor (TLR)2 and TLR4, and/or mannose-binding lectin, leading to the production of nitric oxide, inflammatory cytokines, IL-10, and PGE2 (Ramaswamy et al. 2000; Pivarcsi et al. 2004). Additionally, upon entry in the dermis, skin-stage schistosomula secrete a *S. mansoni*-derived apoptosis-inducing factor of 23 kDa, which elicits apoptosis in the CD4+ and CD8+ T lymphocytes surrounding the larvae in the skin of naive and irradiated cercariae-vaccinated mice (Chen et al. 2002). Blood cell cultures of humans infected with *S. mansoni* and/or *S. haematobium* stimulated with cercarial excretory–secretory products (ESP) also released large amounts of IL-10 (Turner et al. 2013). Indeed, skin schistosomula ESP-mediated immune responses lead to both immune priming and regulation (Mounntford and Trottein 2004).

Katayama syndrome is a systemic immune-complex-mediated hypersensitivity reaction against migrating schistosomula and early egg deposition. The symptoms of Katayama syndrome manifest 14–84 days after individuals are first exposed to schistosome infection or following heavy reinfection. Acute schistosomiasis due to *S. mansoni* or *S. haematobium* infection is common among individuals exposed for the first time such as travellers or migrants but is rare among endemic populations. In contrast, acute disease due to *S. japonicum* is common in endemic communities and is associated with severe and persistent manifestations that may rapidly progress to

hepatosplenomegaly and portal hypertension (Gryseels et al. 2006; Ross et al. 2007; Burke et al. 2009).

5.4.2 Chronic Schistosomiasis

The course of chronic schistosomiasis is variable and is dependent on the anatomical location of adult schistosomes within the vasculature of the mammalian host. In murine models, the immune responses to schistosome antigens manifest a striking shift from a moderate Th1 to a robust Th2-dominated response. Fibrosis and much of the pathology are primarily mediated by Th2, while Th1 responses are presumed to be protective (Reiman et al. 2006). However, recent evidence suggests that maintaining a balanced and controlled Th1 or Th2 response is critical in the case of schistosomiasis for protective granuloma formation without excessive pathology (Wilson et al. 2007).

During the first 4–6 weeks of infection in the mouse, a moderate T helper type 1 (Th1) response is induced against migrating schistosomula and immature adult worms. This response exhibited increased levels of circulating pro-inflammatory cytokines including TNF-α, IL-1, IL-6, and IFN-γ (Pearce and MacDonald 2002; Wynn et al. 2004; Wilson et al. 2007). High levels of these cytokines have also been associated with the development of Katayama syndrome in humans (Caldas et al. 2008). The immune response then polarizes to a T helper type 2 (Th2) response with the start of egg-laying, characterized by heightened expression of IL-4, IL-5, IL-10, and IL-13. The Th2 response reaches a peak at approximately 8 weeks postinfection and is then downregulated with progression to chronic infection (Pearce and MacDonald 2002; Wynn et al. 2004; Wilson et al. 2007).

The situation is more complex in humans as the cytokine profile in chronic cases is typically a variable *mix* of Th1 and Th2 cytokines. Different clinical entities of schistosomiasis are associated with characteristic cytokine patterns (Caldas et al. 2008). For example, one study revealed that whole blood cultures of approximately 340 Egyptian schoolchildren, patently infected with *S. mansoni*, produced large amounts of IFN-γ and IL-17 in response to soluble adult worm antigen (SAWA) and soluble egg antigen (SEA), challenging the dogma that the immune responses to schistosome antigens are dominated by type 2 cytokines, principally IL-4 and IL-5, while Th1 responses are downregulated in human schistosomiasis. Furthermore, the blood cultures of approximately 60% of these children produced IL-4 or IL-5 to SAWA and SEA only following PZQ treatment (Barakat et al. 2015). These findings are in accord with previous reports showing that plasma IL-5, IL-13, and IL-33, serum IgE binding to adult worm antigens, and circulating eosinophil numbers significantly increased only upon treatment in children and adult patients with patent schistosomiasis mansoni (Joseph et al. 2004b; deMorais et al. 2008) or haematobium (Wilson et al. 2013a, b). In other studies of humans with chronic schistosomiasis mansoni, whole blood cultures of adults (Joseph et al. 2004a) and 4–17-year-old

children (Wilson et al. 2008) produced significantly higher levels of IL-4, IL-5, and IL-13 in response to SAWA than to SEA.

In sum, research has indicated that patently infected mice and PZQ-treated humans produce large amounts of type 2 cytokines and the immunosuppressive IL-10 in response to SEA (Joseph et al. 2004a, b; Wilson et al. 2008), likely leading to downregulation of granuloma formation and liver fibrosis. On the other hand, persistent production of low levels of IL-10 and IFN-γ with high levels of inflammatory cytokines appears to be associated with severe periportal fibrosis, hepatosplenomegaly, and portal hypertension (Hoffmann et al. 2000; Booth et al. 2004; Wilson et al. 2008; Barsoum et al. 2013).

5.4.2.1 Granuloma Formation

The eggs of *Schistosoma* deposited in the tissues induce granuloma formation. Upon full maturation, the living embryo, the miracidium, secretes SEA which exit via the microscopic pores of the egg shell (Ashton et al. 2001). For *S. mansoni*, the egg-derived antigens include Sm-40, cytoskeletal proteins like tubulin, egg-secreted protein 15, proteins of the micro-exon gene 3 (MEG-3) family, as well as the IL-4-inducing factors of *S. mansoni* eggs, IL-4-inducing principal of *S. mansoni* eggs (IPSE), and the ribonuclease domain-containing omega 1 (Fitzsimmons et al. 2005; Jang-Lee et al. 2007; Mathieson and Wilson 2010). Extensive studies of experimental schistosomiasis, mostly on murine *S. mansoni*, have revealed that granuloma formation is attributable to a vigorous CD4 + Th2-driven response, akin to a form of delayed-type hypersensitivity, that is tightly regulated by various cell populations, cytokines, and chemokines (Wynn et al. 2004).

Although *Schistosoma* peri-ovular granulomas seem detrimental to the host, it is evident that they serve an indispensible host-protective function, especially during *S. mansoni* infection. Schistosome eggs and their secreted materials are a continuous antigenic stimulus for the immune system. If these antigens are not sequestered or neutralized effectively, they can harm the surrounding tissues, with hepatocytes being particularly sensitive to toxins secreted by the eggs. Hence granuloma formation seems to be a compromise, which allows the host to live with the infection for a long time. Hypothetically, the negative aspects associated with granulomas (fibrosis, portal hypertension) represent a better alternative, for host and parasite, than that of the host dying soon after parasite egg production (Pearce and MacDonald 2002; Wilson et al. 2007; Burke et al. 2009).

Histologically, five stages of granuloma could be identified during the evolution of *Schistosoma*-induced granulomatous reaction irrespective of the anatomical site (Fig. 5.2), as indicated by early studies in mice, rhesus monkeys (Hsu et al. 1972), and, later, pigs (Hurst et al. 2000): the weakly reactive, exudative, exudative–productive, productive, and involutional (Hurst et al. 2000). Initially, there is accumulation of mononuclear cells, neutrophils, and eosinophils around the eggs (weakly reactive), which increases to form a microabscess in the exudative stage.

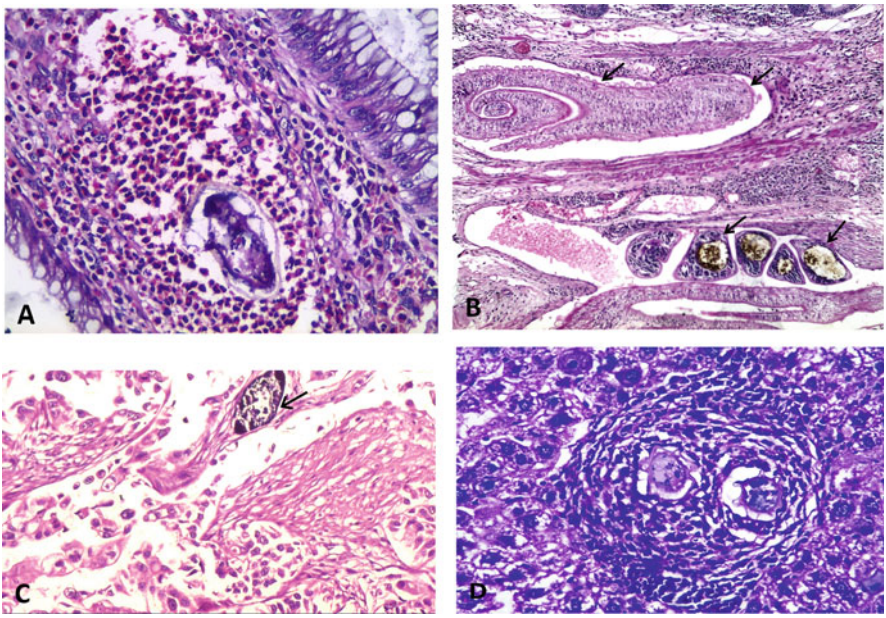

Fig. 5.2 (**a**) Early "exudative" peri-ovular schistosomal granuloma in colonic mucosa. Note the prominent eosinophilic infiltrate (×400). (**b**) Sections of intravascular adult *Schistosoma* worms (arrows) in a resected colonic polyp from an Egyptian patient (×200). (**c**) Carcinoma of urinary bladder on top of schistosomiasis in an Egyptian patient. Note calcified *Schistosoma* egg (arrows) (×400). (**d**) Mature hepatic peri-ovular granuloma (×400) (H&E)

Fibrinoid material is deposited around the eggs (Hoeppli–Splendore reaction) (Lucas 2002). In the exudative–productive stage, histiocytes, epithelioid cells, and foreign body giant cells begin to replace the leucocytic zone. Fibroblasts form a rim around the granuloma. In the productive granuloma, the egg shells are disintegrated, and the cellular elements are replaced by fibroblasts with deposition of collagen. Macrophages, lymphocytes, plasma cells, and few eosinophils are found at the periphery. Finally, the involutional stage is characterized by marked shrinkage of granuloma which is replaced by hyalinized collagen fibres. Eggs may, by then, become calcified (Hsu et al. 1972; Hurst et al. 2000).

It should be noted that the cellular composition of schistosome-induced hepatic granulomas is dependent upon *Schistosoma* species and the host. For example, the *S. japonicum* egg-induced granulomas are mainly composed of neutrophils, whereas *S. mansoni*-induced granulomas consist of a higher ratio of mononuclear cells and eosinophils, with lower numbers of neutrophils. These differences could be attributed to the secreted specific leukocyte-associated chemokines at the site of inflammation (Chuah et al. 2014).

An effective T-cell response is known to be crucial for the development of the granulomatous response and host survival. Nude mice infected with a Chinese strain of *S. japonicum* supported normal parasite survival and fecundity, although

transitory growth retardation was observed during the early stage of infection (Cheng et al. 2008). Moreover, these T-cell-deprived mice developed severe necrosis around the eggs in the liver, a situation similar to T-cell-deprived mice infected with *S. mansoni*. Interestingly, B-cell function is required for development of *S. japonicum* egg-induced granuloma in early infection (Ji et al. 2008). OBF-1 knockout mice and μMT mice, both with impaired B-cell development, developed significantly smaller hepatic granulomas at 5 weeks postinfection compared to their wild-type counterparts. In contrast, they displayed no significant difference in granuloma pathology at 8 weeks postinfection. This is in agreement with some studies on *S. mansoni*, also using B-cell-deficient mouse models, which have suggested that B-cells are required for Th2 T-cell responses but not for granuloma formation late in infection (Ji et al. 2008).

Elegant studies with IL-13- and IL-4-deficient and IL-13/IL-4 doubly deficient mice have demonstrated that IL-4 launches the development of granulomatous inflammation, whereas IL-13 is the central profibrotic cytokine in the development of schistosome-induced liver fibrosis (Fallon et al. 2000). Likewise, there are correlations between severity of hepatic fibrosis and levels of IL-13 expressed by peripheral blood mononuclear cells from individuals with chronic schistosomiasis mansoni (Alves Oliveira et al. 2006). Typically, IL-4 determines the granuloma size, induces the proliferation of Th2 cytokine-producing lymphocytes, and is important for the production of IL-5 and IL-13 by granuloma-associated cells (Cheever et al. 1994). Furthermore, IL-4 is not required for the development of fibrosis but enhances the fibrogenic effects of IL-13. As well as enhancing fibrosis, IL-13 has an additive effect with IL-4 in the development of the Th2-dominant, eosinophil-rich, granulomatous reaction (Fallon et al. 2000). Additionally, IL-5 is required for the recruitment of eosinophils to the granulomatous response as granulomas in mice deficient in IL-5 are virtually devoid of these cells (Cheever et al. 1991). Eosinophils are an important source of Th2 cytokines such as IL-13, and, thus, IL-5 indirectly contributes to the polarization of the immune response through the recruitment of these cells (Rumbley et al. 1999: Burke et al. 2009). In contrast, the egg-induced Th2 immune responses and hepatic fibrosis are counter-regulated by IL-10, IL-12, and IFN-γ (Hoffmann et al. 2002).

Granuloma formation is also associated with high levels of IL-17. It was found that egg antigens, but not adult worm antigens, preferentially induce the generation of Th17 cells. The role of Th17 cells during egg-induced granuloma formation was shown from experiments using knockout mice. These mice were unable to produce IL-23, which drives the production of IL-17 by Th17 cells (Harrington et al. 2005). Experimental studies showed that treatment with anti-IL-17 antibody markedly inhibited hepatic granulomatous inflammation through reducing the pro-inflammatory cytokines and infiltrating neutrophils (Zhang et al. 2012). Recently, T follicular helper (Tfh) cells were found to differentiate from Th2 cells in response to SEA. They promote humoral immune responses and long-lived memory B-cells and enhance the development of hepatic granulomas and fibrogenesis in mice infected with *S. japonicum* via Tfh phenotypic molecule, Bcl-6, and the Tfh-type cytokine, IL-21 (Chen et al. 2014; Wang et al. 2017).

5.4.2.2 Immunomodulation of the Granulomatous Response

In chronic human infections, two paradoxical situations exist: on the one hand, the older indigenous individuals would harbour fewer worms probably due to development of concomitant immunity, and the fact that the fecundity of female worms diminishes over time. Also, the newly formed granulomas become smaller in size. On the other hand, the granulomas will heal by fibrosis that could, over time, be dense enough to cause morbidity and irreversible sequelae (Butterworth and Thomas 1999).

Strict regulation of the Th1, Th2, and possibly Th17 cytokine responses generated during schistosome infection is essential to prevent excessive pathology. In experimental *S. mansoni* infection, Foxp3+ Treg cells appear to regulate schistosome egg-induced immunopathology (Singh et al. 2005; Taylor et al. 2006). Thus, at 8 weeks after infection by *S. mansoni*, Foxp3 gene expression of splenocytes was similar to that of naive mice but increased fourfold by 16 weeks. In contrast, granulomatous livers at 8 and 16 weeks showed 10- and 30-fold increase, respectively, in gene expression compared with normal liver. The percentage of granuloma CD4+ CD25+ T cells rose from 12% at 8 weeks to 88% at 16 weeks of the infection. Moreover, retroviral transfer of the Foxp3 gene at the onset of granuloma formation enhanced fourfold Foxp3 expression in the granuloma CD4+ CD25+ T cells and strongly suppressed full granuloma development (Singh et al. 2005).

Several other mechanisms are thought to be involved in the down-modulatory process during *Schistosoma* infection. Well-designed studies in murine schistosomiasis have revealed that IL-13Rα2 is essential for the downregulation of the granulomatous response and is pivotal in the control of IL-13-mediated fibrosis. IL-13Rα2 acts as a potent decoy receptor, competing with IL-13Rα1 for binding of IL-13 and preventing signaling through the IL-4/IL-13Rα1 receptor complex (Wilson et al. 2007; Burke et al. 2009). A role for apoptosis, particularly apoptosis by neglect of CD4+ T cells, has been suggested to contribute to the down-modulation of the granulomatous response (Rutitzky et al. 2003). Furthermore, B-cell-mediated FcR-dependent signaling has also been implicated in the downregulation of the Th2 response as mice deficient in B lymphocytes or the Fc receptor exhibited marked exacerbation of granulomatous inflammation (Jankovic et al. 1998). Likewise, Ndlovu et al. (2018) indicate that within the B-cell compartment, IL-4Rα-expressing B-cells in particular down-modulate the deleterious egg-driven tissue granulomatous inflammation to enable host survival during schistosomiasis in mice. Interestingly, some sort of immunomodulation, regarding the future immune and fibrogenic responses, occurs in offspring born to experimentally *Schistosoma*-infected hosts in case of postnatal exposure to the parasite (Othman et al. 2010). Perinatal immunological sensitization may occur via transplacental and/or transmammary passage of schistosome antigens, anti-idiotypic antibodies, or immune complexes to the offspring.

5.5 Pathogenesis and Clinical Features of Schistosomiasis

5.5.1 Stage of Invasion (Cercarial Dermatitis)

Penetration of the human skin by cercariae of human schistosomes causes allergic reaction and dermatitis. There is erythema, accompanied by maculopapular, and sometimes vesicular eruption. Scratching and secondary bacterial infection may lead to pustule formation. Exposure to cercariae of avian or bovine schistosomes, even for the first time, may lead to severe dermatitis (swimmer's itch). Treatment consists mainly of avoidance. Local and systemic antihistamines may be needed, as well as antibiotics (Butterworth and Thomas 1999).

Passage of schistosomula through the lungs and the liver may cause fever, cough, pneumonitis, and abdominal symptoms.

5.5.2 Stage of Maturation (Katayama Fever; Acute Schistosomiasis)

This stage coincides with maturation of adult worms and start of deposition of eggs. The patient may experience fever, rigors, headache, dry cough, muscle and joint pains, hepatosplenomegaly, abdominal pain, lymphadenopathy, skin rash, eosinophilia, and patchy pulmonary infiltrates on chest radiographs. Severe central nervous system involvement may occur. The diagnosis relies on serological tests and finding eggs in the excreta (Butterworth and Thomas 1999; Gryseels et al. 2006; Ross et al. 2007). Treatment consists of schistosomicidal drugs with or without steroids. Artemether treatment given early after exposure may decrease the risk of Katayama fever (Ross et al. 2007).

5.5.3 Stage of Established Infection

The eggs of *Schistosoma* deposited in the tissues result in granuloma formation. In *S. haematobium* infection, deposition of eggs in the wall of urinary bladder results in granulomatous inflammatory reaction and appearance of pseudotubercles. The patient suffers from cystitis and haematuria which is typically terminal but may be total. Haematuria is considered a normal developmental event in adolescent males in some regions of Africa. Symptoms of cystitis include suprapubic discomfort or pain, dysuria, and frequency (Bourée 2005). In *S. mansoni* and *S. japonicum*, deposition of eggs in the wall of the large intestine especially in the mucosa and submucosa results in granuloma formation and ulceration. The patient may suffer from chronic or intermittent abdominal pain and discomfort, loss of appetite, diarrhoea, and passage of blood and mucus in stool (schistosomal dysentery). This stage,

unfortunately, may be asymptomatic or oligo-symptomatic; thereby the disease may progress unchecked until complications supervene (Butterworth and Thomas 1999; Gryseels et al. 2006; Bustinduy and King 2014).

5.5.4 Stage of Late Infection and Sequelae

The complications are mainly due to healing of schistosomal lesions by fibrosis. The damage produced at this stage is irreversible due to organ damage by fibrosis and vascular changes.

5.5.4.1 Systemic Non-specific Morbidity

Research has recently focused on exploring the subtle and underrecognized yet significant morbidity during the course of chronic schistosomiasis with special emphasis on vulnerable groups such as children and pregnant females. Typically, these long-term subtle morbidities occur irrespective of the intensity of schistosome infections; yet, their expression levels are proportional to the intensity of infection (Fig. 5.3).

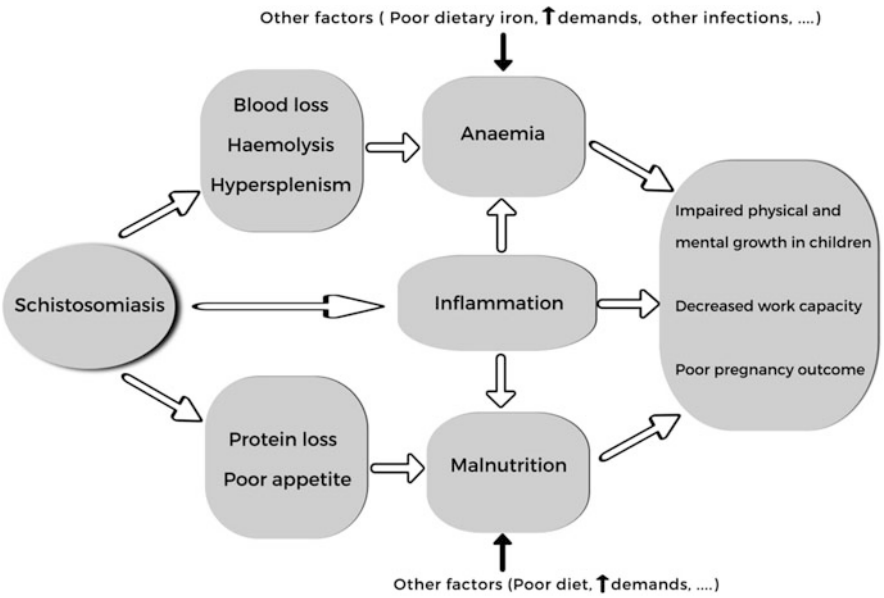

Fig. 5.3 Systemic manifestations of schistosomiasis

Chronic Anaemia

There is a plethora of reports on the association between schistosomiasis and anaemia. *Schistosoma*-induced anaemia prevails in populations with fragile nutritional status, especially those with poor dietary iron, such as growing children, adolescents, and pregnant women, and the cause is thought to be multifactorial. Elevated levels of pro-inflammatory cytokines, such as TNF-α, IL-1, and IL-6, as well as C-reactive protein, resulting in anaemia of inflammation, are deemed to play a central role (Coutinho et al. 2006; Butler et al. 2012). Moreover, for intestinal schistosome species, hypochromic anaemia may occur due to extracorporeal blood loss in the stool and consumption of blood by adult worms. Colonic polyposis also results in bleeding, protein loss, and anaemia. Hypersplenism, bleeding oesophageal varices, malnutrition, and autoimmune haemolysis are additional factors (Friedman et al. 2005; Bustinduy and King 2014). In schistosomiasis haematobium, anaemia may develop due to blood loss in urine (Friedman et al. 2005). Interestingly, *S. mansoni* SEA has been found to trigger erythrocyte cell death in an animal model, thus contributing to the development of anaemia (Kasinathan and Greenberg 2010). Anaemia in turn has been associated with reduced work capacity, reduced ability to execute daily activities, poor pregnancy outcomes, and cognitive impairment in children (Friedman et al. 2005).

Health authorities regard the prevention of anaemia as the most compelling reason to actively deworm children in schistosomiasis-endemic areas (Othman and Soliman 2015). Anaemia has also been proposed as a marker for morbidity reduction in schistosomiasis control programmes because it is one of the most easily assessed of the subtle morbidities and it can reflect the intensity of schistosome infection. However, in many endemic settings, it is difficult to dissect schistosome-associated anaemia from other causes of anaemia, especially malaria and soil-transmitted helminthiases (Valice et al. 2018). In two double-blind controlled studies, the haemoglobin levels increased over 6 months in all school-aged children living in a schistosomiasis-endemic community following PZQ therapy (McGarvey et al. 1996; Olds et al. 1999).

Impact on Children

Clinico-epidemiological evidence indicates that 10–15-year-old children, an age period of growth spurt and high dietary demands, typically show the peak prevalence and intensity of schistosomal infection as well as the highest exposure to inflammation-related disease associated with the infection. Growth deficits are well documented during chronic intestinal and urinary schistosomiasis, and these are likely related to the impact of schistosomiasis on the nutritional status of children (Osakunor et al. 2018). For example, an Egyptian study showed that all examined schistosome-infected schoolchildren showed hindered growth parameters in comparison to noninfected children in the form of low height, weight, and body mass index for age Z-score (Abdel-Motaleb et al. 2013). A Brazilian study demonstrated impaired growth parameters such as height, weight, and others in *S. mansoni*-infected children as compared to control children. The growth stunting was more evident in girls (Parraga et al. 1996).

Besides its impact on physical growth, schistosomiasis affects cognitive abilities of children as well as school performance (King and Dangerfield-Cha 2008; Ezeamama et al. 2018). Chronic schistosomiasis is associated with less than optimal school performance with increased school absenteeism (Gurarie et al. 2011). The study of Kimura et al. (1992) in Kenya showed that even the light schistosomal infection elicited negative effects on mental activities, which were detectable using simple mental tests. These changes were reversible with PZQ treatment. In Egypt, a study, investigating the effects of *S. mansoni* infection on cognitive functions of schoolchildren aged 9–12, demonstrated a significantly lower performance IQ as well as poorer performance on comprehension, vocabulary, and picture completion subtests on Wechsler Intelligence Scale for Children (WISC) and the verbal fluency test (Nazel et al. 1999). Moreover, Ezeamama et al. (2012) showed that school-aged children who were free of or cured from schistosomiasis japonicum for >12 months post-treatment scored higher in three of four cognitive tests. The authors suggest that sustained deworming and control for schistosomiasis could improve children's ability to take advantage of educational opportunities in helminth-endemic regions.

The association between schistosomiasis and growth impairment is complex with many factors at play, such as protein energy malnutrition and chronic anaemia. Extracorporeal blood loss, presence of ongoing inflammation, and poor appetite are all involved. Again the synergy with other growth-limiting factors such as poor diet and other infections is not overstated (Friedman et al. 2005; Ezeamama et al. 2012). True cases of infantilism are rare but described in the literature in severe cases of schistosomiasis. These are attributed, at least partly, to hypopituitarism with decreased levels of somatomedins (Bustinduy and King 2014).

No less alarming is the finding that, in young children, chronic prenatal exposure/sensitisation to helminth infection is associated with reduced efficacy of childhood vaccines through induction of a persistent Th2 response phenotype. Chronic exposure is also thought to be associated with environmental enteropathy, which affects the efficacy of vaccines at infancy (Osakunor et al. 2018). In addition, chronic schistosomiasis is thought to impair immunity against a variety of bacterial and viral childhood diseases (King 2010).

All these effects, taken together, have rendered schoolchildren the main target for population-based mass treatment. In fact, evidence from randomized chemotherapeutic intervention trials indicates that children had augmented physical fitness 1–2 months post-treatment; their skinfold thickness (a measure that assesses subcutaneous body fat) increased 1–8 months post-treatment, they gained weight 3–-12 months after treatment, and they exhibited better cognitive abilities 3 months post-treatment. At the same time, levels of haemoglobin were higher at a 6-month treatment follow-up (McManus et al. 2018).

Schistosomiasis and Pregnancy

It was estimated that schistosomes infect approximately 40 million women of childbearing age in endemic regions (Friedman et al. 2007). The question of the influence exerted by *Schistosoma* infection upon maternal health and birth outcomes has been recently raised. Experimental studies on *S. mansoni* have demonstrated the

detrimental effects of schistosome infection on the mother and offspring (el-Nahal et al. 1998a, b). Also, several anecdotal reports in humans have linked maternal schistosome infections with poor birth outcomes (Friedman et al. 2007). Moreover, two cross-sectional studies indicate that maternal infection by *Schistosoma* might be associated with low birth weight (Siegrist and Siegrist-Obimpeh 1992; Qunhua et al. 2000).

Several mechanisms were proposed to explain the negative impact of schistosomiasis on maternal, foetal, and neonatal health. First, placental inflammation may result with increased expression of pro-inflammatory cytokines in the placenta, contributing to intrauterine growth retardation. This inflammatory state may arise directly from deposition of eggs of *Schistosoma* or, more likely, indirectly via exposure to schistosome antigens (Kurtis et al. 2011). Second, schistosome-induced extracorporeal blood loss results in iron deficiency anaemia, detrimentally affecting maternal health and birth outcome. Finally, the anaemia of inflammation and anorexia are other contributing factors (Friedman et al. 2007).

Impact on Work Capacity
Reduced aerobic capacity (an impaired ability to deliver oxygen to the tissues) has been demonstrated in various epidemiological studies in *Schistosoma*-endemic areas both in children and in adults. This deficit, or relevant reduction in physical work capacity, is greatly correlated with chronic anaemia and malnutrition in patients with schistosomiasis (King et al. 2005; Bustinduy and King 2014).

5.5.4.2 Genitourinary Schistosomiasis

Obstructive Uropathy Healing of granulomatous lesions in the wall of urinary bladder and lower ends of ureters results in fibrosis and urinary obstruction. Various characteristic pathological lesions may appear such as ulceration, sandy patches, cystitis cystica, and calcification. The bladder may become rigid and contracted with reduced capacity. Moreover, urinary stasis predisposes to urinary tract infection and calculus formation which further aggravate the urinary obstruction. The end result is hydroureter and hydronephrosis. The progressive obstructive uropathy (Fig. 5.4) could ultimately lead to end-stage renal failure (Butterworth and Thomas 1999; Bourée 2005; Barsoum et al. 2013).

Cystoscopy may identify different lesions in the urinary bladder such as schistosomal pseudotubercles, nodules or masses, sandy patches, ulceration, cystitis cystica, fibrotic lesions, and malignant ulcers or masses. The technique is invasive and not needed in most patients (Barsoum et al. 2013). In contrast, ultrasound is a simple non-invasive method for evaluation of urinary schistosomiasis. With the exception of hydroureter, ureteral calculi, and bladder calcification, it has, in comparison with other diagnostic procedures, high specificity and sensitivity and is the best technique for grading hydronephrosis, urinary bladder wall lesions, and renal and bladder stones (Bustinduy and King 2014). WHO has published consensus

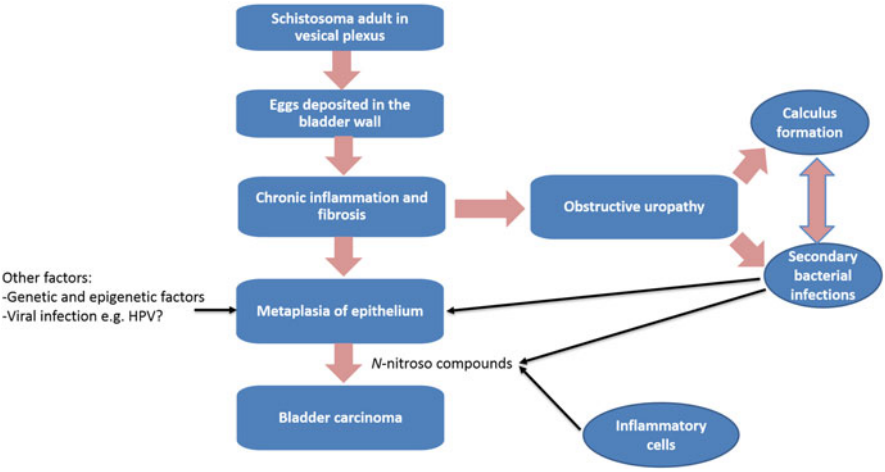

Fig. 5.4 Main pathogenetic events during urinary schistosomiasis. HPV, human papilloma virus (From: Ashour DS, Othman AA (2020) Parasite–bacteria interrelationship. Parasitol Res 119 (10): 3145–3164)

guidelines (Niamey protocol) for ultrasound examination in genitourinary schistosomiasis (Akpata et al. 2015).

Bladder Cancer *S. haematobium* is a biological carcinogen. The mucosa of the urinary bladder undergoes squamous metaplasia due to chronic inflammation. This predisposes to squamous cell carcinoma of the bladder. The typical histopathological lesion reported in many studies over the years is a squamous cell carcinoma in roughly 60% of cases. Other histological types include transitional cell carcinoma (20%), adenocarcinoma (10%), and mixed (10%). Schistosomal ova were detected in more than 85% of bladder cancers in an Egyptian series of 1026 cases subjected to surgical cystectomy (Ghoneim et al. 1997). The tumour, particularly when of the squamous cell type, remains localized for a long time before spreading to the surrounding pelvic tissues or to a distant site, owing to the occlusion of lymphatics by the fibrotic process (Barsoum et al. 2013).

Characteristically, *S. haematobium* extracts induce cancer-like phenotypes such as loss of p27, increased expression of Bcl-2, proliferation, inhibition of apoptosis, migration, and tumorigenesis in cultured epithelial cells (Botelho et al. 2009), and a carcinogenic and mutagenic activity on CD-1 mice normal bladders (Botelho et al. 2011). Several genetic and epigenetic abnormalities have been described in *Schistosoma*-associated bladder cancer (Khaled 2013). Moreover, concomitant bacterial and viral infections, rather than parasitic products, are suggested to be key factors in the pathogenesis of bladder cancer. Associated infection with human papillomavirus has received considerable recent attention in this respect, being encountered in about one-fourth of cases (Barsoum et al. 2013). Various schistosome-induced epithelial changes were attributed to the effect of inflammatory cell-generated reactive oxygen radicals, the cleavage of conjugated urinary

carcinogens, or the production of nitrosamines by bacterial enzymes (Khaled 2013) (Fig. 5.4).

Immune-Mediated Glomerulonephritis Immune-complex glomerulonephritis may supervene in all types of *Schistosoma* infection but most commonly with *S. mansoni*, and the basic lesion is mesangioproliferative or membranoproliferative glomerulonephritis. Immunofluorescence and electron microscopy reveal the presence of immune complexes containing IgM, IgG, IgA, IgE, complement components, and schistosomal antigens in the mesangium and along the endothelial side of the capillary wall (van Velthuysen and Florquin 2000). Genetic and environmental factors, e.g. chronic salmonellosis, are implicated in the pathogenesis of renal disease. The disease is manifested by proteinuria, hypertension, or nephrotic syndrome. It occurs years after the development of hepatosplenic disease, and it may be attributable, at least in part, to loss of immune-complex clearing function of hepatic macrophages due to portosystemic shunting. Amyloid deposition in the kidney occurs infrequently in all types of schistosomiasis and may lead to nephrotic syndrome (van Velthuysen and Florquin 2000; Barsoum et al. 2013).

Other Lesions Eggs may be deposited in the seminal vesicles, prostate, and spermatic cord in males leading to funiculitis, epididymitis, prostatitis, haemospermia, and rarely infertility. In females the eggs may reach the vagina, uterine cervix, and fallopian tubes resulting in inflammation and fibrosis that may predispose to infertility or ectopic pregnancy. Schistosomal lesions of the genital organs may increase chances of transmission of sexually transmitted pathogens including HIV. Moreover, lesions in the uterine cervix may be mistaken for carcinoma (Bourée 2005; Barsoum et al. 2013). Finally, *S. haematobium* may cause osteomalacia resulting from tubular lesions in association with obstructive uropathy (Butterworth and Thomas 1999).

5.5.4.3 Intestinal Schistosomiasis

Schistosomiasis mansoni causes patchy fibrosis of the wall of the intestine. Strictures, sinuses, and fistulae may occur. It may lead also to colonic polyposis resulting in bleeding, anaemia, protein loss, and hypoproteinemia. Polyps range in size from 2 to 20 mm and may be sessile, pedunculated, or showing a cauliflower appearance. They are mainly concentrated in the distal colon, and they range from few to very numerous. The overlying mucosa is usually redder than the surrounding mucosa due to severe congestion and due to focal haemorrhages. Ulceration is common in rectal polyps; the ulcerated areas appear dusky to blackish grey in colour caused by superficial haemorrhage and are frequently secondarily infected (El-Garem 1998; Elbaz and Esmat 2013).

S. japonicum affects similarly the large intestine as well as the stomach. *S. mekongi* gives manifestations that are similar to those of *S. japonicum* but usually milder. *S. intercalatum* infection is usually asymptomatic or causes mild intestinal

symptoms. The latter may also infrequently give rise to urinary manifestations (Bustinduy and King 2014).

There are contradictory reports on the contribution of *S. japonicum* to the etiopathogenesis of colorectal or primary liver cancer (Bustinduy and King 2014). The situation is more ambiguous for *S. mansoni*. The anecdotal reports of association of schistosomiasis mansoni and colorectal carcinoma do not differentiate between direct causal relationship and mere accompaniment (Salim et al. 2010). To date, the carcinogenicity of both species is not proved, and the issue awaits further well-designed research for clarification.

5.5.4.4 Hepatosplenic Schistosomiasis

In case of *S. mansoni* and *S. japonicum*, more than 50% of eggs are not passed in the faeces and are retained in the tissues. Eggs swept to the liver via the portal circulation induce granuloma formation and periportal hepatic fibrosis, ending, in some cases, in presinusoidal portal hypertension (Fig. 5.5). The liver is initially enlarged and non-tender with firm smooth surface, but may become shrunken in the advanced stages. Portal hypertension leads to opening of portosystemic anastomotic venous channels resulting in oesophageal (and gastric) varices, secondary haemorrhoids, and ascites. Bleeding from oesophageal varices may lead to fatal haematemesis and/or melena.

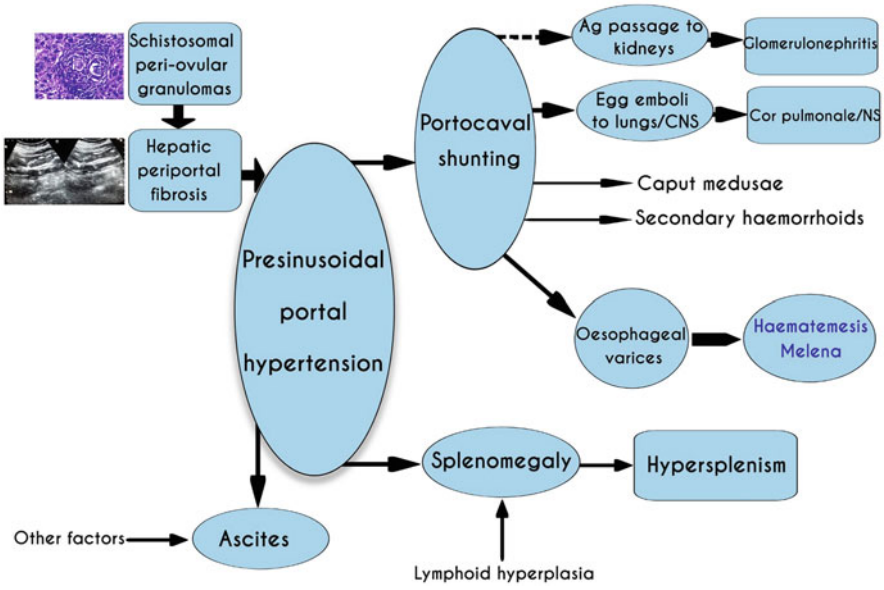

Fig. 5.5 Pathogenesis of hepatosplenic schistosomiasis. Ag: Schistosomal antigens; NS: Neuroschistosomiasis

Splenomegaly occurs due to passive congestion secondary to portal hypertension, and reticuloendothelial and lymphoid hyperplasia. The spleen is markedly enlarged, firm, smooth, and non-tender. A huge spleen may cause discomfort and dyspepsia, or pain in case of perisplenitis or infarction. Hypersplenism may also develop (Butterworth and Thomas 1999; Elbaz and Esmat 2013; Bustinduy and King 2014).

Radiographic studies indicate amputation of the large portal veins, development of collateral veins, arterioportal venous shunts, and diminished hepatic arterial diameters (Da Silva and Carrilho 1992). When portal fibrosis is established, with its associated distortion of vascular architecture, the incoming eggs can pass through collateral veins around the large portal veins and via the granuloma/fibrosis sequence; this results in progressively expanding tracts of collagen that characterize the clay-pipestem pattern of fibrosis, originally described by Symmers (Lucas 2002). *S. japonicum*, owing to smaller size of its eggs, produces periportal fibrosis that affects the peripheral and central zones of the liver, whereas *S. mansoni* affects only the central zones (Burke et al. 2009).

Hepatic function is well preserved in pure schistosomal fibrosis until very late. Decompensated hepatosplenic disease with stigmata of liver cell failure, especially ascites, may supervene. This may occur due to malnutrition, marked collagen deposition in the space of Disse, ischaemic damage from repeated variceal bleeding, and severe distortion of arteriovenous relationships in the portal tracts. Alcohol abuse and viral hepatitis types B, C, D, and E are additional complicating factors (Watt et al. 1991; Bustinduy and King 2014).

Fibrosis is the ultimate sequel and a major culprit of chronic hepatic schistosomiasis. Apparently, hepatic stellate cells (HSCs) play a major role in the process of fibrous tissue formation in the liver. They are responsible for synthesis of components of extracellular matrix and several types of collagen as well as fibrogenic cytokines, matrix metalloproteinases, and tissue inhibitors of metalloproteinases that contribute to the remodelling of fibrous tissue (Parola and Robino 2001). HSCs reside in the spaces of Disse of the liver sinusoids (Fig. 5.6), and they constitute a minor cell type, roughly 5–8% of the total liver cells (Maubach et al. 2006).

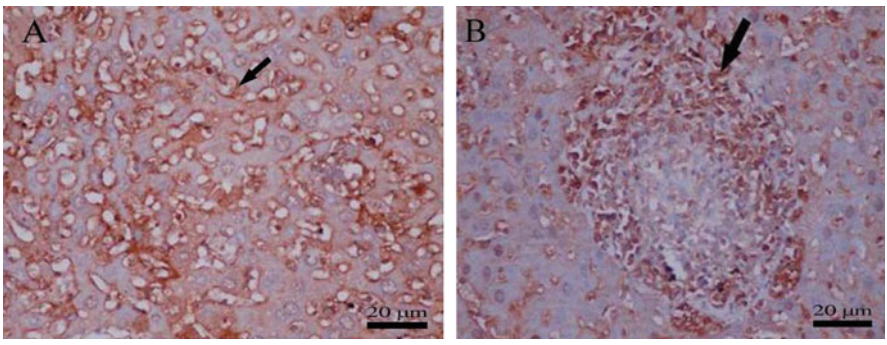

Fig. 5.6 Hepatic immunohistochemical stain for HSCs in *Schistosoma*-infected mouse showing (**a**) sinusoidal "arrow", (**b**) mesenchymal "arrow" HSCs (Glial fibrillary acidic protein (GFAP) immunoperoxidase ×400)

Following chronic injury, HSCs differentiate into myofibroblast-like cells, acquiring contractile and fibrogenic properties (Zhang et al. 2006). Remarkably, HSCs affect adversely the hepatic microcirculation. When activated, they transform into myofibroblasts that contract around the hepatic sinusoids, increasing the vascular resistance and contributing to portal hypertension (Friedman 2000). The different stimuli that initiate and perpetuate HSC activation in chronic liver disease are poorly understood, but the role of oxidative stress seems crucial. This explains the multitude of trials of administration of exogenous antioxidants (e.g. coenzyme Q10, melatonin, vitamin E, silymarin, and molecular hydrogen) in an attempt to reverse or limit *Schistosoma*-induced liver fibrosis (Gharib et al. 2001; El-Sokkary et al. 2002; Othman et al. 2008).

Not all *Schistosoma*-infected people have the propensity to develop serious periportal hepatic fibrosis; only some unlucky individuals develop severe hepatic fibrosis with its attendant sequelae. Several immunological and non-immunological determinants govern the progression of schistosomal fibrosis. These determinants were recently reviewed by Kamdem et al. (2018). The severity of liver fibrosis is dependent upon the relative weight of several opposing pro- and antifibrotic drives. Interestingly, the genetic constitution of the host was found to determine the propensity to fibrosis. In Egypt, patients with human leucocyte antigen (HLA)-A1 and HLA-B5 have been associated with severe hepatosplenic schistosomiasis mansoni, whereas in the case of *S. japonicum* infection, HLA-DR and HLA-DQ have been implicated in the differential regulation of immune responses to egg antigens (Butterworth and Thomas 1999). Positive and negative associations between different HLA class II alleles and the susceptibility of patients to develop moderate to severe hepatic fibrosis following *S. japonicum* infection have been described (Kamdem et al. 2018).

The immunological factors include several cytokines and cytokine receptors; chemokines; growth factors; immunoglobulins; and cells such as eosinophils, macrophages, and Tregs. As noted before, T helper 2 cytokines such as Il-4, IL-5, and IL-13 as well as TNF-α exert potent profibrotic force on HSC, whereas regulatory cytokines IL-10 and Il-12 have the opposite effect (Henri et al. 2002; Wilson et al. 2007; Othman et al. 2010). INF-γ is a potent antifibrotic agent, and higher levels were associated with a marked reduction of the risk of fibrosis during schistosomiasis in Sudanese patients (Henri et al. 2002). INF-γ inhibits the transdifferentiation of HSCs, reduces the production of matrix proteins, and increases the collagenase in the liver (Mallat et al. 1995). Similarly, IL-6 is found to be protective against severe schistosomal fibrosis (Mutengo et al. 2018). CCL3 and CCL24 are two chemokines that exhibit profibrotic effects, whereas the chemokine RANTES (CCL5) has the opposite effect (Kamdem et al. 2018). Osteopontin is a profibrogenic cytokine that is recently identified as crucial for granuloma formation and development of fibrosis (Pereira et al. 2015). Alternatively activated macrophages (aaMΦ) are found to contribute to schistosome-induced fibrosis in murine schistosomiasis (Wynn et al. 2004; Wilson et al. 2007; Stavitsky 2004). Alternative activation of macrophages is induced by Th2 responses and promotes collagen synthesis and fibrogenesis via the metabolism of L-arginine to proline and polyamine by arginase-1. Finally, the

eosinophil cationic protein of eosinophils is thought to be positively associated with the progression of fibrosis during schistosomiasis (Eriksson et al. 2007). Some of these factors or their inhibitors could be potential targets for development of antifibrotic therapies.

Research has indicated that over time schistosomal fibrosis becomes established owing to cross-linking of collagen fibres, rather than the difference in collagen isotypes deposited early or late in infection (Ricard-Blum et al. 1992). When the fibrosis is recent in early infection, a process of collagen breakdown occurs with therapy. This breakdown is almost complete, as well as rapid and abrupt. On the other hand, long-standing fibrosis in chronic infection could also undergo regression with therapy, but the process is slow and gradual. Regression of fibrosis entails collagen degradation and vascular remodelling which includes neovascularization (Andrade 2008). The process of regression of schistosomal fibrosis has been demonstrated both in humans and experimental animals. In humans, it can be revealed by regression of splenomegaly and oesophageal varices.

Ultrasonography has greatly facilitated the assessment of schistosomal hepatic fibrosis, splenomegaly, portal vein dimensions, and the presence of collateral vessels (Lambertucci et al. 2008). It helps to assess the degree of periportal fibrosis by measuring portal tract thickness: Grade I if thickness is 3–5 mm, Grade II if it is 5.1–7 mm, and Grade III if it is more than 7 mm. This method reflects the hemodynamic changes and provides a good estimate of the clinical status of patients who have periportal fibrosis (Abdel-Wahab et al. 1992). Notably, in an attempt to harmonize the practices in clinical trials and evaluation of therapeutic measures, WHO elaborated a standard ultrasound scoring protocol (Niamey-Belo Horizonte) which includes a qualitative assessment of liver parenchyma (according to reference patterns A to F) (King et al. 2003). Portal hypertension is suspected when dilatation of one or more of the portal, mesenteric, and splenic veins is detected. For the collateral vessels, the most commonly described are the left and right gastric, short gastric, parumbilical, splenointercostal, and splenorenal veins (Lambertucci et al. 2008; Pinto-Silva et al. 2010). Lastly, the hepatic veins in schistosomiasis can be assessed ultrasonographically. They remain patent with normal phasic flow as the disease evolves, which is different from liver cirrhosis (Elbaz and Esmat 2013).

Unlikely to replace ultrasonography, biological markers can play an important role in assessing the progress of liver fibrosis and portal hypertension and the efficacy of therapy. Direct markers are less useful because their measurement is costly and not readily available. These include procollagen type I and type II, collagen type IV, and laminin (Domingues et al. 2011). Hyaluronic acid (HA) is one of the best direct biomarkers. It is a component of the extracellular matrix and is synthesized by HSCs. Two studies by Pascal et al. (2000) and Eboumbou et al. (2005) have confirmed that serum HA levels rise in advanced form of schistosomal liver disease. In contrast, being available in all laboratories at reasonable cost, the indirect markers are more practical. They include platelet count, aspartate aminotransferase to platelet ratio (APR) index, specific IgG4 level, and gamma-glutamyl transpeptidase (Domingues et al. 2011). The reduction of platelet counts correlates with the progress of liver fibrosis, and a count of 130,000/μl can accurately

differentiate between patients with and without portal hypertension (Souza et al. 2000). The enhanced liver fibrosis (ELF) test has been found useful in assessment of schistosomal liver disease. ELF is a blood test, measuring HA, procollagen type III, and tissue inhibitor of metalloproteinase 1 (Olveda et al. 2017).

MicroRNAs (miRNAs) are small non-coding RNAs (18–25 nt), which act as subtle gene expression regulators of a variety of cellular processes, including fibrogenesis (Cai et al. 2018). Recent research indicates that miRNAs play a role in schistosomiasis, in at least three domains. First, miRNAs are involved in the pathogenesis of schistosomal liver fibrosis. Dysregulation of miRNAs in liver tissue during schistosome infection has been reported in mice and human subjects (Cai et al. 2013; Cabantous et al. 2017). Typically, MiR-12 and miR-96 enhance schistosomiasis-associated liver fibrosis through activation of the SMAD signaling pathway. MiR-351 is also a profibrogenic agent during schistosomiasis, targeting the vitamin D receptor. In contrast, other miRNAs such as miR-203-3p exert an antifibrotic effect in schistosomal liver disease (Chen et al. 2019). Second, circulating miRNAs are potentially useful as biomarkers for the progress of schistosomal liver fibrosis: four miRNAs (miR-150-5p, let-7a-5p, let-7d-5p, and miR-146a-5p) were able to distinguish patients with mild versus severe fibrosis. The diagnostic performance of miR-150-5p in discriminating mild from severe fibrosis is similar to serum HA level (Cai et al. 2018). Finally, these agents are potential targets for therapy via the delivery of their antagonists or mimics. They require a vehicle for their delivery inside the cells such as vectors or nanoparticles. After the era of the large-scale vaccination against COVID-19 using nucleic acid constructs, the use of these agents in human therapy may be witnessed in the near future.

Interactions with Viral Hepatitis Coinfection with either hepatitis B virus (HBV) or hepatitis C virus (HCV) is very common since the regions with a high prevalence of schistosomiasis usually have a high endemicity of chronic viral hepatitis as well (Elbaz and Esmat 2013). An important cause of the extraordinary high prevalence of HCV in Egypt was the establishment of a large reservoir of infection as a result of the mass parenteral chemotherapy campaigns against schistosomiasis that ended in the 1980s (Frank et al. 2000). The association between both schistosomiasis and HCV is known to cause earlier deterioration of hepatic functions and severer morbidity. The liver is the principal site for both HCV replication and egg deposition. The latter downregulates the local immune responses in the liver (Lundy et al. 2001) and results in suppression of the intrahepatic bystander immune response to HCV. Moreover, this coinfection can produce a unique clinical, virological, and histological pattern manifested by viral persistence with high HCV RNA titres, higher necroinflammatory and fibrosis scores in liver biopsy specimens as well as poor response to interferon therapy, and rapid progression of liver fibrosis (Kamal et al. 2006; Omar 2019).

5.5.4.5 Neuroschistosomiasis

Although uncommon in comparison to the total toll of schistosomal disease, neuroschistosomiasis is not uncommon and is probably underrecognized. Spinal cord schistosomiasis, especially due to *S. mansoni*, is considered as a primary cause of spinal cord parasitic infection in Egypt (Badr et al. 2011).

CNS disease may occur in all *Schistosoma* spp. during the initial stage of maturation (acute schistosomiasis) particularly in case of *S. japonicum*. The disease tends to be more common and severe in non-endemic exposed individuals. It is probably due to immunologically mediated vasculitis. In the chronic stage, the pathology is due to passage of eggs of *Schistosoma* to CNS where they induce space-occupying granulomatous reactions (Ferrari et al. 2008; Ferrari and Moreira 2011).

Schistosoma eggs (or occasionally adults) may reach the CNS via two routes: the first is through Batson's vertebral venous plexus which connects the portal venous system and inferior venae cavae to the spinal cord and cerebral veins. This route permits both anomalous migrations of adult worms in copula to sites close to the CNS followed by in situ deposition and, occasionally, massive embolization of eggs from the portal mesenteric and pelvic venous system towards the CNS. High intra-abdominal pressure, e.g. during defecation and coughing, increases chance of retrograde flow. The second route is via the arterial system either directly as in *S. haematobium* or after development of portocaval anastomoses secondary to portal hypertension in *S. mansoni* (Katchanov and Nawa 2010; Ferrari and Moreira 2011).

In the chronic stage of infection, cerebral disease is more common in *S. japonicum* than in other species and most commonly presents with seizures. On the other hand, *S. mansoni* and *S. haematobium* (to a lesser extent) affect more commonly the spinal cord leading to paresis, radicular symptoms, sphincteric problems, and cauda equina syndrome (Hughes and Biggs 2002). Schistosomal myelopathy has been reported to occur 38 days to 6 years after infection and is acute or subacute with rapidly progressive neurological deficit over the first 24 h. In a review of 26 patients with spinal cord disease, 11.5% died, 34.6% remained paraplegic, and 54% showed moderate to good improvement with therapy (Scrimgeour and Gajdusek 1985).

The diagnosis relies on clinical presentation, demonstration of schistosomal infection by microscopy and serological methods, imaging features, and finally exclusion of other causes of myelopathy (Lambertucci et al. 2008). CSF examination may be normal but may show increased protein and pleocytosis in the majority but eosinophilia in only one-quarter of cases. Eggs were never found in CSF (Hughes and Biggs 2002). Histopathology of specimens obtained after surgical removal or biopsy provides definitive diagnosis.

In cerebral disease, CT and MRI usually show a non-specific tumour-like lesion surrounded by oedema, associated with mass effect and heterogeneous contrast enhancement. The borders are often irregular and poorly defined. Further, MRI is very sensitive in the detection of abnormalities in patients with spinal disease, but the

alterations are non-specific. The most common findings are signal hyperintensity on T2-weighted images, enlargement of the spinal cord (particularly lower cord and conus medullaris), thickening of the spinal roots (especially cauda equina roots), and a heterogeneous pattern of contrast enhancement on T1-weighted images (Silva et al. 2004; Lambertucci et al. 2008).

5.5.4.6 Cardiopulmonary Schistosomiasis

Embolization of eggs into the capillary bed of the lungs may occur via systemic circulation in *S. haematobium* infection, and in intestinal species especially *S. mansoni* after development of portosystemic venous anastomoses secondary to portal hypertension. In an early study in Upper Egypt, pulmonary schistosomiasis was associated with *S. haematobium* infection in 58% of cases, *S. mansoni* infection in 31% of cases, and mixed infection in 11% of cases (Shaw and Ghareeb 1938). Furthermore, the prevalence of pulmonary hypertension in the patients with schistosomal liver fibrosis was found to be 10.7% in one Brazilian study (Ferreira et al. 2009).

The eggs induce pulmonary granuloma formation and fibrosis, and vascular changes occur in the adjacent vessels, resulting in necrotizing arteritis, thrombi in the affected vessels, medial hypertrophy, and intimal proliferation. Plexiform lesions develop in severe cases where dilated arterioles and venules are detected. The loss of vascular structures can lead eventually to pulmonary hypertension with or without cor pulmonale (Gutierrez 2000). Clinically, the patient may complain of fatigue, exertional dyspnoea, chest pain, cough with occasional haemoptysis, oedema, and later central cyanosis and clubbing of fingers. Electrocardiographic and radiological abnormalities are observed.

5.5.4.7 Coinfection of *Schistosoma* and Endemic Pathogens

***Schistosoma–Salmonella* Interactions** The morbid association between *Schistosoma* spp. and *Salmonella* has long been recognized where entero-invasive salmonellae reach and attach to the integument or gut of adult schistosomes via the circulation (LoVerde et al. 1980; Melhem and LoVerde 1984; Bustinduy and King 2014). The schistosomes apparently serve as a safe intravascular niche in which *Salmonella* can evade systemic antibiotic therapy. The bacteria bind to schistosomes by means of a specific fimbrial protein (FimH) present on the surface of the bacteria. This same fimbrial protein is the molecular mechanism by which *Salmonella* can bind to mammalian cells (Barnhill et al. 2011). Chronic bacteraemic salmonellosis is an individualized clinical entity characterized by prolonged fever with enlargement of the liver and spleen that occurs in *Schistosoma*-infected individuals who are coinfected with *Salmonella*.

Moreover, administration of a schistosomicidal agent can result in a massive release of schistosome-dwelling salmonellae causing bacteraemia if the appropriate

antibacterial drug is not co-administered (Melhem and LoVerde 1984; Barnhill et al. 2011). This may explain the severe side effects observed infrequently after PZQ treatment in some patients. Finally, the use of ineffective antibiotics or failure to cure associated *Schistosoma* infection may lead to bacterial persistence and development of bacterial resistance to chemotherapy (Barnhill et al. 2011).

Schistosomiasis and Malaria Research indicates that *Schistosoma* infection upregulates the protective antimalarial immune response; therefore, children coinfected with schistosomiasis have fewer symptomatic malarial attacks (Wilson and Khalife 2012). Most of the coinfection studies focused on IgG3 directed towards the two parasites. Interestingly, it was found that IgG3 response is specific to *Plasmodium falciparum* schizont (PFS) while IgG3 to SAWA and SEA are due to cross-reactivity with PSF. In other words, IgG3 levels to antimalarial antigens are higher in patients coinfected with *Schistosoma*, accounting for the less severe malarial paroxysms (Naus et al. 2003).

INF-γ and TNF-α involved in pro-inflammatory responses are increased in the plasma of coinfected individuals. Moreover, IL-10 showed higher levels in schistosome/malaria coinfection than in case of single infection. The increased level of this regulatory cytokine reflects the immediate need for controlling the damaging pro-inflammatory responses. As a result, it reduces cellular adhesion molecules, leading to protection from cerebral pathology in case of coinfection (Diallo et al. 2010).

Immuno-epidemiological studies showed that Th2 immune response to SEA is lower in children coinfected with malaria. Similar results were detected in experimental studies where lower levels of IL-4 and IL-5 were produced by splenocytes from mice with schistosome/malaria coinfection. Moreover, mice infected with *P. berghei* have smaller schistosomal liver granulomas and reduced eosinophil recruitment compared to non-malaria-infected mice (de Jesus et al. 2004). Likewise, a recent study in Burkina Faso revealed lower levels of *S. haematobium*-specific anti-SEA IgG in a particular population known to be malaria-resistant (Mangano et al. 2020).

On the other hand, the incidence of hepatosplenomegaly in many children where *S. mansoni* and malaria are co-endemic is increased as a response to the higher plasma levels of pro-inflammatory cytokines. Therefore, it is important to perform well-designed combined field and laboratory studies to identify the impact of coinfection on disease progress and severity (Wilson and Khalife 2012).

***Schistosoma* and Soil-Transmitted Helminths** In sub-Saharan Africa, which is endemic for schistosomiasis, the greatest number of soil-transmitted helminth (STH) infections occurs particularly in preschool-aged and school-aged children. Schistosomiasis-STH infection co-endemicity is commonly reported in sub-Saharan countries (Njaanake et al. 2016; Molvik et al. 2017).

The occurrence of both schistosomiasis and STH including *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms is highly related to the environment and population behaviours such as limited access to safe water, sanitary facilities, or adequate health facilities (Boko et al. 2016; Dejon-Agobé et al. 2020).

Immunological studies from Brazil and Venezuela reported that sera from patients infected with hookworms and/or *A. lumbricoides* showed a significant degree of cross-reactivity with antigens from *S. mansoni*. For example, peripheral blood mononuclear cells from mono-infected hookworm or *A. lumbricoides* patients readily responded to stimulation with schistosomal antigens in patients from a schistosomiasis endemic area (Corrêa-Oliveira et al. 2002). However, the degree of the humoral immune response against schistosomal antigens was not altered in schistosomiasis patients with a hookworm coinfection when compared with a *S. mansoni* mono-infection (Webster et al. 1997). Therefore, further immunological investigations of patients coinfected with multiple helminth species are required.

***Schistosoma* and HIV** Africa has approximately 70% of global HIV infections and 90% of global schistosome infections. Among approximately 36.7 million HIV infections worldwide, an estimated 6 million individuals are schistosome coinfected (Downs et al. 2017; Colombe et al. 2018).

In coinfected individuals, immunological studies suggested that chronic HIV-1 infection could affect *S. mansoni*-related morbidities, resulting in differences in the prevalence and intensity of *S. mansoni* infection, the efficiency of parasite egg excretion, and the response to anthelmintic treatment (Kallestrup et al. 2005, 2006; Secor 2012). However, Mazigo et al. (2013) showed that HIV-1 infection may not affect *S. mansoni* disease development and excretion of eggs from the coinfected individuals in spite of the reduction in CD4+ cell counts in HIV-infected patients.

Four different cross-sectional studies from Tanzania and Zimbabwe had shown approximately threefold increase of HIV-1 infection in women with schistosomiasis compared to non-schistosomal infections (Kjetland et al. 2006; Downs et al. 2012, 2017; Brodish and Singh 2016). Local physical and immunological changes caused by schistosome eggs in the mucosal tissue of the vagina and cervix are thought to increase susceptibility to the virus during sexual HIV exposure (Kallestrup et al. 2005; Jourdan et al. 2011). An experimental study on *S. mansoni*-infected rhesus macaques showed an increase in the replication of HIV and faster disease progression than in animals without *S. mansoni* infection (Ayash-Rashkovsky et al. 2007). Moreover, Colombe et al. (2018) suggested that schistosome infection could impair antiviral immune response.

On the other hand, a community-based study in Tanzania from 2006 to 2017 reported that people with schistosome infection at the time of HIV seroconversion developed adverse HIV outcomes more slowly than those without (Colombe et al. 2018). Moreover, some studies have reported transitory increases in HIV-1 viral loads following treatment of schistosome infections (Brown et al. 2005; Kallestrup et al. 2005). Thus, the protective effect of schistosomes against HIV was suggested. One possible immunological mechanism is the induction of Th17 and Tregs by chronic schistosome infection that leads to delayed HIV-1 disease progression (Colombe et al. 2018). Still, more immunological and epidemiological studies have to be conducted to explore further possible interaction between HIV-1 and *Schistosoma* infections.

Schistosomiasis and COVID-19 Coronavirus disease 2019 (COVID-19), which became a pandemic early in the year 2020, has caused a significant global morbidity and mortality (Singhal 2020). The disease is currently rapidly spreading in many African countries most affected by the neglected tropical diseases (NTDs) with over five million active cases and about 600,000 deaths (Oyeyemi et al. 2020). Therefore, concomitant COVID-19 infection with schistosomiasis and parasitic NTDs might have a significant impact on the risks and severity of clinical manifestations of COVID-19 (Ludvigsson 2020).

Th2 and regulatory immune response in schistosomiasis limit the pro-inflammatory cytokine-related immunopathology and may impair immunity to COVID-19, thus resulting in increased susceptibility and higher incidence of COVID-19 in schistosomiasis-endemic areas of Africa, and may result in treatment failure in COVID-19 active cases (Oyeyemi et al. 2020).

In contrast, Ssebambulidde et al. (2020) demonstrated an inverse correlation between the number of COVID-19 cases and deaths and the endemicity of parasitic infections globally in Africa. They showed that COVID-19 cases were reduced with endemicity of malaria, schistosomiasis, or STH infections, suggesting that these parasitic infections may confer some protection against COVID-19. In other words, lower proportion of COVID-19 confirmed clinical cases and deaths in Africa may be related to the immunomodulatory effects occasioned by parasitic infections endemic in this region resulting in improvement of COVID-19 clinical severity.

Surprisingly, better COVID-19 outcomes have been observed in countries with at least 75% coverage of schistosomiasis MDA of PZQ, sustained for at least the past 10 years, suggesting the possible role of schistosomiasis preventive chemotherapy in modulating the immune system against SARS-CoV-2 (Oyeyemi et al. 2020).

On the other hand, WHO COVID-19 guidelines recommend countries to postpone the ongoing NTD intervention programmes including those for schistosomiasis (World Health Organization 2021). Therefore, it is important to note that we might encounter an increase in *Schistosoma* infections in the post-COVID era. Many factors are involved such as the postponement of MDA, especially after closure of schools because school-aged children are the main targets of MDA. In addition, PZQ production and supply chains of MDA may be disrupted due to travel restrictions (Kura et al. 2021).

5.6 Diagnosis

5.6.1 Parasitological Techniques

Detection of eggs in stool or urine remains the "gold standard" for schistosomiasis diagnosis despite its lack of sensitivity, because of its unsurpassed level of specificity (Bärenbold et al. 2021; Chen et al. 2021). The shape, size, and spine of the eggs of the three major schistosome species are useful diagnostic features. The eggs of *S. japonicum* are round with a reduced lateral spine and are smaller in size (60 by

100 mm) than those of *S. mansoni* (61 by 140 mm; prominent lateral spine) and *S. haematobium* (62 by 150 mm, prominent terminal spine), which are both ovoid. The 10 ml urine sedimentation or filtration techniques are reliable field diagnostic methods for *S. haematobium* (Dazo and Biles 1974). The Kato–Katz technique (Endriss et al. 2005) remains the method of choice for diagnosis of intestinal schistosomiasis, despite its low sensitivity in areas of low endemicity and the conspicuous intra- and inter-specimen variation of egg distribution and aggregation in faeces (Krauth et al. 2012). However, Berhe et al. (2004) reported that "examination of five Kato−Katz thick smears from one stool specimen using 41.7 mg template or three Kato−Katz thick smears from one stool specimen, and if these are negative, followed by examination of additional triplet Kato−Katz thick smears from subsequent day stool specimen can adequately assess individuals for infection status with *S. mansoni*". Similar findings were recorded for *S. japonicum* parasitological diagnosis (Lin et al. 2008; Utzinger et al. 2011). The formol-ether concentration technique (Allen and Ridley 1970) is slightly more sensitive than triplet Kato–Katz slides (Ebrahim et al. 1997) but less sensitive than the FLOTAC method (Cringoli 2006), which is recommended for highly accurate coprological diagnosis and for ascertaining cure following chemotherapy (Glinz et al. 2010; Utzinger et al. 2011). The Percoll separation technique (Eberl et al. 2002) could be used in hospitals and laboratories dedicated to research and clinical investigations.

In light infection, examination of rectal mucosa snips taken with a curette through a proctoscope is more sensitive than stool examination. Material thus obtained is examined as a crush preparation. Viability of eggs is confirmed by movement of the cilia of flame cells or of the miracidia within the egg shell (Bustinduy and King 2014).

5.6.2 Immunological Techniques

Schistosomes elicit immune responses immediately upon host skin invasion, and during development, maturation, copulation, and egg-laying in blood vessels. Accordingly, antibody responses to different SAWA or SEA or selected antigens, e.g. species-specific microsomal antigens, cathepsin B, etc., have been used for immunodiagnosis by indirect hemagglutination assay (IHA), enzyme-linked immunosorbent assay (ELISA), and/or dipstick dye immunoassay, all of which showed high sensitivity (Xiang et al. 2003; Zhou et al. 2007, 2008, 2011b; Cai et al. 2019). IHA is among a few commercial serodiagnostic kits for schistosomiasis and was shown to be ideal for assessing IgM and IgG responses in travellers to, but not residents of, endemic regions (Van Gool et al. 2002; Yu et al. 2007; Zhou et al. 2007, 2008; Zhao et al. 2012). Indeed, IHA showed a sensitivity of 57.45% and specificity of 48.10% in detection of active *S. mansoni* infection among 173 Egyptian adults (Saftawy 2021). Antibody detection methods do not allow discrimination between current and previous infection or reinfection, and are thus not useful for assessing follow-up after chemotherapy (Zhou et al. 2011b).

Immunodiagnostic methods based on antigen detection have overcome some of these limitations. Worm antigens and egg-derived molecules are readily detectable in serum or plasma, faeces, urine, and even saliva, provided the availability of specific capture and detecting antibodies (Cai et al. 2017; Qokoyi et al. 2021). Such immunodiagnostic methods are of importance not only for assessment of cure after treatment, but even more importantly as the most reliable way for diagnosis of chronically infected patients who fail to excrete eggs in stool or urine (Enk et al. 2008; Zhou et al. 2011b). Research has primarily focused on two glycoproteins, circulating anodic antigen (CAA) and circulating cathodic antigen (CCA), which are released from the gut of viable developing larvae and adult worms, and may, thus, provide information on active infection and intensity (Van Dam et al. 1996). The assays require provision of CAA- and CCA-specific antibodies (Al-Sherbiny et al. 1999); yet, commercial kits are becoming available and showed exquisitely high sensitivity and specificity for diagnosis of *S. mansoni* but not *S. haematobium* (Coulibaly et al. 2013; Erko et al. 2013; Utzinger et al. 2011). Urine-CCA cassette test has recently showed *S. mansoni* high diagnostic specificity (91.14%); yet sensitivity was remarkably low (Saftawy 2021). Serum and urine CAA assays are being continuously improved for reliable diagnosis of active *S. mansoni* and *S. haematobium* infections in low-prevalence settings (Corstjens et al. 2020).

5.6.3 Molecular Techniques

Conventional or real-time polymerase chain reaction (PCR) amplification of repetitive elements specific to each schistosome species has been used for diagnosis of *S. mansoni* (Pontes et al. 2003; Allam et al. 2009), *S. haematobium* (Obeng et al. 2008; Aryeetey et al. 2013), and *S. japonicum* (Xu et al. 2010; Zhao et al. 2012) using stool, urine, or serum samples. The statistical method of latent class analysis (LCA) that included Kato–Katz technique (two thick stool smears), urine-CCA cassette assay, and schistosome-specific real-time (Rt)-PCR determined that the Rt-PCR test had a sensitivity of 98.7% and the highest specificity (81.2%) in detection of *S. mansoni* infection in school-aged Tanzanian children (Fuss et al. 2018). This method was, however, found to be highly reliable in diagnosis of S. *mansoni* in serum, but not urine, of adult residents in endemic areas before and after chemotherapy (Fuss et al. 2020). Recently, a DNA dipstick technology was developed allowing successful, speedy, and simple detection of DNA from adult worms, eggs, and infected snails, justifying considering this method for point-of-care detection of *S. japonicum*, and other schistosomes (Aula et al. 2021). Simple, one-tube, Rt-PCR targeting DNA repeats Dra1 of *S. haematobium* and Sm1–7 of *S. mansoni* in human serum has recently proved highly specific and sensitive in schistosomiasis diagnosis (Frickmann et al. 2021).

5.6.4 Other Useful Non-specific Tests

The frequency of haematuria and proteinuria among individuals with urinary schistosomiasis has led to the use of urine dipsticks for detection of microhaematuria. The test is found to be 90% sensitive and hence a cost-effective means for estimating the prevalence of *S. haematobium* infection (Bustinduy and King 2014; Osakunor et al. 2018). Faecal occult blood (FOB) test depends on the presence of cryptic blood in stool, resulting when *S. mansoni* eggs penetrate the bowel mucosa and cause a slight discharge of blood into the lumen of the gut. Another marker for intestinal schistosomiasis is calprotectin which is released by leucocytes in response to the attendant inflammation (Osakunor et al. 2018). FOB and calprotectin have been evaluated in children in Uganda (Betson et al. 2010, 2012). In these studies, FOB and calprotectin seem to correlate positively with *S. mansoni* infection before and after treatment. Notably, blood eosinophilia is marked (>20%) during the acute stage of schistosomiasis but diminishes significantly and becomes inconstantly low in the chronic phase.

5.7 Treatment of Schistosomiasis

5.7.1 Schistosomicidal Agents

5.7.1.1 Praziquantel

PZQ is a pyrazino-isoquinoline derivative that is practically insoluble in water, sparingly soluble in ethanol, but very soluble in chloroform and dimethylsulfoxide. PZQ is the mainstay of antischistosomal therapy; it is effective against all five species of human schistosomes in a single oral dose of 40–60 mg/kg body weight, leading to cure rates of 60–90% in different epidemiological settings (Davis 1993; Kumar and Gryseels 1994). It has the great advantage of being administered as a single oral dose. Other advantages include high efficacy, excellent tolerability, few and transient adverse effects, low cost, effectiveness in mass treatment control programmes, and broad therapeutic profile (Abaza 2013). WHO includes it on their list of essential medicines (Timson 2020).

Despite that the initial effects of the drug included a rapid influx of calcium into the worm and calcium-dependent muscle contraction and paralysis, the exact mode of action of PZQ is not known as yet (Greenberg 2005), and the PZQ receptor on schistosomes remains elusive. However, in vivo, PZQ-induced muscle contraction and tegumental lesions produce loss of attachment to the endothelial lining of veins and dislodgment to the liver. Host cells of the defence system attach to the tegumental vacuoles and start to penetrate to the interior of the parasite early after treatment (Mehlhorn et al. 1981; Day et al. 1992). It has been recently shown that PZQ binds and polymerizes adult schistosome actin, which may account for some of

its effects (Tallima and El Ridi 2007). Adverse effects of PZQ include direct and dose-related effects such as nausea and abdominal pain, headache, and dizziness, as well as indirect effects attributable to death of worms such as fever, urticaria, pruritis, rashes, arthralgia, myalgia, and eosinophilia (Tracy and Webster 2001). PZQ can be administered to pregnant women at any stage of pregnancy and during lactation, as benefit of treatment outweighs risk. Also, the drug can safely be administered to school-aged children (WHO 2006).

It is a matter of controversy how long the *Schistosoma*-induced pathological lesions would take to regress after PZQ therapy. One study found that the *Schistosoma*-induced hepatic granulomas were reduced to half its original size at 5–12 weeks after PZQ treatment in mice (Mehlhorn et al. 1982). On the other hand, other experimental studies on *S. mansoni* and *S. japonicum* demonstrated that the pathological lesions persisted after 1 year of PZQ therapy (Cheever and Deb 1989; Cheever et al. 1992). Taken together, these experimental studies provide evidence that the resolution of schistosomal lesions occurs but as a long-term insidious process, and to an extent that is governed by the preexisting pathology at the time of therapy (Chai 2013). A recent randomized clinical trial showed that the repeated application of PZQ has a better therapeutic efficacy in the treatment of hepatic fibrosis due to schistosomiasis (Hong-Bao and Ming 2016).

Some limitations are noteworthy with PZQ. First, the emergence of drug resistance is a real threat, given the fact that the drug is the only one available for treatment and is used extensively in mass treatment programmes. PZQ resistance has been induced in experimental animals for *S. mansoni* and, to a less extent, for *S. japonicum*, usually by exposure of schistosomes to subcurative doses of PZQ over several successive generations (Vale et al. 2017). In the field, the situation is less clear as it is difficult to differentiate between true resistance and therapeutic failure due to other causes such as worm immaturity, or reinfection in areas of intense transmission. Remarkably, *S. mansoni* isolates with reduced sensitivity to PZQ have been reported from different epidemiological settings especially in Africa, e.g. in Egypt, Kenya, and Senegal (Ismail et al. 1996; Cioli et al. 2004; Melman et al. 2009). Sensitivity of *S. japonicum* and *S. haematobium* to PZQ seems to remain unchanged (Vale et al. 2017). The mechanism for resistance is not known, but the induction of ATP-binding cassette (ABC) transporters may be incriminated (Vale et al. 2017).

Second, the drug is not effective against juvenile forms of the parasite. It has little activity against eggs or immature worms (schistosomulae) and cannot abort early infection. Therefore, patients treated early in their infection must be retreated with PZQ after the adult worms have matured (usually in 6–12 weeks) (Kappagoda et al. 2011). Third, there is no appropriate paediatric formula of PZQ available for preschool-aged children who are highly susceptible to *Schistosoma* infections. Finally, PZQ does not prevent reinfection, and does not alter the life cycle of schistosomes (McManus et al. 2018).

Follow-Up of Therapy Parasitological cure has to be confirmed 6–8 weeks and 4–6 months after therapy. Finding viable eggs at 4–6 months indicates either reinfection, therapeutic failure, or that treatment was given too early after infection.

Other parameters of cure include improvement of symptomatology and improvement of radiological, ultrasonographic, and endoscopic findings (Bustinduy and King 2014). Antibody titres, and eosinophilia if present, decline with therapy. However, there may be transient eosinophilia and rise in antibody titres in the first 2 weeks after therapy (Bourée 2005). Antigen detection, if available, is a useful test for establishing cure.

5.7.1.2 Other Drugs

Oxamniquine When administered orally, it is effective against *S. mansoni*, male worms being more affected than females, while it has no effect on *S. haematobium*. A single, two, or three oral doses of 20 mg/kg each are needed for a cure rate of 80–90%, depending on the geographical region. It is now believed that oxamniquine undergoes esterification by a sulfotransferase uniquely present in sensitive schistosomes. The ester spontaneously dissociates, yielding an electrophilic reactant capable of alkylating schistosome DNA, with subsequent inhibition of DNA and RNA synthesis. The absence of this enzyme in mammals, including humans, explains the low toxicity of oxamniquine (Pica-Mattoccia and Cioli 1985). Unfortunately, oxamniquine is no longer readily available (McManus et al. 2018).

Antimalarials The antimalarial drug artemether, a methoxy derivative of artemisinin, has been shown to be active against *S. japonicum*, *S. mansoni*, and *S. haematobium* in experimentally infected animals (Xiao et al. 2002). Mefloquine, another antimalarial drug, was also found to have significant anti-schistosome activity in vitro and in vivo as well, since a single dose (200 or 400 mg/kg), administered orally to mice infected with *S. mansoni*, resulted in worm burden reductions of 72.3–100% (Keiser et al. 2009). It has been shown that artemether interacts with haemin to exert a toxic effect on schistosomes, while mefloquine is believed to inhibit haemozoin formation. Artemisinin derivatives are particularly effective against the immature stages of *S. japonicum*, *S. mansoni*, and possibly *S. haematobium*. Yet there are objections for use of antimalarials in the treatment of schistosomiasis for fear of development of artemisinin-resistant malaria (Gryseels et al. 2006; Utzinger et al. 2007).

Arachidonic Acid An essential fatty acid, a component of our diet and cells, the polyunsaturated fatty acid arachidonic acid (ARA), has been proposed as a remedy for schistosomiasis, due to its ability to activate the parasite tegument-bound neutral sphingomyelinase, with subsequent hydrolysis of the apical lipid bilayer sphingomyelin molecules, allowing access of specific antibody molecules, and eventual worm demise (Tallima et al. 2020a, b). This concept was convincingly supported using larval and adult *S. mansoni* and *S. haematobium* worms during in vitro experiments and in vivo studies in inbred mice and outbred hamsters (El Ridi and Tallima 2013a, b). Moreover, ARA schistosomicidal action was shown to be safe and efficacious in children with light *S. mansoni* infection. A combination of PZQ and ARA showed an outstanding cure rates in children with heavy *S. mansoni* infection (Selim et al. 2014). Additionally, ample evidence was obtained for the

powerful ARA ovicidal potential in vivo and in vitro against *S. mansoni* and *S. haematobium* liver and intestine eggs (Tallima et al. 2020a, b).

5.7.1.3 Novel Therapeutic Approaches

A different approach to therapy of schistosomiasis has relied on plants known for medicinal effects. Extracts and oils of several medicinal plants have been tested for potential therapeutic activity against schistosome infection and have been exhaustively compiled in excellent reviews (e.g. Tagboto and Townson 2001; Yousif et al. 2007). For example, curcumin, the major constituent in the rhizome of *Curcuma longa*, has been shown to display potent schistosomicidal activities in vivo and in vitro against *S. mansoni* worms (El-Ansary et al. 2007; El-Banhawey et al. 2007).

Of great interest is the class of compounds targeting schistosome histone-modifying enzymes, namely, histone acetyltransferases and histone deacetylases, and leading to parasite apoptosis and death in cultures (Pierce et al. 2011). Further, trioxaquines, which are currently in development for malaria, are hybrid molecules consisting of two pharmacophores, a trioxane and a 4-aminoquinoline moiety (Utzinger et al. 2011). These drugs, used at concentrations of 5–50 μg/ml, rapidly kill 21-day-old juvenile and 49-day-old adult *S. mansoni* in vitro. Therefore, these evidence-based findings confirm that peroxidic compounds are active against schistosomes and perhaps other trematodes as well (Keiser and Utzinger 2007; Xiao et al. 2007). HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase, the target for statins (a class of lipid-lowering drugs), was first identified as a metabolic rate-limiting factor in *S. mansoni* and was later shown by chemical and genetic methods to be a validated drug target in schistosomes (Rojo-Arreola et al. 2014).

Recently, there has been interest in multidrug transporters, members of ATP-binding cassette (ABC) superfamily of efflux transporters, such as P-glycoprotein (Pgp; ABCB1), which have been associated with drug resistance in parasites, including helminths such as schistosomes (Greenberg 2013). The role of schistosome ABC transporters in regulating drug susceptibility, and in normal schistosome physiology, including reproduction and excretory activity, has been recently explored. For example, *S. mansoni* expresses higher levels of multidrug resistance-associated protein 1 (SmMRP1) in juvenile worms and in response to PZQ (Kasinathan et al. 2010). Greenberg (2013) postulated that schistosome ABC transporters could be useful targets for compounds that enhance the effectiveness of current therapeutics as well as for agents that act as anti-schistosomes on their own.

5.7.1.4 Adjuvant Therapy

Corticosteroids are usually needed for treatment of acute schistosomiasis and neuroschistosomiasis (McManus et al. 2018). A variety of adjuvant therapies has been proposed for the treatment of schistosomiasis either to enhance anthelmintic therapy or to limit *Schistosoma*-associated inflammatory reactions, but,

unfortunately, most are not validated in clinical trials. Herein, we refer to few examples of these therapies. The immunomodulatory agent β-glucan has shown beneficial effects during murine schistosomiasis (Elgendy et al. 2020). Inhibitors of the renin angiotensin system such as losartan and aliskiren were able to ameliorate *Schistosoma*-induced fibrosis (Parreira et al. 2018). Probiotics have been tried, and the probiotic bacteria *Zymomonas mobilis* provide over 60% protection from the infection of *S. mansoni* in mice (Santos Jde et al. 2004). The proton pump blocker omeprazole has been found useful as adjuvant therapy in experimental *S. mansoni* infections (Almeida et al. 2015; Ellakany et al. 2019). Finally, resveratrol is an anti-inflammatory and antioxidant agent with promising beneficial effects on schistosomiasis (Soliman et al. 2017; Gouveia et al. 2019).

5.7.2 Symptomatic Treatment and Management of Complications

For prevention of bleeding from oesophageal varices due to schistosomal portal hypertension, pharmacotherapy in the form of beta blockers is used together with endoscopic treatment in the form of sclerotherapy, or much better band ligation. For management of acute variceal bleeding, resuscitation is mandatory together with the administration of vasoactive drugs (e.g. terlipressin, somatostatin, or octreotide). Endoscopic treatment particularly band ligation is effective in controlling bleeding (Rajekar et al. 2011). Other procedures for portal hypertension include the minimally invasive transjugular intrahepatic portosystemic shunt (TIPS) (Richter et al. 2015). Surgery is reserved for those patients who fail to respond to endoscopic therapy or in case of symptomatic hypersplenism and includes oesophagogastric devascularization or shunt procedure together with splenectomy (Bosch et al. 2008; Rajekar et al. 2011). Colonoscopic polypectomy is safe and effective and may be required along with medical therapy to achieve complete symptom relief and prevent complications (Mostafa et al. 1990; Elbaz and Esmat 2013).

Obstructive uropathy is usually managed conservatively. However, a damaged kidney may necessitate nephrectomy or nephrostomy. Corrective surgery may be needed for contracted bladder and ureteric obstruction. Carcinoma of the bladder is treated through the conventional procedures of tumour management. Total or partial cystectomy is usually needed. Schistosomal bladder cancer responds less favourably to radiotherapy (Bourée 2005).

5.8 Prognosis

Schistosomiasis remains a major cause of morbidity in tropical and subtropical countries with significant economic, medical, and psychosocial impact (Engels et al. 2002). Clinical observations in schistosomiasis-endemic areas show that

major complications of *Schistosoma* infection develop in approximately 4–12% of the population, while the majority of infected people remain asymptomatic or exhibit mild non-specific symptoms (Tachon and Borojevic 1978). Intensity and duration of the infection are major determinants, but other factors are also involved. These include genetic background of the host, nutritional status, parasite strain differences, and frequency of infection. Maternal infection status—in that offspring of the infected mothers may be primed to mount a modulated type of response at first infection—has been proposed by many authors in order to explain the individual differences (Butterworth and Thomas 1999).

Additionally, schistosomiasis does not cause the annual loss of between 1.7 and 4.5 (Steinmann et al. 2006), but between 24 and 56 (King 2010), and up to 70 (Gray et al. 2010; Siddiqui et al. 2011) million disability-adjusted life years. Unlike malaria, tuberculosis, and HIV/AIDS, schistosomiasis and a host of other helminthic, bacterial, protozoan, and viral diseases remain truly neglected (Utzinger et al. 2011). The close link with poverty and lack of resources, geographical isolation, underestimated global burden, stigmatization, lack of political voice of those affected, and the absence of an established global funding mechanism are some of the factors that explain the general neglect of schistosomiasis (Molyneux 2008; Gray et al. 2010; Utzinger et al. 2011).

At the individual level, antischistosomal therapy given early could lead to resolution of schistosomal lesions and could halt the progression of the disease provided that re-exposure to infection is avoided. In case of established fibrosis, antischistosomal therapy is given to prevent further damage, but the current morbidity has to be addressed appropriately (Butterworth and Thomas 1999). However, there is evidence of regression of hepatosplenic disease with therapy (Butterworth and Thomas 1999; Lucas 2002; Andrade 2008; Hong-Bao and Ming 2016).

5.9 Prevention and Control

The most effective strategy for prevention and control of schistosomiasis is regarded by most authorities as a package of integrated efforts combining several preventive approaches.

5.9.1 Preventive Chemotherapy

WHO (2006) recommended PZQ as the basis of preventive chemotherapy in schistosomiasis. PZQ-based preventive therapy is a cost-effective public health tool that aims at morbidity control: periodic treatment of at-risk populations will cure subtle morbidities and prevent infected individuals from developing severe, late-stage sequelae due to schistosomiasis. For assessment of prevalence of *Schistosoma* infection in a suspected endemic area, school-aged children are examined. A few

schools are selected close to the water and some a little further away, and 50 students of the upper classes in each school are examined either by stool examination (Kato–Katz method) for intestinal schistosomiasis or by search for haematuria (via a questionnaire or urine examination) or urine analysis for ova, for urinary schistosomiasis (WHO 2006, 2013).

According to WHO guidelines, PZQ should be given once yearly to all school-aged children and adults at risk in high-risk communities ($\geq$50% prevalence of infection by parasitological methods); once every 2 years to all school-aged children and adults at risk in moderate-risk communities ($\geq$10% but $<$50% prevalence by parasitological methods); and twice during primary schooling age to all school-aged children and case-directed treatment for adults in low-risk communities ($<$10% prevalence by parasitological methods). Safety of PZQ is not established in children below 4 years; therefore, these children should be excluded from mass treatment but can be treated on an individual basis by medical personnel. The dosage of PZQ is determined according to the height of children. Possible indicators for monitoring preventive chemotherapy interventions are prevalence of infection (by parasitological methods), intensity of infection (proportion of heavy-intensity infections), and prevalence of macrohaematuria, microhaematuria, anaemia, or ultrasound-detectable lesions (urinary tract and liver) (WHO 2006).

5.9.2 Vector Control

Snail control was once the mainstay for control of schistosomiasis. Currently, niclosamide is the only chemical molluscicide in use. Other approaches for snail control such as environmental management, herbal molluscicides, and biological methods were tried with variable success in the field. In order to be effective, molluscicide has to be sustained, a situation which presents financial and administrative difficulties especially for endemic low-income countries. Another disadvantage is the potential harm to the ecosystem such as cross-species toxicity. Finally, the community acceptability of molluscicide remains an issue to be addressed (King 2010; Bustinduy and King 2014; Faust et al. 2020). Anyway, molluscicide seems to continue in use as one of the specific control methods, but techniques have evolved from the old "blanket application" to a much more focal approach dependent upon the epidemiological criteria of high prevalence, high intensity, and rapidity of reinfection in any particular focus or setting of infection (Bustinduy and King 2014). A recent meta-analysis demonstrated that among studies of human infections, molluscicide decreased local prevalence and incidence of schistosome infection in humans in most, but not all, areas. Moreover, estimates from the aggregated studies show that molluscicide (alone) typically decreased reinfections by 64% and local prevalence declined over a period of years. This decline was more rapid and more profound (84% reduction) if chemotherapy was also given (King et al. 2015). Interestingly, Egypt has launched an ambitious national project of lining

all the water canals to minimize water loss. Hopefully, elimination of this ancient long-lasting endemic disease could ensue as a secondary positive effect.

5.9.3 Vaccine Development

5.9.3.1 The Immediate Need of a Vaccine

For combating schistosomiasis, chemical and biological molluscicides have proven efficacy yet may be harmful to the environment (Combes and Cheng 1986; King et al. 2015). Health and hygiene education and improving sanitary and living conditions are required, yet are not available and are not expected to be available in the near future in most countries where schistosomiasis is endemic (Utzinger et al. 2009, 2011; King 2010; Olveda and Gray 2019). Despite the massive distribution and use of the rather safe, effective, and cost affordable schistosomicide, PZQ, it was given to only 30–40 million patients, while hundreds of millions at large remained without treatment. Chemotherapeutic treatment neither delays nor prevents reinfection, requiring repeated administration, and increases the probability of development of parasite resistance to the drug. Accordingly, a safe, efficacious, and cost-effective vaccine should be available to children in endemic rural areas without any delay, in an aim to interrupt transmission and eliminate schistosomiasis (McManus et al. 2018, 2020).

5.9.3.2 The Prospects of the Candidate Vaccine Antigens

During the 1990s, a number of candidate vaccine antigens were found to be the targets of Th1-/Th2-related immune responses in irradiated cercariae-vaccinated mice (reviewed in El Ridi and Tallima 2013a, b, 2015) and residents of endemic countries, such as Egypt and Brazil, that are susceptible or resistant to reinfection after PZQ treatment (Bergquist et al. 2002; Al-Sherbiny et al. 2003). The selected molecules were purified, characterized, and prepared in a recombinant or multiple antigen peptide (MAP) form. The test molecules, likely emulsified in Freund's adjuvant or alum, failed to induce the benchmark of 50% protection in mice in independent trials sponsored by the WHO (Bergquist and Colley 1998; Todd and Colley 2002). IrV5 studies were discontinued. Experiments with triose-phosphate isomerase (TPI) MAP-1 and MAP-2 (Reis et al. 2008) showed that no protection was induced in mice following immunization with TPI together with immunogenic epitopes of glutathione-S-transferase, paramyosin, calpain, and Sm23, assembled as DNA, recombinant protein, or MAP constructs (Yang et al. 2000). Yet, immunization of BALB/c mice with a codon-optimized *S. japonicum* TPI construct elicited higher than 50% reduction in challenge *S. japonicum* worm burden and worm egg counts (Zhu et al. 2010). In contrast, glyceraldehyde 3-phosphate dehydrogenase (SG3PDH) in a recombinant, linear peptide, and di- and tetrabranched MAP

construct, administered to inbred and outbred mice intramuscularly or subcutaneously, without adjuvant or emulsified in Freund's alum or Allison's or RIBI adjuvant led to only 10–35% protection against challenge *S. mansoni* infection (El Ridi 1998; El Ridi and Tallima 2013a, b). Trials with the large chain of calpain Sm-80 in a recombinant or DNA vaccine construct were successful, whereby approximately 50% reductions in worm burdens and worm egg loads were achieved in inbred mice and outbred baboons (Siddiqui et al. 2011).

Regarding paramyosin, a good manufacturing practice-ready, pilot-scale process to produce recombinant full-length *S. japonicum* paramyosin, rSj97, was established, and efficacy and safety studies were conducted in rodents and large animal models (Jiz et al. 2008, 2009). Sm23, a member of the tetraspanin family of molecules located at the host–parasite interface, was used in multiple trials in a recombinant (large extracellular hydrophilic domain) construct in conjunction with alum or Th1-type adjuvants, resulting in limited levels of protection of inbred C57BL/6 mice against subsequent challenge infection (Da'Dara et al. 2003). Vaccination with nucleic acid constructs encoding Sm23 or Sj23 effectively induced parasite-specific IFN-γ and Th1-/Th2-related antibody responses yet failed to evoke other critical responses needed for optimal vaccine efficacy (Da'dara et al. 2002, 2003; Gan et al. 2005; Ganley-Leal et al. 2005). On the contrary, fatty acid-binding protein, Sm14, was produced for use in preclinical and clinical studies (Tendler and Simpson 2008). Members of the tetraspanin (TSP) family of integral membrane proteins, Sm-TSP-1 and Sm-TSP-2, emulsified in Freund's adjuvant were found to induce significant ($P < 0.001$) levels of protection in CBA/CaH mice against schistosomiasis mansoni (Tran et al. 2006). These findings were, however, not reproduced on using *S. japonicum* tetraspanins (Zhang et al. 2011).

During the last decade, tremendous efforts in numerous laboratories succeeded in isolating, producing, and assessing the immunogenicity and protective capacity of several candidate vaccines derived from schistosome apical surface, tegument, cytosol, gut, and ESP (for recent review see Al-Naseri et al. 2021). Notoriously, the location of schistosome candidate vaccines is now of little relevance as the majority, if not all, were detected in adult worms' ESP or extracellular vesicles (EV) (Kifle et al. 2020a; b). This finding explains how the host immune effectors, namely, antibodies, interact with otherwise inaccessible worm surface membrane, tegumental and cytosolic molecules, and activate immune cells capable of chasing and harming the parasite (El Ridi and Tallima 2013a, b; Al-Naseri et al. 2021).

Four candidate vaccines received the support needed for progressing to clinical trials. Glutathione-S-transferase of *S. haematobium* (rShGST) was the first to progress to phase 1 clinical trials, using alum as adjuvant; safety and tolerability were documented (Riveau et al. 2012). Safety, tolerability, and immunogenicity of the vaccine were also demonstrated in adults and children residing in endemic regions (Mo et al. 2014). rShGST is the only schistosomiasis antigen that has reached phase 3 clinical trials. The Senegalese study failed to involve young, parasite-free children and enrolled instead 250 6- to 9-year-old children who had to receive PZQ treatment before immunization. The children were randomized to receive three subcutaneous injections of either rSh28GST/Alhydrogel or Alhydrogel alone with 4-week

intervals. All children were again PZQ treated on week 44 and received a booster immunization 8 weeks later, precisely 1 year after the first injection. The vaccine protective capacity was evaluated by recurrence of natural infection 2 years later. Vaccinated children showed elevated levels of Sh28GST-specific IgG1, IgG2, and IgG4 antibody but a lack of IgG3 and IgA isotypes. Acquired immunity to infection in human populations was characterized by high levels of IgG3 and IgA antibodies to Sh28GST. Failure in achieving protection against urinary schistosomiasis was attributed to the antibody isotype issue (Riveau et al. 2018) or might be consequent to the confounding effects of PZQ administration before the first and last immunization (Alsallaq et al. 2017).

S. mansoni fatty acid-binding protein, Sm14, formulated with glucopyranosyl lipid A (GLA) adjuvant was used to immunize male and female volunteers from a non-endemic area for schistosomiasis in Rio de Janeiro state, Brazil. No adverse reactions were recorded. The vaccine elicited significant increase in Sm14-specific total IgG, with no IgE observed at any time, and stimulated both Th1 and Th2 cytokines (Tendler et al. 2015; Santini-Oliveira et al. 2016). In phase 2a trial, rSm14 vaccine in combination with GLA in a stable squalene-based oil-in-water emulsion (GLA-SE) was safe with long-lasting immunogenicity when administrated to 30 male adults from endemic area for both *S. mansoni* and *S. haematobium* in Senegal River Basin (Tendler et al. 2018). Accordingly, phase 2b and phase 3 trials are planned (Tendler et al. 2018; McManus et al. 2020; https://clinicaltrials.gov/ct2/show/NCT03041766; https://clinicaltrials.gov/ct2/show/NCT03799510).

Along with ShGST and Sm14, *S. mansoni* TSP-2 has reached phase 1 clinical trial. Recombinant TSP-2/alum with or without an aqueous GLA formulation (GLA-AF) was administered to 72 healthy, parasite-free, young people in double-blind, dose-escalation (10, 30, and 100 μg/injection) trial. The three injections given 8 weeks apart of the different vaccine formulations were well tolerated and safe. Serum Sm-TSP-2-specific IgG responses were, however, rather low except in volunteers who received 100 μg of Sm-TSP-2/Al with GLA-AF (Keitel et al. 2019). Nevertheless, phase 1b dose-escalation study has been undertaken to assess the safety and immunogenicity of Sm-TSP-2 with or without AP 10–701 (new nomenclature of GLA-AF) in healthy Ugandan adults (Keitel et al. 2019; https://clinicaltrials.gov/ct2/show/NCT03910972).

S. mansoni p80 the large subunit (heavy chain) of the *S. mansoni* calcium-activated neutral protease, calpain, has been tested for efficacy in different forms, protein, recombinant, or DNA-based constructs, and showed significant protection capacity, had remarkable efficacy in fecundity reduction, and reduced the egg-induced pathology with transmission blocking potential in rodents and baboons (Le et al. 2018; Eyayu et al. 2020). Furthermore, in a double-blind preclinical trial, baboons immunized with Sm-p80/GLA-SE showed considerable reduction in adult female worms (93.4%) and remarkable reduction in tissue egg load (89.9%) (Zhang et al. 2018). These promising results together with evidence of cross-schistosome species prophylactic efficacy supported Sm-p80 vaccine approval for phase 1 clinical trials to begin in early–mid-2021 (Molehin 2020; Tsuji 2020).

5.9.3.3 The Breakthrough Progress

Presently, most if not all schistosomiasis promising vaccines were shown to be readily detectable in worm ESP or EV, supporting the statement formulated as early as 2013 predicting that candidate vaccine antigens should only be sought among worm ESP (El Ridi and Tallima 2013a, b, c). Indeed, schistosomes reside in the bloodstream impervious to immune attacks. If intact parasites were susceptible to killing via antibody-dependent complement activation or cell-mediated cytotoxicity (ADCC), they would not survive a day, least of all a decade, as surface membrane-associated molecules released from dying or dead parasites are readily accessible to the host and are immunogenic (Mahana 2006; Tran et al. 2006). Yet, generated antibodies are not able to access antigens on the apical membrane or the tegument (El Ridi and Tallima 2013a, b and references therein; Migliardo et al. 2014; El Ridi et al. 2017). The immune system effectors may, however, recognize and interact with the "scent" molecules, the ESP and EV of developing and adult worms, resulting into immune cell activation and release of inflammatory and toxic mediators in the vicinity of the parasite. In large vessels, ESP and EV are easily washed away from the parasite. The situation is more difficult for the developing worms in the lung capillaries and liver sinusoids, where the schistosome-derived products might stagnate, attracting the hunting cells in close proximity to the migrating schistosomula. Accordingly, ESP or EV released by developing worms should be excellent vaccine candidates. Indeed, all of the candidate vaccine molecules are parasite ESP or reside in worm-derived EV (El Ridi and Tallima 2009; Kifle et al. 2020a; b).

Most, if not all, promising vaccine candidates elicited preponderance of Th1- and Th2-related cytokines and antibodies in total accord with dependence of human resistance to schistosome reinfection on type 2 immune responses (Hagan et al. 1991; Ganley-Leal et al. 2006; Jiz et al. 2009; Figueiredo et al. 2012; Fitzsimmons et al. 2012). To develop an ESP-based vaccine, it is important to examine the "hunting" team summoned upon parasite invasion (von Lichtenberg et al. 1977). Schistosome ESP induces predominantly Th1-/Th17-related cytokines and antibody responses in mice and humans during natural infection, and in mice following immunization with schistosome antigens and Th1-/Th17-biased adjuvants (El Ridi and Tallima 2012; Abdel Aziz et al. 2016). Accordingly, circulating monocytes and neutrophils would be readily recruited and activated and certainly contribute to demise of a proportion of invading larvae. Yet, eosinophils and basophils are not invited to participate in the chase, and are actually entirely excluded (von Lichtenberg et al. 1977). To recruit and activate eosinophils and basophils, it is necessary to use an adjuvant that skew the larval-induced Th1/Th17 immune responses towards the type 2 phenotype (El Ridi and Tallima 2012, 2013a, b and references therein).

Adjuvant Selection Our efforts for proper adjuvant choice led to selection of the type 2 immune response-inducing thymic stromal lymphopoietin, TSLP (El Ridi and Tallima 2012), and papain. For every experiment, papain injection consistently and

Table 5.1 Effect of papain administration before infection with *S. mansoni* on parasitological parameters

Mean ± SD (*p*-value; reduction) in mice	Untreated	Papain treated
Total worm burden	39.9 ± 9.1	13.1 ± 3.2 (< 0.0001; 67.16%)
Male worm burden	20.6 ± 6.2	7.1 ± 2.6 (< 0.0001; 65.53%)
Female worm burden	19.2 ± 4.0	6.0 ± 0.75 (< 0.0001; 68.75%)
Liver egg counts	36,350 ± 7777	17,187 ± 3575 (< 0.0001; 52.71%)
Small intestine egg counts	31,035 ± 9251	15,187 ± 3835 (0.0002; 51.06%)

Representative of four independent experiments whereby ten mice/groups were subcutaneously injected with 100 μl phosphate-buffered saline, pH 7.1 (PBS), or 50 μg papain in 100 μl PBS 1 h before exposure to *S. mansoni* cercariae via whole body exposure. Parasitological parameters were evaluated 6 weeks after infection and data analysed using Student's *t*-test.

reproducibly elicited highly significant anti-schistosomiasis protection (Table 5.1). Papain has been documented to drive ovalbumin immune responses towards the type 2 axis within a few days after injection in mice (Sokol et al. 2008, 2009; Tang et al. 2010), and that was sufficient, without any previous or additional immunization, to lead to highly significant protection against schistosomiasis mansoni in every test mouse as compared to controls. These findings, which were fully confirmed in schistosomiasis mansoni (Abdel Aziz et al. 2016; Tallima et al. 2019) and schistosomiasis haematobium (Tallima et al. 2017a; Abdel Aziz et al. 2019) protection trials, provided a solid proof that a large proportion of invading *S. mansoni* larvae would succumb if met upon host invasion with type 2 immune responses, and a proof of concept as papain may not be for considered human use. A glucopyranosyl lipid A (GLA) combined with alum (Tendler et al. 2015; Santini-Oliveira et al. 2016; Riveau et al. 2018), or formulated as an aqueous nano-suspension, GLA-AF (Keitel et al. 2019), or stable squalene-based oil-in-water emulsion (Tendler et al. 2018; Zhang et al. 2018) is increasingly used as adjuvant for schistosomiasis vaccines intended for humans and enhanced generation of type 1 and type 2 cytokines and antibodies (Reed et al. 2018).

A Proof of Concept Leads to an Adjuvant-Free Vaccine Formulation: The Cysteine Peptidase-Based Schistosomiasis Vaccine Immunization of outbred mice and hamsters with schistosome antigens that are both ESP and type 2 immune response-inducing, namely the cysteine peptidases, cathepsin B and L, consistently and reproducibly elicited highly significant reduction (60–75%) of challenge *S. mansoni* and *S. haematobium* worm burden and worm egg load in the liver and small intestine as compared to unimmunized mice and hamsters, respectively. It is, thus, fortunate that we developed an adjuvant-free, Th2 immune responses-based vaccine effective in outbred hosts, since it is destined to the outbred human population, where adjuvant use remains a considerable challenge and where immunological correlates of resistance to the three species of schistosomes are associated with type 2 responses (El Ridi et al. 2014; Abdel Aziz et al. 2019; Tallima et al. 2015, 2017a, b, 2020a, b).

Conclusion More efforts are needed to understand why the cysteine peptidase-based vaccines involving calpain or cathepsins fail to achieve sterilizing immunity. Reaching that goal may open the door to preclinical and clinical trials in an aim to develop a safe, efficacious, and commercially available schistosomiasis vaccine.

Acknowledgement The authors are thankful to prof. Dalia S. Ashour, Faculty of Medicine, Tanta, Egypt, for her help during preparation of the manuscript.

References

Abaza SM (2013) Treatment of schistosomiasis: from praziquantel to development of new drug targets. PUJ 6:127–148

Abdel Aziz N, Tallima H, Hafez EA, El Ridi R (2016) Papain-based vaccination modulates *Schistosoma mansoni* infection-induced cytokine signals. Scand J Immunol 83(2):128–138

Abdel Aziz N, Tallima H, Abou El Dahab M, El Ridi R (2019) Immunogenicity and protective capacity of *Schistosoma haematobium* recombinant cathepsin L against infection of hamsters with *S. haematobium*. Vaccine Res 6:1–8

Abdel-Motaleb GS, El-Ghareeb AS, Aly NS, Salama NA (2013) Present situation of *Schistosoma haematobium* infection among primary school aged children in some areas of Qualyobia governorate-Egypt. J Egypt Soc Parasitol 43:577–589

Abdel-Wahab MF, Esmat G, Farrag A, El-Boraey YA, Strickland GT (1992) Grading of hepatic schistosomiasis by the use of ultrasonography. Am J Trop Med Hyg 46:403–408

Adam I, ALhabardi NA, Al-Wutayd O, Khamis AH (2021) Prevalence of schistosomiasis and its association with anemia among pregnant women: a systematic review and meta-analysis. Parasit Vectors 14(1):133

Akpata R, Neumayr A, Holtfreter MC, Krantz I, Singh DD, Mota R, Walter S, Hatz C, Richter J (2015) The WHO ultrasonography protocol for assessing morbidity due to *Schistosoma haematobium*. Acceptance and evolution over 14 years. Systematic review. Parasitol Res 114(4):1279–1289

Allam AF, Kader O, Zaki A, Shehab AY, Farag HF (2009) Assessing the marginal error in diagnosis and cure of *Schistosoma mansoni* in areas of low endemicity using Percoll and PCR techniques. Tropical Med Int Health 14(3):316–321

Allan F, Dunn AM, Emery AM, Stothard JR, Johnston DA, Kane RA, Khamis AN, Mohammed K, Rollinson D (2013) Use of sentinel snails for the detection of *Schistosoma haematobium* transmission on Zanzibar and observations on transmission patterns. Acta Trop 128(2): 234–240. https://doi.org/10.1016/j.actatropica.2013.01.003

Allen AV, Ridley DS (1970) Further observations on the formol-ether concentration technique for faecal parasites. J Clin Pathol 23(6):545–546

Almeida GT, Lage RC, Anderson L, Venancio TM, Nakaya HI, Miyasato PA, Rofatto HK, Zerlotini A, Nakano E, Oliveira G, Verjovski-Almeida S (2015) Synergy of omeprazole and praziquantel in vitro treatment against *Schistosoma mansoni* adult worms. PLoS Negl Trop Dis 9(9):e0004086

Al-Naseri A, Al-Absi S, El Ridi R, Mahana N (2021) A comprehensive and critical overview of schistosomiasis vaccine candidates. J Parasit Dis 45(2):557–580. https://doi.org/10.1007/s12639-021-01387-w. Online ahead of print

Alsallaq RA, Gurarie D, Ndeffo Mbah M, Galvani A, King C (2017) Quantitative assessment of the impact of partially protective anti-schistosomiasis vaccines. PLoS Negl Trop Dis 11:e0005544

Al-Sherbiny MM, Osman AM, Hancock K, Deelder AM, Tsang VC (1999) Application of immunodiagnostic assays: detection of antibodies and circulating antigens in human schistosomiasis and correlation with clinical findings. Am J Trop Med Hyg 60(6):960–966

Al-Sherbiny M, Osman A, Barakat R, El Morshedy H, Bergquist R, Olds R (2003) In vitro cellular and humoral responses to *Schistosoma mansoni* vaccine candidate antigens. Acta Trop 88 (2):117–130

Alves Oliveira LF, Moreno EC, Gazzinelli G, Martins-Filho OA, Silveira AM, Gazzinelli A, Malaquias LC, LoVerde P, Leite PM, Correa-Oliveira R (2006) Cytokine production associated with periportal fibrosis during chronic schistosomiasis mansoni in humans. Infect Immun 74(2): 1215–1221

Andrade ZA (2008) Schistosomiasis and hepatic fibrosis regression. Acta Trop 108:79–82

Aryeetey YA, Essien-Baidoo S, Larbi IA, Ahmed K, Amoah AS, Obeng BB, van Lieshout L, Yazdanbakhsh M, Boakye DA, Verweij JJ (2013) Molecular diagnosis of *Schistosoma* infections in urine samples of school children in Ghana. Am J Trop Med Hyg 88(6):1028–1031

Ashton PD, Harrop R, Shah B, Wilson RA (2001) The schistosome egg: development and secretions. Parasitology 122(Pt 3):329–338

Aula OP, McManus DP, Mason MG, Botella JR, Gordon CA (2021) Rapid parasite detection utilizing a DNA dipstick. Exp Parasitol 224:108098

Ayash-Rashkovsky M, Chenine AL, Steele LN, Lee SJ, Song R, Ong H et al (2007) Coinfection with *Schistosoma mansoni* reactivates viremia in rhesus macaques with chronic simian–human immunodeficiency virus clade C infection. Infect Immun 75:1751–1756

Badr HI, Shaker AA, Mansour MA, Kasem MA, Zaher AA, Salama HH, Safwat MI (2011) Schistosomal myeloradiculopathy due to *Schistosoma mansoni*: report on 17 cases from an endemic area. Ann Indian Acad Neurol 14:107–110

Barakat RMR (2013) Epidemiology of schistosomiasis in Egypt: travel through time. J Adv Res 4(5):425–432

Barakat R, Abou El-Ela NE, Sharaf S, El Sagheer O, Selim S, Tallima H, Bruins MJ, Hadley KB, El Ridi R (2015) Efficacy and safety of arachidonic acid for treatment of school-age children in *Schistosoma mansoni* high-endemicity regions. Am J Trop Med Hyg 92(4):797–804

Bärenbold O, Garba A, Colley DG, Fleming FM, Assaré RK, Tukahebwa EM, Kebede B, Coulibaly JT, N'Goran EK, Tchuem Tchuenté LA, Mwinzi P, Utzinger J, Vounatsou P (2021) Estimating true prevalence of *Schistosoma mansoni* from population summary measures based on the Kato-Katz diagnostic technique. PLoS Negl Trop Dis 15(4):e0009310

Barnhill AE, Novozhilova E, Day TA, Carlson SA (2011) *Schistosoma*-associated *salmonella* resist antibiotics via specific fimbrial attachments to the flatworm. Parasit Vectors 4:123

Barsoum RS, Esmat G, El-Baz T (2013) Human schistosomiasis: clinical perspective. J Adv Res 4(5):433–444

Bergquist NR, Colley DG (1998) Schistosomiasis vaccine: research to development. Parasitol Today 14(3):99–104

Bergquist R, Al-Sherbiny M, Barakat R, Olds R (2002) Blueprint for schistosomiasis vaccine development. Acta Trop 82(2):183–192. Review

Berhe N, Medhin G, Erko B, Smith T, Gedamu S, Bereded D, Moore R, Habte E, Redda A, Gebre-Michael T, Gundersen SG (2004) Variations in helminth faecal egg counts in Kato-Katz thick smears and their implications in assessing infection status with *Schistosoma mansoni*. Acta Trop 92(3):205–212

Betson M, Sousa-Figueiredo JC, Rowell C, Kabatereine NB, Stothard JR (2010) Intestinal schistosomiasis in mothers and young children in Uganda: investigation of field-applicable markers of bowel morbidity. Am J Trop Med Hyg 83(5):1048–1055

Betson M, Sousa-Figueiredo JC, Kabatereine NB, Stothard JR (2012) Use of fecal occult blood tests as epidemiologic indicators of morbidity associated with intestinal schistosomiasis during preventive chemotherapy in young children. Am J Trop Med Hyg 87(4):694–700

Boissier J, Kincaid-Smith J, Berry A, Allienne JF, Fillaux J, Toulza È (2015) Global changes and snail-borne disease: an hybrid schistosome reaches Europe. Med Sci (Paris) 31(11):962–964

Boissier J, Grech-Angelini S, Webster BL, Allienne JF, Huyse T, Mas-Coma S, Toulza E, Barré-Cardi H, Rollinson D, Kincaid-Smith J et al (2016) Outbreak of urogenital schistosomiasis in Corsica (France): an epidemiological case study. Lancet Infect Dis 16(8):971–979

Boko PM, Ibikounle M, Onzo-Aboki A, Tougoue J-J, Sissinto Y, Batcho W et al (2016) Schistosomiasis and soil transmitted helminths distribution in Benin: a baseline prevalence survey in 30 districts. PLoS One 11(9):e0162798. https://doi.org/10.1371/journal.pone.0162798

Booth M, Mwatha JK, Joseph S, Jones FM, Kadzo H, Ireri E, Kazibwe F, Kemijumbi J, Kariuki C, Kimani G, Ouma JH, Kabatereine NB, Vennervald BJ, Dunne DW (2004) Periportal fibrosis in human *Schistosoma mansoni* infection is associated with low IL-10, low IFN-gamma, high TNF-alpha, or low RANTES, depending on age and gender. J Immunol 172(2):1295–1303

Bosch J, Berzigotti A, Garcia-Pagan JC, Abraldes JG (2008) The management of portal hypertension: rational basis, available treatments and future options. J Hepatol 48(Suppl 1):S68–S92

Botelho M, Ferreira AC, Oliveira MJ, Domingues A, Machado JC, Da Costa JM (2009) *Schistosoma haematobium* total antigen indices increased proliferation, migration, and invasion and decreases apoptosis of normal epithelial cells. Int J Parasitol 39:1083–1091

Botelho MC, Oliveira PA, Lopes C, Correia da Costa JM, Machado JC (2011) Urothelial dysplasia and inflammation induced by *Schistosoma haematobium* total antigen instillation in mice normal urothelium. Urol Oncol 29(6):809–814

Bourée P (2005) Parasitoses urinaires (Urinary parasitosis). Ann Urol 39:232–246

Brodish PH, Singh K (2016) Association between *Schistosoma haematobium* exposure and human immunodeficiency virus infection among females in Mozambique. Am J Trop Med Hyg 94(5): 1040–1044

Brown M, Mawa PA, Joseph S, Bukusuba J, Watera C, Whitworth JA, Dunne DW, Elliott AM (2005) Treatment of *Schistosoma mansoni* infection increases helminth-specific type 2 cytokine responses and HIV-1 loads in coinfected Ugandan adults. J Infect Dis 191(10):1648–1657

Budiono NG, Satrija F, Ridwan Y, Handharyani E, Murtini S (2019) The contribution of domestic animals to the transmission of schistosomiasis japonica in the Lindu Subdistrict of the Central Sulawesi Province, Indonesia. Vet World 12(10):1591–1598

Burke ML, Jones MK, Gobert GN, Li YS, Ellis MK, McManus DP (2009) Immunopathogenesis of human schistosomiasis. Parasite Immunol 31(4):163–176

Bustinduy AL, King CH (2014) Schistosomiasis. In: Cook GC, Zumla AI (eds) Manson's tropical diseases. Section 10, Chapter 52, 24th edn. Saunders Elsevier, Edinburgh

Butler SE, Muok EM, Montgomery SP, Odhiambo K, Mwinzi PM, Secor WE, Karanja DM (2012) Mechanism of anemia in *Schistosoma mansoni*-infected school children in Western Kenya. Am J Trop Med Hyg 87(5):862–867

Butterworth AE, Thomas JEP (1999) Schistosomiasis. In: Weatherall DJ, TGG L, Warell DA (eds) Oxford textbook of medicine, vol 1, 3rd edn. Oxford University Press, New York, pp 970–981

Cabantous S, Hou X, Louis L, He H, Mariani O, Sastre X, Daujat-Chavanieu M, Li Y, Dessein A (2017) Evidence for an important role of host microRNAs in regulating hepatic fibrosis in humans infected with *Schistosoma japonicum*. Int J Parasitol 47(13):823–830

Cai P, Piao X, Liu S, Hou N, Wang H, Chen Q (2013) MicroRNA-gene expression network in murine liver during *Schistosoma japonicum* infection. PLoS One 8(6):e67037

Cai P, Weerakoon KG, Mu Y, Olveda DU, Piao X, Liu S, Olveda RM, Chen Q, Ross AG, McManus DP (2017) A parallel comparison of antigen candidates for development of an optimized serological diagnosis of schistosomiasis japonica in the Philippines. EBioMedicine 24:237–246

Cai P, Mu Y, Olveda RM, Ross AG, Olveda DU, McManus DP (2018) Circulating miRNAs as footprints for liver fibrosis grading in schistosomiasis. EBioMedicine 37:334–343

Cai P, Weerakoon KG, Mu Y, Olveda RM, Ross AG, Olveda DU, McManus DP (2019) Comparison of Kato Katz, antibody-based ELISA and droplet digital PCR diagnosis of schistosomiasis japonica: lessons learnt from a setting of low infection intensity. PLoS Negl Trop Dis 13(3): e0007228

Caldas IR, Campi-Azevedo AC, Oliveira LF, Silveira AM, Oliveira RC, Gazzinelli G (2008) Human schistosomiasis mansoni: immune responses during acute and chronic phases of the infection. Acta Trop 108:109–117

Chai JY (2013) Praziquantel treatment in trematode and cestode infections: an update. Infect Chemother 45:32–43

Cheever AW, Deb S (1989) Persistence of hepatic fibrosis and tissue eggs following treatment of *Schistosoma japonicum* infected mice. Am J Trop Med Hyg 40:620–628

Cheever AW, Xu YH, Sher A, Macedonia JG (1991) Analysis of egg granuloma formation in *Schistosoma japonicum*-infected mice treated with antibodies to interleukin-5 and gamma interferon. Infect Immun 59:4071–4074

Cheever AW, Macedonia JG, Deb S, Cheever EA, Mosimann JE (1992) Persistence of eggs and hepatic fibrosis after treatment of *Schistosoma mansoni*-infected mice. Am J Trop Med Hyg 46: 752–758

Cheever AW, Williams ME, Wynn TA et al (1994) Anti-IL-4 treatment of *Schistosoma mansoni*-infected mice inhibits development of T cells and non-B, non-T cells expressing Th2 cytokines while decreasing egg-induced hepatic fibrosis. J Immunol 153:753–759

Chen L, Rao KV, He YX, Ramaswamy K (2002) Skin-stage schistosomula of *Schistosoma mansoni* produce an apoptosis-inducing factor that can cause apoptosis of T cells. J Biol Chem 277(37): 34329–34335

Chen X, Yang X, Li Y, Zhu J, Zhou S, Xu Z et al (2014) Follicular helper T cells promote liver pathology in mice during *Schistosoma japonicum* infection. PLoS Pathog 10:e1004097

Chen Q, Zhang J, Zheng T, Chen H, Nie H, Zheng B, Gong Q (2019) The role of microRNAs inthe pathogenesis, grading and treatment of hepatic fibrosis in schistosomiasis. Parasit Vec 12(1):611

Chen C, Guo Q, Fu Z, Liu J, Lin J, Xiao K, Sun P, Cong X, Liu R, Hong Y (2021) Reviews and advances in diagnostic research on *Schistosoma japonicum*. Acta Trop 213:105743

Cheng YL, Song WJ, Liu WQ et al (2008) The effects of T cell deficiency on the development of worms and granuloma formation in mice infected with *Schistosoma japonicum*. Parasitol Res 102:1129–1134

Chuah C, Jones MK, Burke ML, McManus DP, Gobert GN (2014) Cellular and chemokine-mediated regulation in schistosome-induced hepatic pathology. Trends Parasitol 30:141–150

Cioli D, Botros SS, Wheatcroft-Francklow K, Mbaye A, Southgate V, Tchuenté LA, Pica-Mattoccia L, Troiani AR, El-Din SH, Sabra AN, Albin J, Engels D, Doenhoff MJ (2004) Determination of ED50 values for praziquantel in praziquantel-resistant and -susceptible *Schistosoma mansoni* isolates. Int J Parasitol 34:979–987

Colombe S, Machemba R, Mtenga B, Lutonja P, Kalluvya SE, de Dood CJ et al (2018) Impact of schistosome infection on long-term HIV/AIDS outcomes. PLoS Negl Trop Dis 12(7):e0006613

Combes C, Cheng TC (1986) Control of biomedically important molluscs. Arch Inst Pasteur Alger 55:153–193

Corrêa-Oliveira R, Golgher DB, Oliveira GC, Carvalho OS, Massara CL, Caldas IR, Colley DG, Gazzinelli G (2002) Infection with *Schistosoma mansoni* correlates with altered immune responses to *Ascaris lumbricoides* and hookworm. Acta Tropica 83(2):123–132

Corstjens PLAM, de Dood CJ, Knopp S, Clements MN, Ortu G, Umulisa I, Ruberanziza E, Wittmann U, Kariuki T, LoVerde P, Secor WE, Atkins L, Kinung'hi S, Binder S, Campbell CH, Colley DG, van Dam GJ (2020) Circulating Anodic Antigen (CAA): a highly sensitive diagnostic biomarker to detect active *Schistosoma* infections-improvement and use during SCORE. Am J Trop Med Hyg 103(1_Suppl):50–57

Coulibaly JT, Fürst T, Silué KD, Knopp S, Hauri D, Ouattara M, Utzinger J, N'Goran EK (2012) Intestinal parasitic infections in schoolchildren in different settings of Côte d'Ivoire: effect of diagnostic approach and implications for control. Parasit Vectors 5:135

Coulibaly JT, N'gbesso YK, Knopp S, N'guessan NA, Silué KD, van Dam GJ, N'goran EK, Utzinger J (2013) Accuracy of urine circulating cathodic antigen test for the diagnosis of Schistosoma mansoni in preschool-aged children before and after treatment. PLoS Negl Trop Dis 7(3):e2109

Coutinho HM, Leenstra T, Acosta LP, Su L, Jarilla B, Jiz MA, Langdon GC, Olveda RM, McGarvey ST, Kurtis JD, Friedman JF (2006) Pro-inflammatory cytokines and C-reactive

protein are associated with undernutrition in the context of Schistosoma japonicum infection. Am J Trop Med Hyg 75(4):720–726
Cox FE (2002) History of human parasitology. Clin Microbiol Rev 15:595–612
Cringoli G (2006) FLOTAC, a novel apparatus for a multivalent faecal egg count technique. Parassitologia 48(3):381–384
Da Silva LC, Carrilho FJ (1992) Hepatosplenic schistosomiasis. Pathophysiology and treatment. Gastroenterol Clin N Am 21:163–177
Da'dara AA, Skelly PJ, Fatakdawala M, Visovatti S, Eriksson E, Harn DA (2002) Comparative efficacy of the *Schistosoma mansoni* nucleic acid vaccine, Sm23, following microseeding or gene gun delivery. Parasite Immunol 24(4):179–187
Da'Dara AA, Skelly PJ, Walker CM, Harn DA (2003) A DNA-prime/protein-boost vaccination regimen enhances Th2 immune responses but not protection following *Schistosoma mansoni* infection. Parasite Immunol 25(8–9):429–437
Davis A (1993) Antischistosomal drugs and clinical practice. In: Jordan P, Webbe G, Sturrock RF (eds) Human schistosomiasis. CAB International, Wallingford, pp 367–404
Day TA, Bennett JL, Pax RA (1992) Praziquantel: the enigmatic antiparasitic. Parasitol Today 8: 342–344
Dazo BC, Biles JE (1974) Two new field techniques for detection and counting of Schistosoma haematobium eggs in urine samples, with an evaluation of both methods. Bull World Health Organ 51(4):399–408
de Jesus AR, Magalhães A, Miranda DG, Miranda RG, Araújo MI, de Jesus AA, Silva A, Santana LB, Pearce E, Carvalho EM (2004) Association of type 2 cytokines with hepatic fibrosis in human *Schistosoma mansoni* infection. Infect Immun 72(6):3391–3397
Dejon-Agobé JC, Honkpehedji YJ, Zinsou JF, Edoa JR, Adégbitè BR, Mangaboula A, Agnandji ST, Mombo-Ngoma G, Ramharter M, Kremsner PG, Lell B, Grobusch MP, Adegnika AA (2020) Epidemiology of Schistosomiasis and Soil-Transmitted Helminth Coinfections among Schoolchildren Living in Lambaréné, Gabon. Am J Trop Med Hyg 103(1):325–333
deMorais CN, Souza JR, Melo WG, Aroucha ML, Miranda P, Domingues AL, Abath FG, Montenegro SM (2008) Cytokine profile associated with chronic and acute human schistosomiasis mansoni. Mem Inst Oswaldo Cruz 103(6):561–568
Diallo TO, Remoue F, Gaayeb L, Schacht AM, Charrier N, De Clerck D, Dompnier JP, Pillet S, Garraud O, N'Diaye AA, Riveau G (2010) Schistosomiasis coinfection in children influences acquired immune response against plasmodium falciparum malaria antigens. PLoS One 5(9): e12764
Domingues AL, Medeiros TB, Lopes EP (2011) Ultrasound versus biological markers in the evaluation of periportal fibrosis in human *Schistosoma mansoni*. Mem Inst Oswaldo Cruz 106(7):802–807
Downs JA, van Dam GJ, Changalucha JM, Corstjens PL, Peck RN, de Dood CJ et al (2012) Association of schistosomiasis and HIV infection in Tanzania. Am J Trop Med Hyg 87:868–873
Downs JA, de Dood CJ, Dee HE, McGeehan M, Khan H, Marenga A et al (2017) Schistosomiasis and human immunodeficiency virus in men in Tanzania. Am J Trop Med Hyg 96(4):856–862
Eberl M, Al-Sherbiny M, Hagan P, Ljubojevic S, Thomas AW, Wilson RA (2002) A novel and sensitive method to monitor helminth infections by faecal sampling. Acta Trop 83(2):183–187
Eboumbou C, Steghens JP, Abdallahi OM, Mirghani A, Gallian P, van Kappel A, Qurashi A, Gharib B, De Reggi M (2005) Circulating markers of oxidative stress and liver fibrosis in Sudanese subjects at risk of schistosomiasis and hepatitis. Acta Trop 94(2):99–106
Ebrahim A, El-Morshedy H, Omer E, El-Daly S, Barakat R (1997) Evaluation of the Kato-Katz thick smear and formol ether sedimentation techniques for quantitative diagnosis of *Schistosoma mansoni* infection. Am J Trop Med Hyg 57(6):706–708
El Ridi R (1998) More on the search for a schistosomiasis vaccine. Parasitol Today 14(10):436
El Ridi R, Tallima H (2009) *Schistosoma mansoni* ex vivo lung-stage larvae excretory-secretory antigens as vaccine candidates against schistosomiasis. Vaccine 27(5):666–673

El Ridi R, Tallima H (2012) Adjuvant selection for vaccination against murine schistosomiasis. Scand J Immunol 76(6):552–558

El Ridi RAF, Tallima HA-M (2013a) Novel therapeutic and prevention approaches for schistosomiasis. J Adv Res 4(5):467–478

El Ridi R, Tallima H (2013b) Solving the riddle of the lung-stage schistosomula paved the way to a novel remedy and an efficacious vaccine for schistosomiasis. In: El Ridi R (ed) Parasitic diseases—schistosomiasis. InTech, Crotia. ISBN 978-953-51-0942-6, http://www.intechopen.com/articles/show/title/solving-the-riddle-of-the-lung-stage-schistosomula-paved-the-way-to-a-novel-remedy-and-an-efficaciou

El Ridi R, Tallima H (2013c) Vaccine-induced protection against murine schistosomiasis mansoni with larval excretory-secretory antigens and papain or type-2 cytokines. J Parasitol 99(2): 194–202

El Ridi R, Tallima H (2015) Why the radiation-attenuated cercarial immunization studies failed to guide the road for an effective schistosomiasis vaccine: a review. J Adv Res 6(3):255–267

El Ridi R, Tallima H, Selim S, Donnelly S, Cotton S, Gonzales Santana B, Dalton JP (2014) Cysteine peptidases as schistosomiasis vaccines with inbuilt adjuvanticity. PloS One 9: e85401El

El Ridi R, Tallima H, Migliardo F (2017) Biochemical and biophysical methodologies open the road for effective schistosomiasis therapy and vaccination. Biochim Biophys Acta Gen Subj 1861(1 Pt B):3613–3620

El-Ansary AK, Ahmed SA, Aly SA (2007) Antischistosomal and liver protective effects of *Curcuma longa* extract in *Schistosoma mansoni* infected mice. Indian J Exp Biol 45:791–801

El-Banhawey MA, Ashry MA, El-Ansary AK, Aly SA (2007) Effect of *Curcuma longa* or parziquantel on *Schistosoma mansoni* infected mice liver–histological and histochemical study. Indian J Exp Biol 45:877–889

Elbaz T, Esmat G (2013) Hepatic and intestinal schistosomiasis. J Adv Res 4(5):445-452

El-Garem AA (1998) Schistosomiasis. Digestion 59(5):589–605

Elgendy DI, Othman AA, El-Guindy DM, Soliman NA, Elmehy DA (2020) Synergy between beta-1,3-glucan and praziquantel in the treatment of experimental chronic schistosomiasis mansoni. J Egypt Soc Parasitol 50(3):565–574

Ellakany AR, Elgendy DI, Alshenawy HA, Abdel Ghaffar AE (2019) Assessment of the potential therapeutic effects of omeprazole in *Schistosoma mansoni* infected mice. Parasitol Res 118(12): 3399–3408

Elmorshedy H, Bergquist R, Fayed A, Guirguis W, Abdel-Gawwad E, Eissa S, Barakat R (2020) Elimination of schistosomiasis requires multifactorial diagnostics: evidence from high- and low-prevalence areas in the Nile Delta, Egypt. Infect Dis Poverty 9(1):31

el-Nahal HM, Hassan SI, Kaddah MA, Ghany AA, Mostafa EA, Ibrahim AM, Ramzy RM (1998a) Mutual effect of *Schistosoma mansoni* infection and pregnancy in experimental C57 BL/6 black mice. J Egypt Soc Parasitol 28(1):277–292

el-Nahal HM, Kaddah MA, Hassan SI, Abdel Ghany A, Ibrahim AM, Ramzy RM, Mostafa EA (1998b) Effect of *Schistosoma mansoni* infection on offsprings born from infected mothers. J Egypt Soc Parasitol 28(2):523–238

El-Sokkary GH, Omar HM, Hassanein AM, Cuzzocera S, Reiter RJ (2002) Melatonin reduces oxidative damage and increases survival of mice infected with *Schistosoma mansoni*. Free Radic Biol Med 32:319–332

Endriss Y, Escher E, Rohr B, Rohr H, Weiss N (2005) Kato-Katz technique for helminth eggs. Methods in parasitology. Swiss Tropical Institute, Basel. Chapter 8. www.tropeduweb.ch/Parasitology_Methods_PDF/8_Stool_Kato-Katz.pdf

Engels D, Chitsulo L, Montresor A, Savioli L (2002) The global epidemiological situation of schistosomiasis and new approaches to control and research. Acta Trop 82:139–146

Enk MJ, Lima AC, Drummond SC, Schall VT, Coelho PM (2008) The effect of the number of stool samples on the observed prevalence and the infection intensity with *Schistosoma mansoni* among a population in an area of low transmission. Acta Trop 108(2–3):222–228

Eriksson J, Reimert CM, Kabatereine NB, Kazibwe F, Ireri E, Kadzo H, Eltahir HB, Mohamed AO, Vennervald BJ, Venge P (2007) The 434(G>C) polymorphism within the coding sequence of Eosinophil Cationic Protein (ECP) correlates with the natural course of *Schistosoma mansoni* infection. Int J Parasitol 37(12):1359–1366

Erko B, Medhin G, Teklehaymanot T, Degarege A, Legesse M (2013) Evaluation of urine-circulating cathodic antigen (urine-CCA) cassette test for the detection of *Schistosoma mansoni* infection in areas of moderate prevalence in Ethiopia. Trop Med Int Health 18(8):1029–1035. https://doi.org/10.1111/tmi.12117

Eyayu T, Zeleke AJ, Worku L (2020) Current status and future prospects of protein vaccine candidates against *Schistosoma mansoni* infection. Parasite Epidemiol Control 11:e00176

Ezeamama AE, McGarvey ST, Hogan J, Lapane KL, Bellinger DC, Acosta LP, Leenstra T, Olveda RM, Kurtis JD (2012) Friedman JF (2012) Treatment for *Schistosoma japonicum*, reduction of intestinal parasite load, and cognitive test score improvements in school-aged children. PLoS Negl Trop Dis 6(5):e1634

Ezeamama AE, Bustinduy AL, Nkwata AK, Martinez L, Pabalan N, Boivin MJ, King CH (2018) Cognitive deficits and educational loss in children with schistosome infection-A systematic review and meta-analysis. PLoS Negl Trop Dis 12(1):e0005524

Fallon PG, Richardson EJ, McKenzie GJ, McKenzie AN (2000) Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. J Immunol 164:2585–2591

Faust CL, Osakunor DNM, Downs JA, Kayuni S, Stothard JR, Lamberton PHL, Reinhard-Rupp J, Rollinson D (2020) Schistosomiasis control: leave no age group behind. Trends Parasitol 36(7): 582–591

Ferrari TC, Moreira PR (2011) Neuroschistosomiasis: clinical symptoms and pathogenesis. Lancet Neurol 10:853–864

Ferrari TC, Gazzinelli G, Correa-Oliveira R (2008) Immune response and pathogenesis of neuroschistosomiasis mansoni. Acta Trop 108:83–88

Ferreira RC, Domingues AL, Bandeira AP, Markman Filho B, Albuqerque Filho ES, Correiade de Araújo AC, Batista LJ, Markman M, Campelo AR (2009) Prevalence of pulmonary hypertension in patients with schistosomal liver fibrosis. Ann Trop Med Parasitol 103(2):129–143

Figueiredo JP, Oliveira RR, Cardoso LS, Barnes KC, Grant AV, Carvalho EM, Araujo MI (2012) Adult worm-specific IgE/IgG4 balance is associated with low infection levels of *Schistosoma mansoni* in an endemic area. Parasite Immunology 34(12):604–610

Fitzsimmons CM, Schramm G, Jones FM, Chalmers IW, Hoffmann KF, Grevelding CG, Wuhrer M, Hokke CH, Haas H, Doenhoff MJ, Dunne DW (2005) Molecular characterization of omega-1: a hepatotoxic ribonuclease from *Schistosoma mansoni* eggs. Mol Biochem Parasitol 144(1):123–127

Fitzsimmons CM, Jones FM, Pinot de Moira A, Protasio AV, Khalife J, Dickinson HA, Tukahebwa EM, Dunne DW (2012) Progressive cross-reactivity in IgE responses: an explanation for the slow development of human immunity to schistosomiasis? Infect Immun 80(12):4264–4270

Fornaciari G, Gaeta R (2014) Paleoparasitology of helminths. In: Bruschi F (ed) Helminth infections and their impact on global public health. Springer-Verlag, Wien, pp 29–47

Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS et al (2000) The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet 355: 887–891

Frickmann H, Lunardon LM, Hahn A, Loderstädt U, Lindner AK, Becker SL, Mockenhaupt FP, Weber C, Tannich E (2021) Evaluation of a duplex real-time PCR in human serum for simultaneous detection and differentiation of *Schistosoma mansoni* and *Schistosoma haematobium* infections - a cross-sectional study. Travel Med Infect Dis 25:102035

Friedman SL (2000) Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. J Biol Chem 275:2247–2250

Friedman JF, Kanzaria HK, McGarvey ST (2005) Human schistosomiasis and anemia: the relationship and potential mechanisms. Trends Parasitol 21:386–392

Friedman JF, Mital P, Kanzaria HK, Olds GR, Kurtis JD (2007) Schistosomiasis and pregnancy. Trends Parasitol 23:159–164

Fuss A, Mazigo HD, Tappe D, Kasang C, Mueller A (2018) Comparison of sensitivity and specificity of three diagnostic tests to detect *Schistosoma mansoni* infections in school children in Mwanza region, Tanzania. PLoS One 13(8):e0202499

Fuss A, Mazigo HD, Mueller A (2020) Evaluation of serum-based real-time PCR to detect *Schistosoma mansoni* infection before and after treatment. Infect Dis Poverty 9(1):74

Gad YZ, Ahmad NA, El-Desoky I, Arafa MM, Farag RE (2011) Colorectal schistosomiasis: Is it still endemic in delta Egypt, early in the third millennium? Trop Parasitol 1(2):108–110

Gan Y, Shi YE, Bu LY, Ning CX, Zhu HG (2005) Vaccination of mice with recombinant nucleic acid vaccine encoding the integral membrane protein Sj23 and cytokine IL-12 elicits specific immune responses against *Schistosoma japonica*. Zhonghua Yi Xue Za Zhi 85(3):193–198

Ganley-Leal LM, Guarner J, Todd CW, Da'Dara AA, Freeman GL Jr, Boyer AE, Harn DA, Secor WE (2005) Comparison of *Schistosoma mansoni* irradiated cercariae and Sm23 DNA vaccines. Parasite Immunol 27(9):341–349

Ganley-Leal LM, Mwinzi PN, Cetre-Sossah CB, Andove J, Hightower AW, Karanja DM, Colley DG, Secor WE (2006) Correlation between eosinophils and protection against reinfection with *Schistosoma mansoni* and the effect of human immunodeficiency virus type 1 coinfection in humans. Infect Immun 74(4):2169–2176

Garjito TA, Sudomo M, Abdullah DM, Nurwidayati A (2008) Schistosomiasis in Indonesia: past and present. Parasitol Int 57(3):277–280

Gharib B, Hanna S, Abdallahi OM, Leidi H, Gardette B, De Reggi M (2001) Antiinflammatory properties of molecular hydrogen: investigation on parasite induced liver inflammation. CR Academie des Sciences III 324:719–724

Ghoneim MA, El-Mekresh MM, El-Baz MA, El-Attar IA, Ashamallah A (1997) Radical cystectomy for carcinoma of the bladder, critical evaluation of the results in 1026 cases. J Urol 158(2):393–399

Glinz D, Silué KD, Knopp S, Lohourignon LK, Yao KP, Steinmann P, Rinaldi L, Cringoli G, N'Goran EK, Utzinger J (2010) Comparing diagnostic accuracy of Kato-Katz, Koga agar plate, ether-concentration, and FLOTAC for *Schistosoma mansoni* and soil-transmitted helminths. PLoS Negl Trop Dis 4(7):e754

Gouveia MJ, Brindley PJ, Azevedo C, Gärtner F, da Costa JMC, Vale N (2019) The antioxidants resveratrol and N-acetylcysteine enhance anthelmintic activity of praziquantel and artesunate against *Schistosoma mansoni*. Parasit Vectors 12(1):309

Gray DJ, McManus DP, Li Y, Williams GM, Bergquist R, Ross AG (2010) Schistosomiasis elimination: lessons from the past guide the future. Lancet Infect Dis 10:733–736

Greenberg RM (2005) Ca2+ signalling, voltage-gated Ca2+ channels and praziquantel in flatworm neuromusculature. Parasitology 131(Suppl):S97–S108

Greenberg RM (2013) ABC multidrug transporters in schistosomes and other parasitic flatworms. Parasitol Int 62(6):647–653

Gryseels B (1996) Uncertainties in the epidemiology and control of schistosomiasis. Am J Trop Med Hyg 55(5 Suppl):103–108

Gryseels B, Polman K, Clerinx J, Kestens L (2006) Human schistosomiasis. Lancet 368:1106–1118

Gurarie D, Wang X, Bustinduy AL, King CH (2011) Modeling the effect of chronic schistosomiasis on childhood development and the potential for catch-up growth with different drug treatment strategies promoted for control of endemic schistosomiasis. Am J Trop Med Hyg 84(5):773–781

Gutierrez Y (2000) Diagnostic pathology of parasitic infections with clinical correlation, 2nd edn. Oxford University Press, New York, pp 559–562

Habib MR, Lv S, Rollinson D, Zhou XN (2021) invasion and dispersal of *Biomphalaria* species: increased vigilance needed to prevent the introduction and spread of schistosomiasis. Front Med (Lausanne) 8:614797

Hagan P, Blumenthal UJ, Dunn D, Simpson AJ, Wilkins HA (1991) Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. Nature 349(6306):243–245

Hailegebriel T, Nibret E, Munshea A (2020) Prevalence of *Schistosoma mansoni* and *S. haematobium* in snail intermediate hosts in Africa: a systematic review and meta-analysis. J Trop Med 2020:8850840

Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM et al (2005) Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 6:1123–1132

Harrop R, Jennings N, Mountford AP, Coulson PS, Wilson RA (2000) Characterization, cloning and immunogenicity of antigens released by transforming cercariae of *Schistosoma mansoni*. Parasitology 121(Pt 4):385–394

Henri S, Chevillard C, Mergani A, Paris P, Gaudart J, Camilla C, Dessein H, Montero F, Elwali NE, Saeed OK, Magzoub M, Dessein AJ (2002) Cytokine regulation of periportal fibrosis in humans infected with *Schistosoma mansoni*: IFN-gamma is associated with protection against fibrosis and TNF-alpha with aggravation of disease. J Immunol 169(2):929–236

Hoffmann KF, Cheever AW, Wynn TA (2000) IL-10 and the dangers of immune polarization: excessive type 1 and type 2 cytokine responses induce distinct forms of lethal immunopathology in murine schistosomiasis. J Immunol 164(12):6406–6416

Hoffmann KF, Wynn TA, Dunne DW (2002) Cytokine-mediated host responses during schistosome infections; walking the fine line between immunological control and immunopathology. Adv Parasitol 52:265–307

Hong-Bao L, Ming S (2016) Efficacy of repeated application of praziquantel in treatment of hepatic fibrosis due to schistosomiasis. Zhongguo Xue xi Chong Bing fang Zhi Za Zhi 29(2):241–242

Hsu SY, Hsu HF, Davis JR, Lust GL (1972) Comparative studies on the lesions caused by eggs of *Schistosoma japonicum* and *Schistosoma mansoni* in livers of albino mice and rhesus monkeys. Ann Trop Med Parasitol 66:89–97

Hughes AJ, Biggs BA (2002) Parasitic worms of the central nervous system: an Australian perspective. Intern Med J 32:541–553

Hurst MH, Willingham AL, Lindberg R (2000) Tissue responses in experimental schistosomiasis japonica in the pig: a histopathologic study of different stages of single low- or high-dose infections. Am J Trop Med Hyg 62:45–56

Ismail M, Metwally A, Farghaly A, Bruce J, Tao LF, Bennett JL (1996) Characterization of isolates of *Schistosoma mansoni* from Egyptian villagers that tolerate high doses of praziquantel. Am J Trop Med Hyg 55:214–218

Jang-Lee J, Curwen RS, Ashton PD, Tissot B, Mathieson W, Panico M, Dell A, Wilson RA, Haslam SM (2007) Glycomics analysis of *Schistosoma mansoni* egg and cercarial secretions. Mol Cell Proteomics 6(9):1485–1499

Jankovic D, Cheever AW, Kullberg MC et al (1998) CD4+ T cell mediated granulomatous pathology in schistosomiasis is downregulated by a B cell-dependent mechanism requiring fc receptor signaling. J Exp Med 187:619–629

Ji F, Liu Z, Cao J et al (2008) B cell response is required for granuloma formation in the early infection of *Schistosoma japonicum*. PLoS One 3:e1724

Jiz M, Wu HW, Meng R, Pond-Tor S, Reynolds M, Friedman JF, Olveda R, Acosta L, Kurtis JD (2008) Pilot-scale production and characterization of paramyosin, a vaccine candidate for schistosomiasis japonica. Infect Immun 76(7):3164–3169

Jiz M, Friedman JF, Leenstra T, Jarilla B, Pablo A, Langdon G, Pond-Tor S, Wu HW, Manalo D, Olveda R, Acosta L, Kurtis JD (2009) Immunoglobulin E (IgE) responses to paramyosin predict resistance to reinfection with *Schistosoma japonicum* and are attenuated by IgG4. Infect Immun 77(5):2051–2058

Joseph S, Jones FM, Kimani G, Mwatha JK, Kamau T, Kazibwe F, Kemijumbi J, Kabatereine NB, Booth M, Kariuki HC, Ouma JH, Vennervald BJ, Dunne DW (2004a) Cytokine production in whole blood cultures from a fishing community in an area of high endemicity fo*r Schistosoma mansoni* in Uganda: the differential effect of parasite worm and egg antigens. Infect Immun 72(2):728–734

Joseph S, Jones FM, Walter K, Fulford AJ, Kimani G, Mwatha JK, Kamau T, Kariuki HC, Kazibwe F, Tukahebwa E, Kabatereine NB, Ouma JH, Vennervald BJ, Dunne DW (2004b) Increases in human T helper 2 cytokine responses to *Schistosoma mansoni* worm and worm-tegument antigens are induced by treatment with praziquantel. J Infect Dis 190(4):835–842

Jourdan PM, Roald B, Poggensee G, Gundersen SG (2011) Kjetland EF (2011) increased vascularity in cervicovaginal mucosa with *Schistosoma haematobium* infection. PLoS Negl Trop Dis 5:1–7

Jumawan JC, Estaño LA (2021) Prevalence of *Schistosoma japonicum* in bovines and *Oncomelania hupensis quadrasi* from ricefields surrounding Lake Mainit, Philippines. J Parasit Dis Mar 15:1–8. https://doi.org/10.1007/s12639-021-01372-3

Kallestrup P, Zinyama R, Gomo E, Butterworth AE, Mudenge B, vanDam GJ et al (2005) Schistosomiasis and HIV-1 infection in rural Zimbabwe: effect of treatment of schistosomiasis on CD4 cell count and plasma HIV-1 RNA load. J Infect Dis 192:1956–1961

Kallestrup P, Zinyama R, Gomo E, Butterworth AE, van Dam GJ, Gerstoft J, Erikstrup C, Ullum H (2006) Schistosomiasis and HIV in rural Zimbabwe: efficacy of treatment of schistosomiasis in individuals with HIV coinfection. Clin Infect Dis 42(12):1781–1789

Kamal SM, Turner B, He Q, Rasenack J, Bianchi L, Al Tawil A et al (2006) Progression of fibrosis in hepatitis C with and without schistosomiasis: correlation with serum markers of fibrosis. Hepatology 43:771–779

Kamdem SD, Moyou-Somo R, Brombacher F, Nono JK (2018) Host regulators of liver fibrosis during human schistosomiasis. Front Immunol 9:2781

Kappagoda S, Singh U, Blackburn BG (2011) Antiparasitic therapy. Mayo Clin Proc 86(6): 561–583

Kasinathan RS, Greenberg RM (2010) Schistosoma mansoni soluble egg antigens trigger erythrocyte cell death. Cell Physiol Biochem 26(4–5):767–774

Kasinathan RS, Morgan WM, Greenberg RM (2010) *Schistosoma mansoni* express higher levels of multidrug resistance-associated protein 1 (SmMRD1) in juvenile worms and in response to praziquantel. Mol Biochem Parasitol 173:25–31

Katchanov J, Nawa Y (2010) Helminthic invasion of the central nervous system: many roads lead to Rome. Parasitol Int 59:491–496

Keiser J, Utzinger J (2007) Advances in the discovery and development of novel trematocidal drugs. Expert Opin Drug Discov 2(Suppl. 1):9–23

Keiser J, Chollet J, Xiao SH, Mei JY, Jiao PY, Utzinger J et al (2009) Mefloquine – an aminoalcohol with promising antischistosomal properties in mice. PLoS Negl Trop Dis 3:e350

Keitel WA, Potter GE, Diemert D, Bethony J, El Sahly HM, Kennedy JK, Patel SM, Plieskatt JL, Jones W, Deye G, Bottazzi ME, Hotez PJ, Atmar RL (2019) A phase 1 study of the safety, reactogenicity, and immunogenicity of a *Schistosoma mansoni* vaccine with or without glucopyranosyl lipid an aqueous formulation (GLA-AF) in healthy adults from a non-endemic area. Vaccine 37:6500–6509

Khaled H (2013) Schistosomiasis and cancer in Egypt: review. J Adv Res 4:461–466

Kifle DW, Chaiyadet S, Waardenberg AJ, Wise I, Cooper M, Becker L, Doolan DL, Laha T, Sotillo J, Pearson MS, Loukas A (2020a) Uptake of *Schistosoma mansoni* extracellular vesicles by human endothelial and monocytic cell lines and impact on vascular endothelial cell gene expression. Int J Parasitol 50:685–696

Kifle DW, Pearson MS, Becker L, Pickering D, Loukas A, Sotillo J (2020b) Proteomic analysis of two populations of *Schistosoma mansoni*-derived extracellular vesicles: 15k pellet and 120k pellet vesicles. Mol Biochem Parasitol 236:111264

Kimura E, Moji K, Uga S, Kiliku FM, Migwi DK, Mutua WR, Muhoho ND, Aoki Y (1992) Effects of *Schistosoma haematobium* infection on mental test scores of Kenyan school children. Trop Med Parasitol 43(3):155–158

King CH (2010) Parasites and poverty: the case of schistosomiasis. Acta Trop 113(2):95–104

King CH, Dangerfield-Cha M (2008) The unacknowledged impact of chronic schistosomiasis. Chronic Illn 4(1):65–79

King CH, Magak P, Salam EA, Ouma JH, Kariuki HC, Blanton RE, World Health Organization (2003) Measuring morbidity in schistosomiasis mansoni: relationship between image pattern, portal vein diameter and portal branch thickness in large-scale surveys using new WHO coding guidelines for ultrasound in schistosomiasis. Tropical Med Int Health 8:109–117

King CH, Dickman K, Tisch DJ (2005) Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. Lancet 365(9470): 1561–1569

King CH, Sutherland LJ, Bertsch D (2015) Systematic review and meta-analysis of the impact of chemical-based molluscicide for control of *Schistosoma mansoni* and *S. haematobium* transmission. PLoS Negl Trop Dis 9:e0004290

King CH, Yoon N, Wang X, Lo NC, Alsallaq R, Ndeffo-Mbah M, Li E, Gurarie D (2020) Application of schistosomiasis consortium for operational research and evaluation study findings to refine predictive modeling of *Schistosoma mansoni* and *Schistosoma haematobium* control in Sub-Saharan Africa. Am J Trop Med Hyg 103(1_Suppl):97–104

Kjetland EF, Ndhlovu PD, Gomo E, Mduluza T, Midzi N, Gwanzura L et al (2006) Association between genital schistosomiasis and HIV in rural Zimbabwean women. AIDS 20:593–600

Koraitim K (1994) The history of bilharziasis. In: Ikinger U et al (eds) Schistosomiasis: new aspects of an old disease. Ullstein Mosby, Berlin, pp 11–21

Krauth SJ, Coulibaly JT, Knopp S, Traoré M, N'Goran EK, Utzinger J (2012) An in-depth analysis of a piece of shit: distribution of *Schistosoma mansoni* and hookworm eggs in human stool. PLoS Negl Trop Dis 6(12):e1969

Kumar V, Gryseels B (1994) Use of praziquantel against schistosomiasis: a review of current status. Int J Antimicrob Agents 4:313

Kura K, Ayabina D, Toor J, Hollingsworth TD, Anderson RM (2021) Disruptions to schistosomiasis programmes due to COVID-19: an analysis of potential impact and mitigation strategies. Trans R Soc Trop Med Hyg 115(3):236–244

Kurtis JD, Higashi A, Wu HW, Gundogan F, McDonald EA, Sharma S, PondTor S, Jarilla B, Sagliba MJ, Gonzal A, Olveda R, Acosta L, Friedman JF (2011) Maternal Schistosomiasis japonica is associated with maternal, placental, and fetal inflammation. Infect Immun 79(3): 1254–1261

Lambertucci JR, dos Santos Silva LC, Andrade LM, de Queiroz LC, Carvalho VT, Voieta I, Antunes CM (2008) Imaging techniques in the evaluation of morbidity in schistosomiasis mansoni. Acta Trop 108:209–217

Le L, Molehin AJ, Nash S, Sennoune SR, Ahmad G, Torben W, Zhang W, Siddiqui AA (2018) Schistosoma egg-induced liver pathology resolution by Sm-p80-based schistosomiasis vaccine in baboons. Pathology 50:442–449

Leblanc C, Brun S, Bouchaud O, Izri A, Ok V, Caseris M, Sorge F, Pham LL, Paugam A, Paris L, Jaureguiberry S, Bloch-Queyrat C, Boubaya M, Faye A, Mariani P, de Pontual L (2021) Imported schistosomiasis in Paris region of France: a multicenter study of prevalence and diagnostic methods. Travel Med Infect Dis 27:102041

Leiper RT (1916) On the relation between the terminal-spined and lateral-spined eggs of bilharzia. Br Med J i:411

Leonardo L, Rivera P, Saniel O, Antonio Solon J, Chigusa Y, Villacorte E, Christoper Chua J, Moendeg K, Manalo D, Crisostomo B, Sunico L, Boldero N, Payne L, Hernandez L, Velayudhan R (2013) New endemic foci of schistosomiasis infections in the Philippines. Acta Trop 141:354–360. https://doi.org/10.1016/j.actatropica.2013.03.015

Lin DD, Liu JX, Liu YM, Hu F, Zhang YY, Xu JM, Li JY, Ji MJ, Bergquist R, Wu GL, Wu HW (2008) Routine Kato-Katz technique underestimates the prevalence of *Schistosoma japonicum*: a case study in an endemic area of the People's Republic of China. Parasitol Int 57(3):281–286

LoVerde P, Amento C, Higashi G (1980) Parasite–parasite interaction of *salmonella typhimurium* and *Schistosoma*. J Infect Dis 141:177–185

Lucas SB (2002) Other viral and infectious diseases and HIV-related liver diseases. In: RM MS et al (eds) Pathology of the liver, 4th edn. Harcour Publishers Limited, Churchill Livingstone, London. Chapter 8, pp 389–392

Ludvigsson JF (2020) Systematic review of COVID-19 in children shows milder cases and a better prognosis than adults. Acta Paediatr 109:1088–1095

Lundy SK, Lerman SP, Boros DL (2001) Soluble egg antigen stimulated T-helper lymphocyte apoptosis and evidence for cell death mediated by FasL+T and B cells during murine *Schistosoma mansoni* infection. Infect Immun 69:271–280

Mahana NA (2006) Human and murine immune responses to the *Schistosoma mansoni* glucose transporter. Ph.D. Thesis, Faculty of Science, Cairo University. 240 p

Mallat A, Preaux AM, Blazejewski S, Rosenbaum J, Dhumeaux D, Mavier P (1995) Interferon alfa and gamma inhibit proliferation and collagen synthesis of human Ito cells in culture. Hepatology 21(4):1003–1010

Mangano VD, Bianchi C, Ouedraogo M, Kabore Y, Corran P, Silva N, Sirima SB, Nebie I, Bruschi F, Modiano D (2020) Antibody response to *Schistosoma haematobium* and other helminth species in malaria-exposed populations from Burkina Faso. Acta Trop 205:105381

Mathieson W, Wilson RA (2010) A comparative proteomic study of the undeveloped and developed *Schistosoma mansoni* egg and its contents: the miracidium, hatch fluid and secretions. Int J Parasitol 40(5):617–628

Maubach G, Lim MCC, Zhang CY, Zhuo L (2006) GFAP promoter directs lacZ expression specifically in a rat hepatic stellate cell line. World J Gastroenterol 12:723–730

Mazigo HD, Nuwaha F, Kinung'hi SM, Morona D, Pinot de Moira A, Wilson S, Heukelbach J, Dunne DW (2012) Epidemiology and control of human schistosomiasis in Tanzania. Parasit Vectors 5:274

Mazigo HD, Nuwaha F, Wilson S, Kinung'hi SM, Morona D, Waihenya R, Heukelbach J, Dunne DW (2013) Epidemiology and interactions of human immunodeficiency virus - 1 and *Schistosoma mansoni* in sub-Saharan Africa. Infect Dis Poverty 2(1):2

Mazigo HD, Uisso C, Kazyoba P, Nshala A, Mwingira UJ (2021) Prevalence, infection intensity and geographical distribution of schistosomiasis among pre-school and school aged children in villages surrounding Lake Nyasa, Tanzania. Sci Rep 11(1):295

McGarvey ST, Aligui A, Graham K et al (1996) Schistosomiasis japonica and childhood nutritional status in North-Eastern Leyte, the Philippines: a randomized trial of praziquantel versus placebo. Am J Trop Med Hyg 54:498–502

McManus DP, Dunne DW, Sacko M, Utzinger J, Vennervald BJ, Zhou XN (2018) Schistosomiasis. Nat Rev Dis Primers 4(1):13

McManus DP, Bergquist R, Cai P, Ranasinghe S, Tebeje BM, You H (2020) Schistosomiasis-from immunopathology to vaccines. Semin Immunopathol 42(3):355–371

Mehlhorn H, Becker B, Andrews P, Thomas H, Frenkel JK (1981) In vivo and in vitro experiments on the effects of praziquantel on *Schistosoma mansoni*. a light and electron microscopic study. Arzneim Forsch/Drug Res 31:544–554

Mehlhorn H, Frenkel JK, Andrews P, Thomas H (1982) Light and electron microscopic studies on Schistosoma mansoni granulomas of mouse livers following treatment with praziquantel. Tropenmed Parasitol 33:229–239

Melhem R, LoVerde P (1984) Mechanism of interaction of *salmonella* and *Schistosoma* species. Infect Immun 44:274–281

Melman SD, Steinauer ML, Cunningham C, Kubatko LS, Mwangi IN, Barker Wynn N, Mutuku MW, Karanja DMS, Colley DG, Black CL, Secor WE, Mkoji GM, Loker ES (2009) Reduced susceptibility to praziquantel among naturally occurring Kenyan isolates of *Schistosoma mansoni*. PLoS Negl Trop Dis 3:e504

Migliardo F, Tallima H, El Ridi R (2014) Is there a sphingomyelin-based hydrogen bond barrier at the mammalian host-schistosome parasite interface? Cell Biochem Biophys 68(2):359–367

Mo AX, Agosti JM, Walson JL, Hall BF, Gordon L (2014) Schistosomiasis elimination strategies and potential role of a vaccine in achieving global health goals. Am J Trop Med Hyg 90:54–60

Molehin AJ (2020) Schistosomiasis vaccine development: update on human clinical trials. J Biomed Sci 27(1):28

Molvik M, Heiland E, Zulu SG, Kleppa E, Lillebo K, Gundersen SG, Kvalsvig JD, Taylor M, Kjetland EF, Vennervald BJ (2017) Coinfection with *Schistosoma haematobium* and soil-transmitted helminths in rural South Africa. South Afr J Sci 113:1–6

Molyneux DH (2008) Combating the "other diseases" of MDG 6 changing the paradigm to achieve equity and poverty reduction? Trans R Soc Trop Med Hyg 102:509–519

Mostafa IM, Zakaria S, Khalil A, El-Kaluoby A (1990) The effect of medical treatment and endoscopic polypectomy on clinicopathological, immunological and endoscopic aspects of schistosomal colonic polyposis in Egypt. In: Proceedings of the world congresses of gastroenterology Sydney, Australia; 1260

Mostafa OM, Bin Dajem SM, Al-Qahtani A, Ibrahim EH, Al-Quraishy SA (2012) Developing species-specific primers to identify *Bulinus truncatus* and *Bulinus beccari*, the intermediate hosts of *Schistosoma haematobium* in Saudi Arabia. Gene 499(2):256–261

Mounntford AP, Trottein F (2004) Schistosomes in the skin: a balance between immune priming and regulation. Trends Parasitol 20(5):221–226

Mutengo MM, Mduluza T, Kelly P, Mwansa JCL, Kwenda G, Musonda P, Chipeta J (2018) Low Il-6, Il-10, and TNF-α and high Il-13 cytokine levels are associated with severe hepatic fibrosis in *Schistosoma mansoni* chronically exposed individuals. J Parasitol Res 2018:9754060

Naus CW, Jones FM, Satti MZ, Joseph S, Riley EM, Kimani G, Mwatha JK, Kariuki CH, Ouma JH, Kabatereine NB, Vennervald BJ, Dunne DW (2003) Serological responses among individuals in areas where both schistosomiasis and malaria are endemic: cross-reactivity between *Schistosoma mansoni* and *Plasmodium falciparum*. J Infect Dis 187(8):1272–1282

Nazel MW, El-Morshedy H, Farghaly A, Shatat H, Barakat R (1999) *Schistosoma mansoni* infection and cognitive functions of primary school children, in Kafr El Sheikh, Egypt. J Egypt Public Health Assoc 74(1–2):97–119

Ndlovu H, Nono JK, Abdel Aziz N, Nieuwenhuizen NE, Brombacher F (2018) Interleukin-4 receptor alpha expressing B cells are essential to down-modulate host granulomatous inflammation during schistosomasis. Front Immunol 9:2928

Njaanake KH, Vennervald BJ, Simonsen PE, Simonsen PE, Madsen H, Mukoko DA, Kimani G, Jaoko WG, Estambale BB (2016) *Schistosoma haematobium* and soil-transmitted helminths in Tana delta district of Kenya: infection and morbidity patterns in primary schoolchildren from two isolated villages. BMC Infect Dis 16:57

Obeng BB, Aryeetey YA, de Dood CJ, Amoah AS, Larbi IA, Deelder AM, Yazdanbakhsh M, Hartgers FC, Boakye DA, Verweij JJ, van Dam GJ, van Lieshout L (2008) Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of *Schistosoma haematobium* in urine samples from Ghana. Ann Trop Med Parasitol 102(7):625–633

Olds GR, King C, Hewlett J et al (1999) Double-blind placebo-controlled study of concurrent administration of albendazole and praziquantel in schoolchildren with schistosomiasis and geohelminths. J Infect Dis 179:996–100

Olveda RM, Gray DJ (2019) Schistosomiasis in the Philippines: Innovative control approach is needed if elimination is the goal. Trop Med Infect Dis 4(2):66

Olveda DU, Inobaya M, Olveda RM, Vinluan ML, Ng SK, Weerakoon K, McManus DP, Ramm GA, Harn DA, Li Y, Lam AK, Guevarra JR, Ross AG (2017) Diagnosing schistosomiasis-induced liver morbidity: implications for global control. Int J Infect Dis 54:138–144

Omar HH (2019) Impact of chronic schistosomiasis and HBV/HCV co-infection on the liver: current perspectives. Hepat Med 11:131–136

Osakunor DNM, Woolhouse MEJ, Mutapi F (2018) Paediatric schistosomiasis: what we know and what we need to know. PLoS Negl Trop Dis 12(2):e0006144

Othman AA, Soliman RH (2015) Schistosomiasis in Egypt: a never-ending story? Acta Trop 148: 179–190

Othman AA, Shoheib ZS, Abdel-Aleem GA, Shareef MM (2008) Experimental schistosomal hepatitis: protective effect of coenzyme-Q10 against the state of oxidative stress. Exp Parasitol 120:147–155

Othman AA, Shoheib ZS, Saied EM, Soliman RH (2010) Congenital exposure to *Schistosoma mansoni* infection: impact on the future immune response and the disease outcome. Immunobiology 215:101–112

Oyeyemi OT, Okunlola OA, Adebayo AD (2020) Assessment of schistosomiasis endemicity and preventive treatment on coronavirus disease 2019 outcomes in Africa. New Microbe New Infect 38:100821

Parola M, Robino G (2001) Oxidative stress-related molecules and liver fibrosis. J Hepatol 35:297–306

Parraga IM, Assis AM, Prado MS, Barreto ML, Reis MG, King CH, Blanton RE (1996) Gender differences in growth of school-aged children with schistosomiasis and geohelminth infection. Am J Trop Med Hyg 55(2):150–156

Parreira NA, Ramalho FS, Augusto MJ, Silva DM, Prado CM, Elias Júnior J, Rodrigues V, Ramalho LNZ (2018) The comparative efficacy of renin-angiotensin system blockers in schistosomal hepatic fibrosis. Exp Parasitol 191:9–18

Pascal M, Abdallahi OM, Elwali NE, Mergani A, Qurashi MA, Magzoub M, de Reggi M, Gharib B (2000) Hyaluronate levels and markers of oxidative stress in the serum of Sudanese subjects at risk of infection with *Schistosoma mansoni*. Trans R Soc Trop Med Hyg 94(1):66–70

Pearce EJ, MacDonald AS (2002) The Immunobiology of schistosomiasis. Nat Rev Immunol 2: 499–511

Pereira TA, Syn WK, Machado MV, Vidigal PV, Resende V, Voieta I, Xie G, Otoni A, Souza MM, Santos ET et al (2015) Schistosome-induced cholangiocyte proliferation and osteopontin secretion correlate with fibrosis and portal hypertension in human and murine schistosomiasis mansoni. Clin Sci (Lond) 129(10):875–883

Pica-Mattoccia L, Cioli D (1985) Studies on the mode of action of oxamniquine and related schistosomicidal drugs. Am J Trop Med Hyg 34:112–118

Pierce RJ, Dubois-Abdesselem F, Caby S, Trolet J, Lancelot J, Oger F et al (2011) Chromatin regulation in schistosomes and histone modifying enzymes as drug targets. Mem Inst Oswaldo Cruz 106:794–801

Pinto-Silva RA, Queiroz LC, Azeredo LM, Silva LC, Lambertucci JR (2010) Ultrasound in schistosomiasis mansoni. Mem Inst Oswaldo Cruz 105:479–484

Pivarcsi A, Kemény L, Dobozy A (2004) Innate immune functions of the keratinocytes. A review Acta Microbiol Immunol Hungarica 51(3):303–310

Platt TR, Brooks DR (1997) Evolution of the schistosomes (Digenea: Schistosomatoidea): the origin of dioecy and colonization of the venous system. J Parasitol 83(6):1035–1044

Pontes LA, Oliveira MC, Katz N, Dias-Neto E, Rabello A (2003) Comparison of a polymerase chain reaction and the Kato-Katz technique for diagnosing infection with Schistosoma mansoni. Am J Trop Med Hyg 68(6):652–656

Qokoyi NK, Masamba P, Munsamy G, Kappo AP (2021) Diagnostic, prognostic and therapeutic potential of heat shock proteins in schistosomiasis and bladder cancer: a review. Lett Drug Design Disc 18(7):650–665

Qunhua L, Jiawen Z, Bozhao L, Zhilan P, Huijie Z, Shaoying W, Delun M, Hsu LN (2000) Investigation of association between female genital tract diseases and schistosomiasis japonica infection. Acta Trop 77:179–183

Rajekar H, Vasishta RK, Chawla YK, Dhiman RK (2011) Noncirrhotic portal hypertension. J Clin Exp Hepatol 1:94–108

Ramaswamy K, Kumar P, He YX (2000) A role for parasite-induced PGE2 in IL-10-mediated host immunoregulation by skin stage schistosomula of *Schistosoma mansoni*. J Immunol 165(8): 4567–4574

Reed SG, Carter D, Casper C, Duthie MS, Fox CB (2018) Correlates of GLA family adjuvants' activities. Semin Immunol 39:22–29

Reiman RM, Thompson RW, Feng CG, Hari D, Knight R, Cheever AW, Rosenberg HF, Wynn TA (2006) Interleukin-5 (IL-5) augments the progression of liver fibrosis by regulating IL-13 activity. Infect Immun 74:1471–1479

Reis EA, Mauadi Carmo TA, Athanazio R, Reis MG, Harn DA Jr (2008) *Schistosoma mansoni* triose phosphate isomerase peptide MAP4 is able to trigger naïve donor immune response towards a type-1 cytokine profile. Scand J Immunol 68(2):169–176

Ricard-Blum S, Ville G, Grimaud JA (1992) Pyridinoline, a mature collagen cross-link, in fibrotic livers from *Schistosoma mansoni*-infected mice. Am J Trop Med Hyg 47:816–820

Richter J, Bode JG, Blondin D, Kircheis G, Kubitz R, Holtfreter MC, Müller-Stöver I, Breuer M, Hüttig F, Antoch G, Häussinger D (2015) Severe liver fibrosis caused by *Schistosoma mansoni*: management and treatment with a transjugular intrahepatic portosystemic shunt. Lancet Infect Dis 15(6):731–737

Rite EE, Kapalata SN, Munisi DZ (2020) Prevalence, intensity, and factors associated with urogenital schistosomiasis among women of reproductive age in Mbogwe district council, Geita region, Tanzania. Biomed Res Int 2020:5923025

Riveau G, Deplanque D, Remoué F, Schacht AM, Vodougnon H, Capron M, Thiry M, Martial J, Libersa C, Capron A (2012) Safety and immunogenicity of rSh28GST antigen in humans: phase 1 randomized clinical study of a vaccine candidate against urinary schistosomiasis. PLoS Negl Trop Dis 6(7):e1704

Riveau G, Schacht AM, Dompnier JP, Deplanque D, Seck M, Waucquier N, Senghor S, Delcroix-Genete D, Hermann E, Idris-Khodja N, Levy-Marchal C, Capron M, Capron A (2018) Safety and efficacy of the rSh28GST urinary schistosomiasis vaccine: a phase 3 randomized, controlled trial in Senegalese children. PLoS Negl Trop Dis 12:e0006968

Rojo-Arreola L, Long T, Asarnow D, Suzuki BM, Singh R, Caffrey CR (2014) Chemical and genetic validation of the statin drug target to treat the helminth disease, schistosomiasis. PLoS One 9(1):e87594

Ross AG, Vickers D, Olds GR, Shah SM, McManus DP (2007) Katayama syndrome. Lancet Infect Dis 7:218–224

Rothe C, Zimmer T, Schunk M, Wallrauch C, Helfrich K, Gültekin F, Bretzel G, Allienne JF, Boissier J (2021) Developing endemicity of schistosomiasis, Corsica, France. Emerg Infect Dis 27(1):319–321

Rumbley CA, Sugaya H, Zekavat SA, El Refaei M, Perrin PJ, Phillips SM (1999) Activated eosinophils are the major source of Th2-associated cytokines in the schistosome granuloma. J Immunol 162:1003–1009

Rutitzky LI, Mirkin GA, Stadecker MJ (2003) Apoptosis by neglect of CD4+ Th cells in granulomas: a novel effector mechanism involved in the control of egg-induced immunopathology in murine schistosomiasis. J Immunol 171:1859–1867

Saftawy EA (2021) Validity of urine-CCA cassette test and indirect haem-agglutination assay (IHA) in the detection of schistosomiasis-*mansoni* infection relative to microscopic examination. J Parasit Dis 45(1):285–292

Salim OE, Hamid HK, Mekki SO, Suleiman SH, Ibrahim SZ (2010) Colorectal carcinoma associated with schistosomiasis: a possible causal relationship. World J Surg Oncol 8:68

Santini-Oliveira M, Coler RN, Parra J, Veloso V, Jayashankar L, Pinto PM, Ciol MA, Bergquist R, Reed SG, Tendler M (2016) Schistosomiasis vaccine candidate Sm14/GLA-SE: phase 1 safety and immunogenicity clinical trial in healthy, male adults. Vaccine 34:586–594

Santos Jde F, Vasconcelos J, de Souza JR, Coutinho Ede M, Montenegro SM, Azevedo-Ximenes E (2004) The effect of *Zymomonas mobilis* culture on experimental *Schistosoma mansoni* infection. Rev Soc Bras Med Trop 37(6):502–504

Scholte RG, Carvalho OS, Malone JB, Utzinger J, Vounatsou P (2012) Spatial distribution of *Biomphalaria* spp., the intermediate host snails of *Schistosoma mansoni*, in Brazil. Geospat Health 6(3):S95–S101

Scrimgeour EM, Gajdusek DC (1985) Involvement of the central nervous system in *Schistosoma mansoni* and *S. haematobium* infection: a review. Brain 108:1023–1038

Secor WE (2012) The effects of schistosomiasis on HIV/AIDS infection, progression and transmission. Curr Opin HIV AIDS 7(3):254–259
Selim S, El Sagheer O, El Amir A, Barakat R, Hadley K, Bruins MJ, El Ridi R (2014) Efficacy and safety of arachidonic acid for treatment of *Schistosoma mansoni*-infected children in Menoufiya, Egypt. Am J Trop Med Hyg 91(5):973–981
Shaw AFB, Ghareeb AA (1938) The pathogenesis of pulmonary schistosomiasis in Egypt with special reference to Ayerza's disease. J Pathol Bacteriol 46:401–429
Siddiqui AA, Siddiqui BA, Ganley-Leal L (2011) Schistosomiasis vaccines. Hum Vaccin 7:1192–1197
Siegrist D, Siegrist-Obimpeh P (1992) *Schistosoma haematobium* infection in pregnancy. Acta Trop 50:317–321
Silva LCS, Maciel PE, Ribas JG, Souza-Pereira SR, Antunes CM, Lambertucci JR (2004) Treatment of schistosomal myeloradiculopathy with praziquantel and corticosteroids and evaluation by magnetic resonance imaging: a longitudinal study. Clin Infec Dis 39:1619–1624
Singh KP, Gerard HC, Hudson AP, Reddy TR, Boros DL (2005) Retroviral Foxp3 gene transfer ameliorates liver granuloma pathology in *Schistosoma mansoni* infected mice. Immunology 114:410–417
Singhal T (2020) A review of coronavirus disease-2019 (COVID-19). Indian J Pediatr 87(4): 281–286
Sokol CL, Barton GM, Farr AG, Medzhitov R (2008) A mechanism for the initiation of allergen-induced T helper type 2 responses. Nat Immunol 9(3):310–318
Sokol CL, Chu NQ, Yu S, Nish SA, Laufer TM, Medzhitov R (2009) Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. Nat Immunol 10(7):713–720
Sokolow SH, Wood CL, Jones IJ, Lafferty KD, Kuris AM, Hsieh MH, De Leo GA (2018) To reduce the global burden of human schistosomiasis, use 'old fashioned' snail control. Trends Parasitol 34(1):23–40
Soliman RH, Ismail OA, Badr MS, Nasr SM (2017) Resveratrol ameliorates oxidative stress and organ dysfunction in *Schistosoma mansoni* infected mice. Exp Parasitol 174:52–58
Souza MR, Toledo CF, Borges DR (2000) Thrombocytemia as a predictor of portal hypertension in schistosomiasis. Dig Dis Sci 45:1964–1970
Ssebambulidde K, Segawa I, Abuga KM, Nakate V, Kayiira A, Ellis J, Tugume L, Kiragga AN, Meya DB (2020) Parasites and their protection against COVID-19- ecology or immunology? medRxiv. https://doi.org/10.1101/2020.05.11.20098053
Stavitsky AB (2004) Regulation of granulomatous inflammation in experimental models of schistosomiasis. Infect Immun 72:1–12
Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J (2006) Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. Lancet Infect Dis 6:411–425
Stothard JR, Campbell SJ, Osei-Atweneboana MY, Durant T, Stanton MC, Biritwum NK, Rollinson D, Ombede DR, Tchuem-Tchuenté LA (2017) Towards interruption of schistosomiasis transmission in sub-Saharan Africa: developing an appropriate environmental surveillance framework to guide and to support 'end game' interventions. Infect Dis Poverty 6(1):10
Tachon P, Borojevic R (1978) Mother–child relation in human schistosomiasis mansoni: skin test and cord blood reactivity to schistosomal antigens. Trans R Soc Trop Med Hyg 72:605–609
Tagboto S, Townson S (2001) Antiparasitic properties of medicinal plants and other naturally-occurring products. Adv Parasitol 50:199–295
Tallima H, El Ridi R (2007) Praziquantel binds *Schistosoma mansoni* adult worm actin. Int J Antimicrob Agents 29:570–575
Tallima H, Dalton JP, El Ridi R (2015) Induction of protective immune responses against schistosomiasis *haematobium* in hamsters and mice using cysteine peptidase-based vaccine. Front Immunol 6:130

Tallima H, Abou El Dahab M, Kareem S, Dalton JP, El Ridi R (2017a) Protection against *Schistosoma haematobium* infection in hamsters by immunization with *Schistosoma mansoni* gut-derived cysteine peptidases, SmCB1 and SmCL3. Vaccine 135:6977–6983

Tallima H, Dvořák J, Kareem S, Abou El Dahab M, Abdel Aziz N, Dalton JP, El Ridi R (2017b) Protective immune responses against *Schistosoma mansoni* infection by immunization with functionally active gut-derived cysteine peptidases alone and in combination with glyceraldehyde 3-phosphate dehydrogenase. PLoS Negl Trop Dis 11:e0005443

Tallima H, Abou El Dahab M, El Ridi R (2019) Role of T lymphocytes and papain enzymatic activity in the protection induced by the cysteine protease against *Schistosoma mansoni* in mice. J Adv Res 17:73–84

Tallima H, Abou El Dahab M, El Ridi R (2020a) Specific antibodies and arachidonic acid mediate the protection induced by the *Schistosoma mansoni* cysteine peptidase-based vaccine in mice. Vaccine 8:682

Tallima H, Hanna VS, El Ridi R (2020b) Arachidonic acid is a safe and efficacious schistosomicide, and an endoschistosomicide in natural and experimental infections, and cysteine peptidase vaccinated hosts. Front Immunol 11:609994

Tamarozzi F, Fittipaldo VA, Orth HM, Richter J, Buonfrate D, Riccardi N, Gobbi FG (2021) Diagnosis and clinical management of hepatosplenic schistosomiasis: a scoping review of the literature. PLoS Negl Trop Dis 15(3):e0009191

Tang H, Cao W, Kasturi SP, Ravindran R, Nakaya HI, Kundu K, Murthy N, Kepler TB, Malissen B, Pulendran B (2010) The T helper type 2 response to cysteine proteases requires dendritic cell-basophil cooperation via ROS-mediated signaling. Nat Immunol 11(7):608–617

Taylor JJ, Mohrs M, Pearce ED (2006) Regulatory T cell responses develop in parallel to Th responses and control the magnitude and phenotype of the Th effector population. J Immunol 176:5839–5847

Tendler M, Simpson AJ (2008) The biotechnology-value chain: development of Sm14 as a schistosomiasis vaccine. Acta Trop 2–3:263–266

Tendler M, Almeida M, Simpson A (2015) Development of the Brazilian anti schistosomiasis vaccine based on the recombinant fatty acid binding protein Sm14 plus GLA-SE adjuvant. Front Immunol 6:218

Tendler M, Almeida MS, Vilar MM, Pint PM, Limaverde-Sousa G (2018) Current status of the Sm14/GLA-SE schistosomiasis vaccine: overcoming barriers and paradigms towards the first antiparasitic human(itarian) vaccine. Trop Med Infect Dis 3:121

Timson DJ (2020) Praziquantel: an enigmatic, yet effective, drug. In: Schistosoma mansoni: methods and protocols, methods in molecular biology, vol 2151. Springer Science+Business Media, LLC, part of Springer Nature, New York

Todd CW, Colley DG (2002) Practical and ethical issues in the development of a vaccine against schistosomiasis mansoni. Am J Trop Med Hyg 66(4):348–358

Tracy JW, Webster LT (2001) Drugs used in the chemotherapy of helminthiasis. In: Hardman JG, Limbind LE, Gilman AG (eds) Goodman and Gilman's the pharmacological basis of therapeutics, 10th edn. McGraw-Hill, New York, pp 1134–1136

Tran MH, Pearson MS, Bethony JM, Smyth DJ, Jones MK, Duke M, Don TA, McManus DP, Correa-Oliveira R, Loukas A (2006) Tetraspanins on the surface of *Schistosoma mansoni* are protective antigens against schistosomiasis. Nat Med 12(7):835–840

Tsuji N (2020) Schistosomiasis and hookworm infection in humans: Disease burden, pathobiology and anthelmintic vaccines. Parasitol Int 75:102051

Turner JD, Meurs L, Dool P, Bourke CD, Mbow M, Dièye TN, Mboup S, Polman K, Mountford AP (2013) Schistosome infection is associated with enhanced whole blood IL-10 secretion in response to cercarial excretory/secretory products. Parasite Immunol 35(5–6):147–156

Utzinger J, Xiao SH, Tanner M, Keiser J (2007) Artemisinins for schistosomiasis and beyond. Curr Opin Investig Drugs 8:105–116

Utzinger J, Raso G, Brooker S, De Savigny D, Tanner M, Ornbjerg N, Singer BH, N'goran EK (2009) Schistosomiasis and neglected tropical diseases: towards integrated and sustainable control and a word of caution. Parasitology 136(13):1859–1874

Utzinger J, N'goran EK, Caffrey CR, Keiser J (2011) From innovation to application: social-ecological context, diagnostics, drugs and integrated control of schistosomiasis. Acta Trop 120(Suppl 1):S121–S137

Vale N, Gouveia MJ, Rinaldi G, Brindley PJ, Gärtner F, Correia da Costa JM (2017) Praziquantel for schistosomiasis: single-drug metabolism revisited, mode of action, and resistance. Antimicrob Agents Chemother 61(5):e02582–e02516

Valice EM, Wiegand RE, Mwinzi PNM, Karanja DMS, Williamson JM, Ochola E, Samuels A, Verani JR, Leon JS, Secor WE, Montgomery SP (2018) Relative contribution of schistosomiasis and malaria to anemia in western Kenya. Am J Trop Med Hyg 99(3):713–715

Van Dam GJ, Bogitsh BJ, van Zeyl RJ, Rotmans JP, Deelder AM (1996) *Schistosoma mansoni*: in vitro and in vivo excretion of CAA and CCA by developing schistosomula and adult worms. J Parasitol 82(4):557–564

Van Gool T, Vetter H, Vervoort T, Doenhoff MJ, Wetsteyn J, Overbosch D (2002) Serodiagnosis of imported schistosomiasis by a combination of a commercial indirect hemagglutination test with *Schistosoma mansoni* adult worm antigens and an enzyme-linked immunosorbent assay with *S. mansoni* egg antigens. J Clin Microbiol 40(9):3432–3437

van Velthuysen ML, Florquin S (2000) Glomerulopathy associated with parasitic infections. Clin Microbiol Rev 13(1):55–66

von Lichtenberg F, Sher A, McIntyre S (1977) A lung model of schistosome immunity in mice. Am J Pathol 87(1):105–124

Wang YY, Lin C, Cao Y, Duan ZL, Guan ZX, Xu J et al (2017) Up-regulation of interleukin-21 contributes to liver pathology of schistosomiasis by driving GC immune responses and activating HSCs in mice. Sci Rep 7:16682

Watt G, Padre LP, Tuazon M, Wotherspoon A, Adapon B (1991) Hepatic parenchymal dysfunction in *Schistosoma japonicum* infection. J Infect Dis 164(6):1186–1192

Webster M, Correa-Oliveira R, Gazzinelli G, Viana IRC, Fraga LAO, Silveira AMS, Dunne DW (1997) Factors affecting high and low human IgE responses to schistosome worm antigens in an area of Brazil endemic for *Schistosoma mansoni* and hookworm. Am J Trop Med Hyg 57:487–494

WHO (2006) Preventive Chemotherapy in Human Helminthiasis. Coordinated use of anthelmintic drugs in control interventions: a manual for health professionals and programme managers. World Health Organization, Geneva

WHO (2013) Strategic plans 2012–2020. WHO, Geneva

Wilson S, Khalife J (2012) Book chapter: malaria and schistosome. In: Lamb T (ed) Immunity to parasitic infection. Wiley, Hoboken, NJ, pp 375–387

Wilson MS, Mentink-Kane MM, Pesce JT, Ramalingam TR, Thompson R, Wynn TA (2007) Immunopathology of schistosomiasis. Immunol Cell Biol 85:148–154

Wilson S, Jones FM, Mwatha JK, Kimani G, Booth M, Kariuki HC, Vennervald BJ, Ouma JH, Muchiri E, Dunne DW (2008) Hepatosplenomegaly is associated with low regulatory and Th2 responses to schistosome antigens in childhood schistosomiasis and malaria coinfection. Infect Immun 76(5):2212–2218

Wilson S, Jones FM, Fofana HK, Doucouré A, Landouré A, Kimani G, Mwatha JK, Sacko M, Vennervald BJ, Dunne DW (2013a) Rapidly boosted plasma IL-5 induced by treatment of human Schistosomiasis haematobium is dependent on antigen dose, IgE and eosinophils. PLoS Negl Trop Dis 7(3):e2149

Wilson S, Jones FM, Fofana HK, Landouré A, Kimani G, Mwatha JK, Sacko M, Vennervald BJ, Dunne DW (2013b) A late IL-33 response after exposure to *Schistosoma haematobium* antigen is associated with an up-regulation of IL-13 in human eosinophils. Parasite Immunol 35(7–8): 224–228

World Health Organization (2009) Preventive Chemotherapy Databank. http://www.who.int/neglected diseases/preventive chemotherapy/databank/en/index.html
World Health Organization (2011) Elimination of schistosomiasis. http://apps.who.int/gb/ebwha/pdf_files/EB130/B130_20-en.pdf; www.who.int/neglected diseases/preventive chemotherapy/databank/en/index.html
World Health Organization (2020) Schistosomiasis. Fact Sheet. https://www.who.int/news-room/fact-sheets/detail/schistosomiasis
World Health Organization (2021) COVID-19: WHO issues interim guidance for implementation of NTD programmes. https://www.who.int/neglected_diseases/news/COVID19-WHO-interimguidance-implementation-NTD-programmes/en/. Accessed 30 Mar 2021
Wynn TA, Thompson RW, Cheever AW, Mentink-Kane MM (2004) Immunopathogenesis of schistosomiasis. Immunol Rev 201:156–167
Xiang X, Tianping W, Zhigang T (2003) Development of a rapid, sensitive, dye immunoassay for schistosomiasis diagnosis: a colloidal dye immunofiltration assay. J Immunol Methods 280(1–2):49–57
Xiao S, Tanner M, N'Goran EK, Utzinger J, Chollet J, Bergquist R et al (2002) Recent investigations of artemether, a novel agent for the prevention of schistosomiasis japonica, mansoni and haematobia. Acta Trop 82:175–181
Xiao SH, Keiser J, Chollet J, Utzinger J, Dong Y, Endriss Y, Vennerstrom JL, Tanner M (2007) In vitro and in vivo activities of synthetic trioxolanes against major human schistosome species. Antimicrob Agents Chemother 51:1440–1445
Xu J, Rong R, Zhang HQ, Shi CJ, Zhu XQ, Xia CM (2010) Sensitive and rapid detection of *Schistosoma japonicum* DNA by loop-mediated isothermal amplification (LAMP). Int J Parasitol 40(3):327–331
Yang W, Jackson DC, Zeng Q, McManus DP (2000) Multi-epitope schistosome vaccine candidates tested for protective immunogenicity in mice. Vaccine 19(1):103–113
Yousif F, Hifnawy MS, Soliman G, Boulos L, Labib T, Mahmoud S et al (2007) Large-scale in vitro screening of Egyptian native and cultivated plants for schistosomicidal activity. Pharm Biol 45: 501–510
Yu JM, de Vlas SJ, Jiang QW, Gryseels B (2007) Comparison of the Kato-Katz technique, hatching test and indirect hemagglutination assay (IHA) for the diagnosis of *Schistosoma japonicum* infection in China. Parasitol Int 56(1):45–49
Zhang LJ, Zheng WD, Shi MN, Wan XZ (2006) Effects of interleukin-10 on activation and apoptosis of hepatic stellate cells in fibrotic rat liver. World J Gastroenterol 12:1918–1923
Zhang W, Li J, Duke M, Jones MK, Kuang L, Zhang J, Blair D, Li Y, McManus DP (2011) Inconsistent protective efficacy and marked polymorphism limits the value of *Schistosoma japonicum* tetraspanin-2 as a vaccine target. PLoS Negl Trop Dis 5(5):e1166
Zhang W, Molehin JU, Justin R, Sudduth PK, Eunjee G, Kim AJ et al (2018) Sm-p80-based schistosomiasis vaccine: double-blind preclinical trial in baboons demonstrates comprehensive prophylactic and parasite transmission-blocking efficacy. Ann N Y Acad Sci 1425(1):38–51
Zhang Y, Chen L, Gao W, Hou X, Gu Y, Gui L et al (2012) IL-17 neutralization significantly ameliorates hepatic granulomatous inflammation and liver damage in *Schistosoma japonicum* infected mice. Eur J Immunol 42:1523–1535
Zhao GH, Li J, Blair D, Li XY, Elsheikha HM, Lin RQ, Zou FC, Zhu XQ (2012) Biotechnological advances in the diagnosis, species differentiation and phylogenetic analysis of *Schistosoma* spp. Biotechnol Adv 30(6):1381–1389
Zhou YB, Yang MX, Wang QZ, Zhao GM, Wei JG, Peng WX, Jiang QW (2007) Field comparison of immunodiagnostic and parasitological techniques for the detection of schistosomiasis japonica in the People's Republic of China. Am J Trop Med Hyg 76(6):1138–1143
Zhou YB, Yang MX, Tao P, Jiang QL, Zhao GM, Wei JG, Jiang QW (2008) A longitudinal study of comparison of the Kato-Katz technique and indirect hemagglutination assay (IHA) for the detection of schistosomiasis japonica in China, 2001–2006. Acta Trop 107(3):251–254

Zhou YB, Liang S, Chen GX, Rea C, He ZG, Zhang ZJ, Wei JG, Zhao GM, Jiang QW (2011a) An integrated strategy for transmission control of *Schistosoma japonicum* in a marshland area of China: findings from a five-year longitudinal survey and mathematical modeling. Am J Trop Med Hyg 85(1):83–88

Zhou YB, Zheng HM, Jiang QW (2011b) A diagnostic challenge for Schistosomiasis japonica in China: consequences on praziquantel-based morbidity control. Parasit Vectors 4:194

Zhou YB, Liang S, Chen GX, Rea C, Han SM, He ZG, Li YP, Wei JG, Zhao GM, Jiang QW (2013) Spatial-temporal variations of *Schistosoma japonicum* distribution after an integrated national control strategy: a cohort in a marshland area of China. BMC Public Health 13:297

Zhu Y, Lu F, Dai Y, Wang X, Tang J, Zhao S, Zhang C, Zhang H, Lu S, Wang S (2010) Synergistic enhancement of immunogenicity and protection in mice against *Schistosoma japonicum* with codon optimization and electroporation delivery of SjTPI DNA vaccines. Vaccine 28(32): 5347–5355

Ziskind B (2009) La bilharziose urinaire en ancienne Egypte [urinary schistosomiasis in ancient Egypt]. Nephrol Ther 5:658–665

Chapter 6
Fascioliasis

S. Mas-Coma, M. D. Bargues, and M. A. Valero

Abstract Human fascioliasis is an important public health problem in many regions, and livestock infection is a veterinary problem worldwide. This disease is caused by the liver fluke species *Fasciola hepatica* of worldwide distribution and *F. gigantica* restricted to regions of Africa and Asia. Their two-host life cycle is similar, including specific freshwater lymnaeid snails as vectors. The major human health problems are known in Andean countries, the Caribbean, Northern Africa, Near East, Southeast Asia and Western Europe. In human hyperendemic areas, children and females are the most affected. Human fascioliasis shows a marked heterogeneity of epidemiological situations and transmission patterns. Variation of climatic factors and anthropogenic environmental modifications may give rise to different fascioliasis seasonality and long-term disease risk trends. There are many human infection sources, local diet and cultural traditions being important. The rapid and potent ability of fasciolids to suppress the immune response explains why hosts do not develop resistance and the frequency of pathogen coinfections. The disease is chiefly confined to the liver, including hepatic lesions, fibrosis and chronic inflammation. Juvenile flukes may cause ectopic fasciolosis. Clinical manifestations are evident in both invasive and biliary periods. Diagnosis is mainly made by coprological and serological techniques. Among the useful drugs, triclabendazole is of choice at present. Prognosis depends on treatment promptness. Severe complications, sequelae and death causes should be highlighted. New knowledge has allowed to improve individual infection prevention measures and community control. Challenges appear in vaccinology, indicating that a human vaccine is still far from affordable.

S. Mas-Coma (✉) · M. D. Bargues · M. A. Valero
Departamento de Parasitologia, Facultad de Farmacia, Universidad de Valencia, Burjassot, Valencia, Spain
e-mail: S.Mas.Coma@uv.es

F. Bruschi (ed.), *Helminth Infections and their Impact on Global Public Health*,
https://doi.org/10.1007/978-3-031-00303-5_6

6.1 Introduction

Fascioliasis is a well-known veterinary problem worldwide. Moreover, in the last two decades, many surveys have shown it to be an important public health problem as well (Chen and Mott 1990; Mas-Coma et al. 1999c, 2009a), including estimations of 2.4 million, up to 17 million people, or even higher depending from the hitherto unknown situations mainly in Asia and Africa (Mas-Coma 2004).

The number of human case reports is increasing in many countries of the five continents, many human endemic areas have already been assessed (Mas-Coma et al. 2005), and recent results of studies on pathogenicity and immunity underlie the decision to consider fascioliasis an important human parasitic disease henceforth (Mas-Coma et al. 1999b) and include it as a food-borne trematode disease priority within the agenda of the World Health Organization (World Health Organization 2013).

6.2 The Agent

This parasitic disease is caused by two digenean trematodes, *Fasciola hepatica* of worldwide distribution and *F. gigantica* restricted to given regions of Africa and Asia. Their adult stage has a leaf-shaped body and is anatomically characterized by the branching of their caeca, testes and ovary, the very numerous small vitellaria extending bilaterally up to the hindbody and a short uterus located between the ovary and the caecal bifurcation (Fig. 6.1). The eggs are operculated, ovoid, yellow and non-embryonated when laid.

Fasciola gigantica is more elongate and narrower, with lateral walls tending to be parallel and with non-existent or less marked shoulders of the cephalic cone (Fig. 6.1). The adult stage of *F. hepatica* has a maximum length/width of 29.0/14.1 mm, whereas *F. gigantica* is bigger, with a maximum size reaching 52.3/11.8 mm. Hybrid specimens may give rise to intermediate forms in those endemic areas where the two species overlap (Mas-Coma et al. 2009a).

The *F. hepatica* genome has proved to be among the largest known pathogen genomes at 1.3 Gb. The high polymorphism levels found have tentatively been linked to the evolutionary potential for rapid adaptation to changes in host availability, climate change or drug or vaccine interventions (Cwiklinski et al. 2015). Surprisingly, the genome of *F. hepatica* isolated from sheep of North America showed a markedly higher repeat content (55.29%) than the aforementioned genome of *F. hepatica* isolated from sheep of the United Kingdom (32.0%) (McNulty et al. 2017).

Adult worms parasitize the large biliary passages and the gallbladder of ruminants, mainly sheep, goats and cattle, and many other herbivorous domestic and wild animals, including horses, donkeys, mules and also Old and New World camelids.

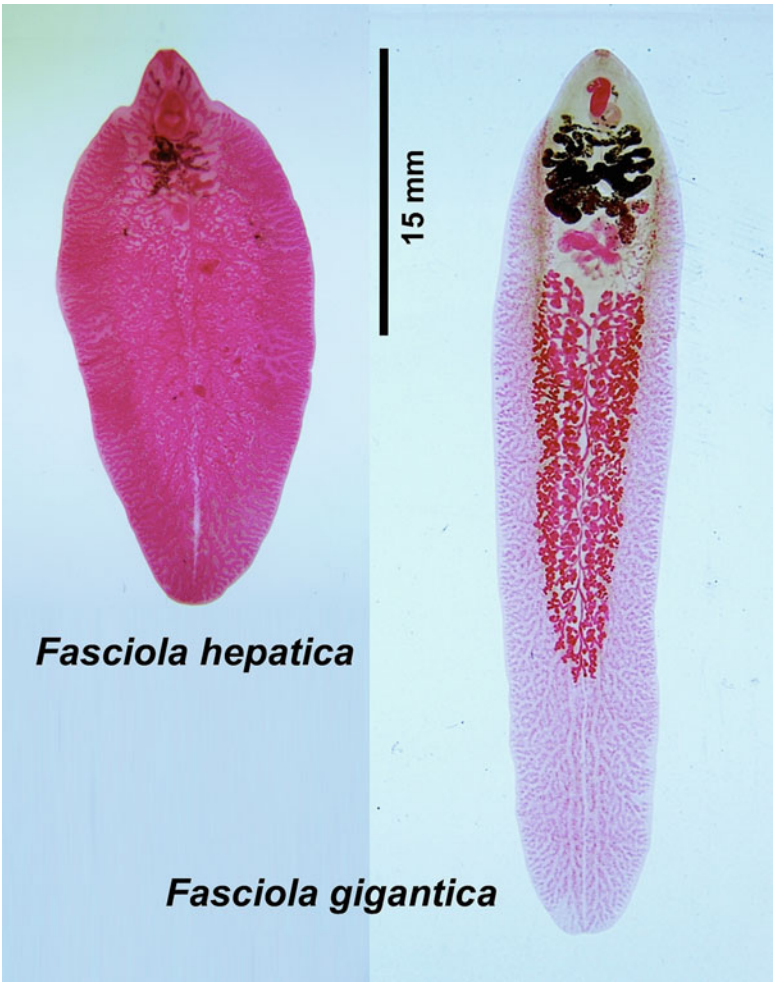

Fig. 6.1 Adult stages of pure *Fasciola hepatica* and pure *F. gigantica*. Note the different size and shape; hybrid forms usually show an intermediate form. The two photographs are at the same scale (Orig. S. Mas-Coma)

Buffalo, deer, wild sheep, wild pig, various marsupials, rabbit, hare and nutria are also susceptible hosts (Mas-Coma and Bargues 1997).

Sheep and cattle are the livestock species most infected by *F. hepatica*, whereas the buffalo appears to be the most important for *F. gigantica*. In human hyperendemic areas, the domestic pig (Mas-Coma et al. 2021) and equines as the donkey (Mas-Coma et al. 2020b) and the mule (Mera y Sierra et al. 2020) should also be considered among the important reservoirs.

Among wild definitive hosts in Europe, *F. hepatica* seems to be less adapted to the roe deer (*Capreolus capreolus*) when compared to other deers (red and fallow

Fig. 6.2 Metacercarial body of *Fasciola hepatica* shortly after detaching from its swimming tail and already starting encystment process (Orig. S. Mas-Coma)

deer), and the introduced nutria (*Myocastor coypus*) has become an important reservoir in France and may also be so in areas of South America from where it is original.

Humans are susceptible hosts for the infection by both *Fasciola* species (Mas-Coma et al. 2009a).

The two-host life cycle of both fasciolids is similar and takes about 14–23 weeks. It comprises four phases (Mas-Coma and Bargues 1997):

(A) The definitive host harbours fluke adults producing eggs which reach the external milieu by way of the bile and intestine; the definitive host is infected by ingestion of metacercariae; in humans, the flukes attain sexual maturity in 3–4 months and their life span is between 9 and 13.5 years.

(B) The transit between definitive mammal host and intermediate snail host includes the long resistance phase of the egg and the short active phase of miracidium; eggs shed with the mammal faeces will continue their development in freshwater of appropriate physicochemical characteristics (mainly temperature of 15–25 °C).

(C) The development at snail level includes miracidium penetration, sporocyst, redial generations, production of cercariae and shedding of the latter into the water; the prepatent period (38–86 days) is dependent on temperature, higher temperatures reducing the period.

(D) The transit between snail and mammal host includes the short swimming phase of cercaria and the long resistance phase of metacercaria; the shedding process takes place between 9 and 26 °C, independently of light or darkness; cercariae swim for a short time until contacting a solid support, mostly leaves of water

plants above or below the water line, to attach and encyst (Fig. 6.2); metacercarial cysts become infective within 24 h.

Liver fluke development is very dependent of the environmental characteristics according to phases B, C and D and markedly influenceable by human activities at phase A.

Vectors of *Fasciola* are freshwater gastropod snails of the family Lymnaeidae. Different lymnaeid species transmit the two fasciolids, which show a marked and different specificity. There are species of Lymnaeidae which cannot transmit fasciolids, other lymnaeid species which transmit *F. hepatica*, other species which transmit *F. gigantica* and a very few which are able to transmit the two fasciolid species.

Fasciola hepatica is mainly transmitted by species of small size belonging to the so-called *Galba/Fossaria* group (Bargues et al. 2007, 2011a), including *Galba truncatula* as the main vector and the only one in Europe but also present in Africa, Asia and South America. Other species of this group act as vectors in the Americas. *Lymnaea tomentosa* is the transmitter in Australia. In South America, the species *Lymnaea neotropica*, also of the *Galba/Fossaria* group, has recently proved to be a very efficient transmitter also involved in the spread of the disease throughout wide regions in the lowlands, whereas *Galba truncatula* is the one involved in the highlands (Bargues et al. 2017).

Fasciola gigantica is transmitted by species of the genus *Radix*, mainly *R. natalensis* in Africa and varieties of *R. auricularia* and *R. viridis* in Asia. In Europe, however, *Radix* species do not show any epidemiological importance, given that only *F. hepatica* is present (Bargues et al. 2001). *Pseudosuccinea* is a monospecific genus including the species *P. columella* which has colonized all continents and appears to be able to transmit both *Fasciola* species (Bargues et al. 2011b).

The presence of lymnaeid vectors defines not only the distribution of fascioliasis but may also explain the distribution of human infection within a country, as has been recently observed in Venezuela (Bargues et al. 2011c) and Chile (Artigas et al. 2011), and within an endemic area, as well as its seasonality or permanent transmission (Mas-Coma et al. 1999a). Similar to other waterborne parasitic diseases, the transmission foci are patchily distributed within a human endemic area and linked to the presence of appropriate water collections, and human prevalences in schoolchildren appear to be related to the distance to water bodies presenting lymnaeids (Mas-Coma et al. 1999a).

6.3 Epidemiology of Infection

A global analysis of the geographical distribution of human cases shows that the expected correlation between animal and human fascioliasis only appears at a basic level. High prevalences in humans are not necessarily related to areas where fascioliasis is a great veterinary problem. The major human health problems are

known in Andean countries (Bolivia, Peru, Chile, Ecuador), the Caribbean (Cuba), Northern Africa (Egypt), Near East (Iran and neighbouring countries), Southeast Asia (Vietnam) and Western Europe (Portugal, France and Spain) (Esteban et al. 1998; Mas-Coma et al. 2009a).

However, the worldwide distribution of fascioliasis also includes human infection risk in developed, high-income countries, in which from individual patients up to small epidemics continue to be reported, with the additional problems of the impacts by the climate and global changes (Mas-Coma 2020).

In the human hyperendemic areas, children are the most affected by the disease, with higher prevalences and intensities (with a peak in the 9–11 age group). In these areas, human infection may occur very early in life, as only a few months after being born. Moreover, child infection has been verified to occur at preschool age more frequently than previously considered (De et al. 2020).

Adult subjects are also infected in the human hyperendemic areas. Adult subjects either maintain the parasites acquired when young or can be newly infected because of the high infection risk. The gender effect in fascioliasis is worth mentioning. Prevalences and/or intensities in human hyperendemic areas appear to be significantly higher in females. In Andean countries, females shed pronouncedly and significantly more eggs than males (Esteban et al. 1999, 2002), whereas in Egypt, the prevalence in females appeared to be significantly higher than in males (Esteban et al. 2003). Interestingly, however, in preschool children, the opposite occurs, with a faster infection increase in males from 2 years onwards (De et al. 2020).

Moreover, it should be considered that the importance of this disease is not only restricted to the health aspects but also to its high economic impact mainly in the endemic rural areas (Espinoza et al. 2010).

6.3.1 Epidemiological Heterogeneity of Human Fascioliasis

After many years of studies on different areas presenting human infection by fasciolid liver flukes throughout the world, the classification of epidemiological situations proposed by Mas-Coma et al. (1999c) still appears to be fully valid and useful. This classification includes the following situations: (1) autochthonous, isolated, non-constant cases; (2) imported cases; endemic situations including (3) hypoendemic, (4) mesoendemic and (5) hyperendemic; and also epidemic situations comprising (6) epidemics in non-human endemic but animal endemic areas and (7) epidemics in human endemic areas.

Fascioliasis presents a very wide spectrum of transmission and epidemiological patterns in human hypo- to hyperendemic areas. These are related to the large diversity of environments, including different human endemic/epidemic situations; different human demographies, races, diets, habits, traditions and religions; different domestic and wild mammal reservoir species; different lymnaeid transmitting species; zones in both the Northern and Southern Hemispheres; altitudes from −27 m up to 4200 m; hot and cold weathers; seasonal and yearly constant temperatures;

scarce to pronounced annual rainfall; low and high mean annual potential evapotranspiration; and from lack of dry period to lack of wet period through different dryness/humidity rates. From the landscape point of view, these areas include from altiplanos to valleys, from islands to mainlands, from natural to artificial irrigations, from lakes to lagoons, from large rivers to small streams and from permanent to temporal water bodies (Mas-Coma et al. 2003).

A classification of transmission patterns has been proposed (Mas-Coma 2005) and is progressively updated to offer a baseline for future research (Mas-Coma et al. 2009a). Up to the present, the following patterns have been distinguished: (1) a very high altitude pattern in Andean countries including the altiplanic pattern and the valley pattern (Valero et al. 2012a); (2) a Caribbean insular pattern; (3) a pattern related to Afro-Mediterranean lowlands; (4) a pattern related to Caspian surrounding areas; and (5) a pattern related to lowland areas in Southeast Asia.

An additional extreme pattern has been found in Argentina, involving desertic-arid and semi-arid conditions with very low yearly precipitation, which are very different from the typical fascioliasis transmission foci. In such a place, lymnaeids are confined to lateral river side floodings and small man-made irrigation systems, with water availability only depending on the rivers flowing from neighbouring mountains. This reminds the transmission foci of schistosomiasis in oases of the Sahara Desert in Africa (Bargues et al. 2016). In Uttar Pradesh, India, infection risk appears concentrated in freshwater collections where the 72.0% rate found in lymnaeids in one transmission focus appears to be the highest worldwide record of fasciolid infection in a lymnaeid population (Sunita et al. 2021).

Thus, well-known situations and patterns of fascioliasis may not always explain the disease characteristics in a given area. Only once epidemiology and transmission characteristics of the new area are sufficiently assessed may appropriate control measures be designed for the endemic area in question.

6.3.2 Seasonality and Long-Term Impacts of Climate and Global Changes

Climatic factors are decisive in the transmission of fascioliasis, mainly temperature, rainfall and/or potential evapotranspiration (Mas-Coma et al. 2009b).

Variation of mainly rainfall and temperature gives rise to different fascioliasis seasonality. In Europe, the transmission of the disease is typically bi-seasonal, due to the activity periods of the lymnaeid vectors in spring and autumn. In the Bolivian Altiplano, however, the transmission takes place throughout the year, lymnaeid vector populations being always present because of inhabiting permanent water bodies instead of temporary ones due to the high evapotranspiration rates at the very high altitude (Mas-Coma et al. 1999a). In other areas, the transmission appears mono-seasonal, due to the existence of only 1-year period with water availability.

Climate change overlaps other anthropogenic and environmental modifications which are included in the broad term of "global change" (Mas-Coma et al. 2009b). Thus, artificial field irrigation appears to be sufficient by its own to allow for fascioliasis transmission in the Peruvian Altiplano (Esteban et al. 2002). In the province of Punjab, in Pakistan, transmission includes bi-seasonality with a peak related to natural rainfall and another peak related to man-made irrigation (Afshan et al. 2014). The Punjab is the first endemic area where the emergence of human infection has been correlated with a significant increase of fascioliasis transmission risk due to an impact of climate change throughout a 20-year period (Afshan et al. 2014).

In the Northern Bolivian Altiplano human hyperendemic area, comparisons of transmission foci data from the 1990s with those of 2018 demonstrated an endemic area expansion. Altitudinal, northward and southward expansions suggest movements of livestock transporting snail vectors, with increasing temperatures transforming previously unsuitable habitats into suitable transmission areas. Important repercussions include the need to widen the area throughout which the preventive chemotherapy programmes are implemented (Bargues et al. 2020).

For the study of climate and environment influences on fascioliasis, three types of approaches are useful, including the assessment from geographical distribution and seasonality up to human and animal infection risk and forecasting methods, namely, mathematical modelling based on climate factors, remote sensing (RS) based on images and information furnished mainly by space satellites and geographic information systems (GIS) based on computer mapping by geo-positioning of different abiotic and biotic factors and characteristics. These methods allow for developments from low to very high resolution adapted to endemic areas of different characteristics (Bergquist et al. 2021).

6.3.3 Sources of Human Infection

Metacercarial infectivity is dependent upon storage time, being lower when metacercariae are older. The maximum longevity was 48 weeks. Moreover, metacercarial viability and infectivity did not show differences between isolates from different reservoir species (Valero and Mas-Coma 2000).

The ingestion of infective metacercariae by humans may occur by different ways. Several infection sources have been distinguished (Mas-Coma 2004; Mas-Coma et al. 2018). These infection sources include foods, water and combinations of both:

- Ingestion of freshwater wild plants: main aspects to be considered are the plant markers of transmission foci, watercress, other freshwater wild plants and wild plants sold in urban markets.
- Ingestion of freshwater cultivated plants, mainly watercress.
- Ingestion of terrestrial cultivated plants needing frequent irrigation.

- Ingestion of terrestrial wild plants: collected in dry habitats but which were submerged in water a few weeks or months before.
- Ingestion of traditional local dishes made with contaminated sylvatic plants.
- Ingestion of raw liver infected with migrating metacercariae which may keep the capacity to restart migration.
- Drinking of contaminated water.
- Drinking of beverages and juices made from local plants.
- Ingestion of dishes and soups made with contaminated water.
- Washing of vegetables, fruits, tubercles, kitchen utensils or other objects with contaminated water.

Cultural traditions prove to be important in given endemic areas. Experimental studies showed the role that plant-made foods may play in human infection in Gilan Province, Iran (Ashrafi et al. 2006). There are three methods to assess infection sources: (1) detection of metacercariae attached to plants or floating in freshwater, (2) anamnesis in individual patients and (3) questionnaire surveys in endemic areas (Mas-Coma et al. 2018).

In Mexican children, an association between fascioliasis and the habit of eating raw vegetables was identified. The link of fascioliasis risk with consumption of raw vegetables other than watercress should be highlighted, as it suggests contamination when washing terrestrial vegetables with untreated water and/or in plant cultures using natural water for irrigation (Zumaquero-Ríos et al. 2013).

6.4 The Host Response to the Parasite

Immunologically, cell- and/or antibody-mediated response varies from host to host and in the same host according to the phase of the infection. Similarly, immunity to reinfection differs greatly from host to host.

In humans, studies on immunity are limited. It is generally believed that humans are not a suitable host, most migrating flukes becoming trapped in the liver parenchyma and dying without reaching the bile ducts. Considerable tissue reaction and calcification of the bile passages due to the flukes have been recorded (Acosta-Ferreira et al. 1979).

6.4.1 Immunological Processes

Fasciolid trematodes promote their own survival through several strategies to downregulate the host's immune response during the early phase of infection (Brady et al. 1999). Another study proved that immune response modulation occurs in advanced chronic fasciolosis too. The results indicated that during early chronic infection, there was a predominance of a Th2 response, which decreased in the

advanced chronic infection characterized by a persistent immune suppression (Girones et al. 2007). Fascioliasis is a potent inducer of Th2 responses which impair the ability to mount any effective Th1 responses against bacteria and other pathogens (Brady et al. 1999; O'Neill et al. 2000; Jaffar et al. 2004).

The rapid and potent ability of fasciolids to suppress the immune response explains why infected hosts do not develop resistance. This underlies the frequent reinfections in human hyperendemic areas where the infection risk is very high (Valero et al. 2017, 2020).

Within 24 h after oral infection, peritoneal macrophages express markers for the Th2-associated phenotype and display a reduced ability to respond to Th1 stimulants. This implies that by the time the newly excysted juveniles have penetrated the intestinal wall and entered the peritoneum, they have already initiated the immune events that will dominate throughout infection. So, these early-stage parasites secrete immunomodulatory molecules that influence the function of innate cells (dendritic cells, macrophages, neutrophils, mast cells, etc.) in the intestinal wall and peritoneal cavity. A systemic antigen-specific Th2 response is firmly established already at 7 days postinfection and is characterized by the secretion of IL-4, IL-5 and IL-13 from splenocytes. As the infection develops (3 weeks), regulatory macrophages (TGF-ß and IL-10 producing) and dendritic cells (IL-10 producing) are recruited to the peritoneum, and dendritic cell maturation is inhibited. Mast cells recruited to the site of infection exhibit impaired Th1-promoting abilities. Most CD4* T cells in the peritoneum secrete IL-10 but not IL-4 or IFN-γ. IL-10-secreting Tregs are induced which exert a suppression of both Th1 and Th2 cells that become non-responsive to parasite-specific antigens, and mesenteric lymph nodes produce IL-10 and IL-5, but not IFN-γ and IL-17, in response to stimulation by parasite antigens (Dalton et al. 2013).

The chronic disease is also typified by Th2 responses and suppressed Th1 responses. Serologically, this polarity of immune response is strikingly displayed in the isotype of circulating antibodies. Fluke-infected animals secrete high titres of IgG1 antibodies and virtually no IgG2. Furthermore, blood macrophages are non-responsive to stimulation with endotoxin and exhibit elevated levels of arginase indicative of a phenotype that metabolize l-arginine and are important in promoting Th2 responses and facilitating tissue repair and fibrosis (Dalton et al. 2013).

6.4.2 Associations with Other Parasites

A consequence of liver fluke infection is the suppression of immune responses directed against concurrent pathogenic infections. The synergistic capacity of fasciolids in coinfection with other pathogenic agents is well known, immunological responses to pathogen antigens being markedly suppressed and concomitant infection being exacerbated following fascioliasis infection. The parasitological spectrum of protozoan and helminthic species found in the inhabitants of the human endemic areas, the multiparasitisms and the associations between liver fluke infection and

infection by other pathogenous parasites, all appear to be similar in the different human endemic zones (Esteban et al. 1997a, 1997b, 1999, 2002, 2003; Gonzalez et al. 2011). These synergistic associations of fascioliasis with other pathogens are believed to underlie the high morbidity and mortality rates of Aymara children inhabiting the Northern Altiplano (Mas-Coma 2004).

In animals, the clinic synergistic capacity of *F. hepatica* in concomitant infection with other pathogenic agents is well known. In humans, the association of *F. hepatica* with other parasites has been assessed in recent large surveys performed in human fascioliasis endemic areas. In the Bolivian Altiplano endemic zone, among the samples positive to *F. hepatica*, the number of other parasite species found varied from one to eight. In 35.5% of the cases with *F. hepatica* eggs, the liver fluke showed to be the sole parasite species present in which a pathogenic capacity is well known. *Fasciola hepatica* infections associated with one (34.9%), two (23.7%), three (4.6%) and four (1.3%) of the other generally recognized pathogenic parasites were recorded. A significant positive association of great health importance in children was found with *Giardia intestinalis*, both in Bolivia and Peru (Esteban et al. 1997a, b, 1999, 2002).

6.5 Pathology

Pathogenesis depends on the number of flukes. However, fluke size has also proved to be an important factor when comparing the pathogenicity of *F. hepatica* with that by *F. gigantica*. Results demonstrated that *F. gigantica* is more pathogenic than *F. hepatica*, due to its bigger size and biomass. The higher *F. gigantica* pathogenicity contrasts with previous studies which only reflected the faster development of *F. hepatica* observed in short-term experiments (Valero et al. 2016).

The penetration of the duodenum or jejunum wall by metacercariae may cause focal haemorrhages and inflammation, although lesions may not be clinically evident. The fluke migration through the liver parenchyma for 4–6 weeks or longer induces the major pathological changes. Parasites digest hepatic tissue and cause extensive parenchymal destruction with intensive haemorrhagic lesions and immunological and inflammatory reactions. Migration tracks are observed in histological sections. Migratory flukes sometimes die leaving cavities filled with necrotic debris, and considerable liver areas may subsequently be replaced by scar tissue (Mas-Coma et al. 1999b, 2000).

A small proportion of the flukes may reach the bile ducts where they may live for even years. Less pathogenic effects may be caused in the bile ducts, although inflammation resulting in fibrosis, thickness and expansion is common (Fig. 6.3) (Chen and Mott 1990). The ultrastructural picture revealed bile ductular hyperplasia, fibrosis of portal tracts, widening of the interhepatic spaces by many microvilli and dilated Disse space with collagen fibres. Bile ductular hyperplasia may be the initial factor to fibrinogenesis, which subsequently enhances the development of the microvilli on the surface of the hepatocytes.

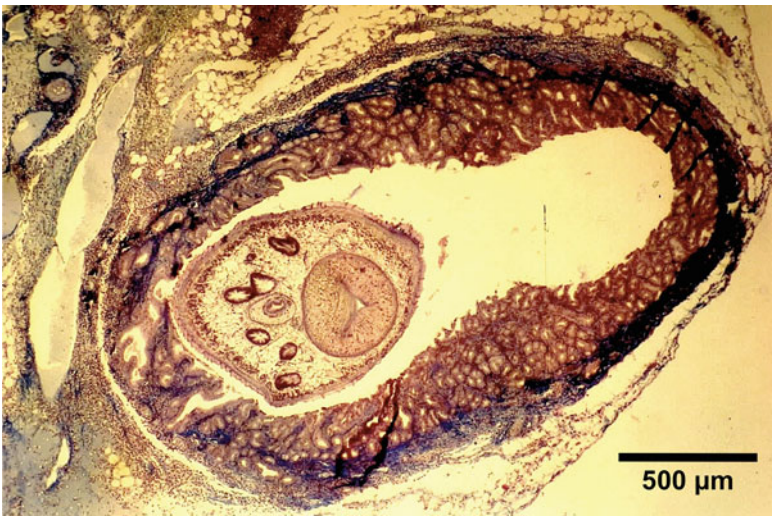

Fig. 6.3 Histopathological section showing a specimen of *Fasciola hepatica* in a rat biliary duct. See section of large sucker inside and prominent spines in the tegumental external surface of the worm (Orig. S. Mas-Coma)

Anaemia is one of the most characteristic symptoms, especially in heavier infections. Blood loss into the bile seems most probably to be an important factor contributing to severe anaemia. An association between anaemia and fluke burden (the most important), epg, fluke body area, presence of blood in faeces, IgG1 and eosinophil levels and per cent of splenic weight was verified in a multivariate analysis. These results lead to the assumption that a high risk of anaemia in subjects with a heavy parasitic burden in human hyperendemic areas is to be expected (Valero et al. 2008). These results are crucial, because although there were several reports listing anaemia in patients from endemic areas, results could only be considered with great caution because coinfections were never excluded in those papers and in fact it becomes very difficult, not to say almost impossible, to find subjects from endemic areas only infected by fascioliasis. And among those parasites coinfecting fascioliasis-affected subjects, many are also known to cause anaemia.

The process may be summarized as follows: (1) the fluke is a blood feeder but may also feed on tissue; (2) haemorrhages may occur from the erosion of the biliary epithelium due to the infection; (3) reticulocytes are increased in the peripheral blood; (4) generalized haemolysis is absent; and (5) there is no evidence of plasma iron or vitamin B_{12} deficiencies, although a significant reduction in serum iron has recently been detected in patients with chronic fasciolosis.

The most important pathogenic sequelae are hepatic lesions and fibrosis and chronic inflammation of the bile ducts, given that the disease is chiefly confined to the liver. Unlike clonorchiasis or opisthorchiasis, no association with biliary carcinoma has been reported (Mas-Coma and Bargues 1997).

6.5.1 Liver and Biliary Tract

The liver appears usually enlarged with a smooth or uneven surface. The most common macroscopic lesions are multiple soft, yellowish or grey-white nodules ranging from 2 to 30 mm in diameter, which correspond to eosinophilic abscesses. Nodules are also observed in the parietal peritoneum proximal to the liver and on the round liver ligament. Haemorrhagic stippling appears at the margin of the nodules. White or yellow striae, surrounded by telangiectasia, are observed on the liver capsule. Close to the nodules, ribbed or vermiform formations with similar colour and consistency as nodules are also observed under laparoscopy. Hepatic capsular thickening of varying degree appears, and in a few cases, the entire hepatic capsule appears thickened. Subcapsular lymphatic vessels are dilated. The lymph nodes near the porta hepatis may be markedly enlarged. Multiple subcapsular cavities filled with necrotic material were observed below the liver capsule in one patient who died. Several reddish-purple tracks radiated from the nodules, whereas others were greyish white and fibrous. The tracks extended from the liver capsules and ended in subcapsular cavities. Most of the lesions are less than 20 mm below the capsules (Acosta-Ferreira et al. 1979). In cases with marked involvement of the peritoneal wall and the liver surfaces, yellow and opalescent ascites was present. Apart from mild splenomegaly in two patients, no significant portal hypertension was found in laparoscopic examinations of several patients.

The common bile ducts are usually large (Fig. 6.3) and dilated, and the wall is thickened on palpation. The gallbladder wall is greatly thickened and oedematous. Multiple, greyish-white subserous nodules are present, and adhesions of the gallbladder to adjacent structures are common. The mucosal folds of the gallbladder appear prominent. The wall of the gallbladder appears thickened owing to muscular hypertrophy and perimuscular fibrosis. There is glandular epithelial hyperplasia. All layers of the wall contain patchy infiltrates with lymphocytes, plasma cells and eosinophils (Acosta-Ferreira et al. 1979).

Lithiasis, often multiple, in the common bile ducts and gallbladders is very frequent (Arjona et al. 1995), whereas cirrhosis does not appear to be so (Marcos et al. 2009). The presence of gallstones was experimentally proved to increase with infection time. Therefore, the lithogenic induction by infection becomes manifest in situations of advanced chronicity. Gallstone presence was strongly associated with the number of flukes located in the bile duct. The risk of pigment stones appears to depend mainly on factors that favour bile duct obstruction (cholangitis, fluke body development versus time, intensity of infection). Situations of undiagnosed cases, as in subjects presenting undistinguishable symptoms or in those keeping their infection for a long time because of non-treatment or of repetitive reinfections, usually in human endemic areas of developing countries, imply a higher lithiasis risk. Thus, a high gallstone risk may be expected in subjects inhabiting human hyperendemic areas where very high egg outputs detected in humans suggest that liver fluke burdens may also be very high (Valero et al. 2003).

Generally, the migration tracks can be found in the liver and other organs. The walls of the tracks in the liver often contain Charcot-Leyden crystals and eosinophils. The cavities of the tracks are filled with necrotic cellular debris, including hepatocytes, fibrin and red cells. A considerable eosinophilic infiltrate surrounds the tracks. Longer tracks can cross several hepatic lobules. In older lesions, macrophages, lymphocytes, eosinophils and fibrous tissue are observed. Focal calcification is sometimes seen in the margin of the necrotic debris. Calcifications may form the outline of a dead fluke (Acosta-Ferreira et al. 1979).

Egg granulomas have been reported. Multinuclear giant cells surround a single egg with subsequent layers of epithelioid cells and fibrous tissue, plasma cells, lymphocytes and eosinophils. The portal triads are dilated and oedematous with infiltrates of lymphocytes and eosinophils. Bile duct proliferation, periductal fibrosis, necrotizing arterial vasculitis and portal venous thrombosis are frequent (Acosta-Ferreira et al. 1979; Chen and Mott 1990).

6.5.2 Other Locations, Ectopic Fascioliasis and at Distance Effects

Juvenile flukes may deviate during migration, enter other organs and cause ectopic fascioliasis. In humans, the most frequent ectopic lesions are those of the gastrointestinal tract (Acosta-Ferreira et al. 1979). Other ectopic locations reported are the subcutaneous tissue; heart, blood vessels, lung and pleural cavity; abdominal wall; appendix; pancreas; spleen; inguinal nodes; cervical node; skeletal muscle; and epididymis. Such ectopic flukes almost never achieve maturity (Mas-Coma et al. 2014). The usual pathological effects of ectopic lesions are due to the migratory tracks causing tissue damage with inflammation and fibrosis. Parasites may be calcified or become incorporated in a granuloma (Arjona et al. 1995; Mas-Coma and Bargues 1997).

Very recently, a wide analysis has shown that neurofascioliasis or intracranial infection by *Fasciola* and ophthalmofascioliasis or direct affection of the eye by migrating flukes may be rare, although not sporadic as previously believed. However, manifestations including a very wide range of neurological symptoms, signs and syndromes, together with meningeal, psychiatric or neuropsychic manifestations, and ocular disorders caused at distance by flukes infecting the liver may be frequent but underestimated due to misdiagnosis, mainly in low-income regions. The impressive clinical pictures should be highlighted. They include from hemiplegia and paraplegia to disturbances and difficulties of walking capacity, speech disorders, convulsions, epilepsy and coma, amnesia or visual hallucinations and permanent blindness, only to mention a few, plus the clinical complexity of the puzzling polymorphisms, the disconcerting multifocality of the manifestations and their changes along the evolution of the disease in a same patient, as well as differences between the clinical pictures shown by different patients. Moreover, these studies

emphasize post-treatment sequelae and mortality in neurological patients and the need to consider neurological fascioliasis when estimating the global burden of this disease (Mas-Coma et al. 2013, 2014).

Recently, proteomic and mass spectrometry analyses of the *Fasciola hepatica* excretome/secretome identified numerous, several new, plasminogen-binding proteins enhancing plasmin generation. This may underlie blood-brain barrier leakage whether by many simultaneously migrating, small-sized juvenile flukes in the acute phase or by breakage of encapsulating formations triggered by single worm tracks in the chronic phase. Blood-brain barrier leakages may subsequently occur due to a fibrinolytic system-dependent mechanism involving plasmin-dependent generation of the proinflammatory peptide bradykinin, after different plasminogen-binding protein agglomeration waves. Interactions between diverse parasitic situations and non-imbalancing fibrinolysis system alterations are for the first time proposed that explain the complexity, heterogeneity and timely variations of neurological disorders (Gonzalez-Miguel et al. 2019).

6.6 Clinical Manifestations

The following clinical periods can be distinguished: incubation period (from the ingestion of metacercariae to the appearance of the first symptoms) and infection periods including the invasive or acute phase (fluke migration up to the bile ducts), the latent phase (maturation of the parasites and starting of oviposition) and the biliary, chronic or obstructive phase. Of these four periods, the invasive or acute phase and the biliary or chronic phase are the most important and in which most of the patients are detected.

The incubation period varies considerably depending on the number of metacercariae ingested and the host's response. The period of incubation in humans has not yet been accurately determined: only "a few" days, 6 weeks, 2–3 months or even more (Mas-Coma et al. 1999b).

The latent phase can last for months or years. The proportion of asymptomatic subjects in this phase is unknown. They are often discovered during family screening after a patient is diagnosed, confirmed after clinical suspicion or in epidemiological surveys by finding the eggs in the duodenal fluid and/or in the stool (Arjona et al. 1995). An unexplained, prominent eosinophilia may already be suggestive of infection (Gil-Benito et al. 1991). These persons may have gastrointestinal complaints or one or more relapses of the acute symptoms during this phase (Mas-Coma and Bargues 1997).

6.6.1 Invasive or Acute Phase

In this phase, the symptomatology is due mainly to the mechanical destruction of the liver tissue and of the abdominal peritoneum by the migrating larvae causing localized or generalized toxic and allergic reactions lasting 2–4 months. However, in endemic areas, *F. hepatica* infection is usually repetitive, and the acute lesions are superimposed on chronic disease. Thus, the acute phase may be prolonged and overlap on to a latent or an obstructive phase.

The major symptoms of this period include (Mas-Coma et al. 1999b):

- **Fever:** it is usually the first symptom, usually low or moderate but may reach 40 °C and in heavily infected cases as high as 42 °C; it may be remittent, intermittent or irregular with higher temperature in the evening; in some cases, a low, recurrent fever lasted for a long time (4–18 months).
- **Abdominal pain:** from mild to excruciating, sometimes vague, it may be generalized at the outset but is usually localized in the right hypochondrium or below the xiphoid.
- **Gastrointestinal disturbances:** loss of appetite, abdominal flatulence, nausea and diarrhoea are common, whereas vomiting and constipation are infrequent.
- **Urticaria**: it is, with dermatographia, a distinctive feature in the early stage of the fluke invasion and may be accompanied with bouts of bronchial asthma.
- **Respiratory symptoms:** cough, dyspnoea, hemoptysis and chest pain occur occasionally but in some cases are the first manifestation of infection.

The following signs may appear in the invasive phase on physical examination (Mas-Coma et al. 1999b):

- **Hepatomegaly and splenomegaly:** the liver is usually enlarged and tender, sometimes reaching down to the right iliac fossa, but it is never hard; the degree of hepatomegaly seems to increase during the course of the disease, and hepatic abscesses are detected; splenomegaly is not common but has been many times reported.
- **Ascites:** this sign has been reported several times; it is yellow with a high leucocyte count, eosinophils predominating; the pathogenesis is considered to be an inflammatory response to a large number of juvenile flukes penetrating the intestinal walls, irritation of the peritoneum and penetration through the liver capsule during their migration rather than hepatic failure per se.
- **Anaemia:** mild to moderate anaemia can be seen; pallor of the skin and mucosa are commonly associated with lassitude, dizziness, palpitation and weakness.
- **Chest signs:** on auscultation, dry or moist rales can occasionally be elicited upon coughing at the base of the right lung probably due to migration of the juvenile flukes; pleural rub with effusion and even spontaneous pneumothorax have been reported; parenchymal infiltrates resembling the Loeffler syndrome and pleural effusion are the most common radiologic manifestations; pyopneumothorax has been also reported.

- **Jaundice:** it is infrequent, and when it appears, it is milder than that seen in the chronic phase.

In human endemic zones, there is usually a decrease of the prevalence from children and young subjects to adult subjects. Despite this, results demonstrate that adult subjects either maintain the parasites acquired when young or can be newly infected as the consequence of inhabiting a zone of high infection risk (Esteban et al. 1999). It must be considered here that the life span of the adult fluke in humans is between 9 and 13.5 years (Mas-Coma and Bargues 1997). Such a picture suggests that, in those areas, the majority of adult subjects should be in the biliary period, acute lesions by repetitive infections being superimposed on chronic disease with relative frequency. Thus, the acute period may be prolonged and overlap with both latent and biliary periods.

6.6.2 Biliary or Chronic Phase

This phase may develop after months to years of infection. Adult flukes in the bile ducts cause inflammation and hyperplasia of the epithelium. There is thickening and dilatation of the ducts, and the gallbladder walls ensue. The resulting cholangitis and cholecystitis, combined with the large body of the flukes, are sufficient to cause mechanical obstruction of the biliary duct which is comparatively small in diameter. The proportion of those whose infection develops into the obstructive phase or their prognosis has not been defined.

In this phase, biliary colic, epigastric pain, fatty food intolerance, nausea, jaundice, pruritus, right upper-quadrant abdominal tenderness, etc. are clinical manifestations indistinguishable from cholangitis, cholecystitis and cholelithiasis of origins other than *Fasciola* infection. Hepatic enlargement may be associated with an enlarged spleen or ascites (Acosta-Ferreira et al. 1979).

The common bile ducts are usually seen as distended and thickened. The diameters are 1.5–3.0 times normal size. The most frequent site of obstruction is the common bile duct. A diverticulum in this duct has been observed, and the head of the pancreas was enlarged and firm.

In case of obstruction, the gallbladder is usually enlarged and oedematous with thickening of the wall. The gallbladder may measure $12 \times 7 \times 7$ cm and the lower edge reaches the umbilicus. Fibrous adhesions of the gallbladder to adjacent organs are common. Lithiasis of the bile duct or the gallbladder is frequent and the stones are usually small and multiple (Chen and Mott 1990; Arjona et al. 1995). The bile duct and the gallbladder may contain blood mixed with bile (haemobilia), blood clots and fibrinous plugs.

Symptomatology in children from human endemic areas of Peru includes abdominal pain localized in the epigastrium, the Murphy symptom and jaundice as the most frequent clinical biliary characteristics, the rest of the symptoms being non-specific (Marcos Raymundo et al. 2002).

The duration and intensity of fasciolid infection and liver damage have been experimentally verified to be associated with bacterobilia by *Escherichia coli* (45% of cases), *Enterococcus faecalis* (45%) and *Klebsiella pneumoniae* (10%). This supports that the obstruction caused by advanced chronic fasciolosis may be related to biliary sepsis. These results lead to a reconsideration of treatment features in human disease, i.e. therapeutic strategies should also consider the possibility of bacterial coinfection (Valero et al. 2006b).

6.6.3 Clinical Laboratory Analyses

The outstanding abnormal laboratory findings concern leucocytosis, eosinophilia, anaemia, erythrocyte sedimentation rate, hepatic functions and serum immunoglobulin levels (Chen and Mott 1990; Mas-Coma et al. 1999b, 2000).

- **Leucocytosis and eosinophilia:** In the acute phase, the leucocyte counts are usually over 10,000 up to 43,000/mm^3. The eosinophil count is nearly always greater than 5% of the total leucocytes and may be as high as 83%. In a developed country, blood eosinophilia and the ingestion of watercress or any other suggestive freshwater plant in anamnesis are extremely useful in guiding towards a fascioliasis diagnosis. Unfortunately, these two aspects are usually not helpful in human endemic areas of developing countries, where eosinophilia may be also caused by other helminth infections and local food traditions including the ingestion of many uncooked plants may mask liver fluke infection sources (Mas-Coma et al. 2014).
- **Anaemia:** Anaemia is common but usually not very severe and mostly between 7.0 and 13.5 g dl^{-1} haemoglobin. Levels as low as 2.8 and 4.0 g dl^{-1} have been reported.
- **Erythrocyte sedimentation rate:** The erythrocyte sedimentation rate may be high in the acute phase, reaching 165 mm in an hour, normal in the latent phase and normal or only moderately high in the obstructive phase.
- **Hepatic functions:** Abnormal results in liver function tests may be found both in the invasive and biliary periods.

  In the invasive period, data are inconsistent. Abnormal results may be obtained in hepatic function tests. In the acute phase, results sometimes include a rise of the two aminotransferases (formerly transaminases) most frequently utilized, namely, alanine aminotransferase (ALT, formerly serum glutamic pyruvate transaminase—SGPT) and aspartate aminotransferase (AST, formerly serum glutamate oxaloacetic transaminase—SGOT), as well as elevated thymol turbidity, zinc sulphate turbidity, serum globulin and serum bilirubin. In other cases, tests give normal results, with the exception of alkaline phosphatase (AKP or ALP). Serum electrophoresis may show an increase of α_2- and γ-globulins. Serum triglycerides and very-low-density lipoproteins have been seen to increase, while total serum cholesterol, high-density lipoprotein

cholesterol and low-density lipoprotein cholesterol exhibited a significant decrease. These changes were due to the degenerative necrotic damage of the hepatocytes. Other reported findings include abnormally high levels of β-glucuronidase.

In the biliary period, jaundice is a prominent feature. Serum bilirubin levels between 2.0 and 8.6 have been reported. Biliary colic is usually followed by a higher level of serum bilirubin as well as dark urine positive for bilirubin. Serum bilirubin may be normal in this phase and between attacks of biliary colic. AKP, GPT, GOT and serum globulin (mainly γ-globulin) are often elevated in this phase, while albumin is decreased.

- **Immunoglobulins:** Levels for IgG, IgM and IgE are usually elevated. Specific IgE antibodies were detected in 48% of the patients. Total and specific IgE levels have been shown to be positively correlated with the egg burden, age, clinical features and degree of eosinophilia. IgA levels are usually normal but may be sometimes elevated.

6.7 Diagnosis

Several suggestive clinical presentation aspects may be useful, mainly in human endemic areas where physicians are aware about liver fluke infection risk in humans. However, verification needs the use of at least one among the direct parasitological techniques or indirect immunological tests. Other non-invasive diagnostic techniques presently available may be additionally helpful. Non-invasive diagnostic techniques which can be used for human diagnosis are radiology, radioisotope scanning, ultrasound, computed tomography and magnetic resonance (see reviews in Esteban et al. 1998 and Hillyer 1999).

For the differential diagnosis of *F. hepatica* and *F. gigantica*, clinical, pathological, coprological or immunological methods are useless. This is a problem in overlapping areas because this differential diagnosis is very important owing to the different pathological, transmission and epidemiological characteristics of the two fasciolids, as well as due to intermediate forms in which egg measurements may overlap. Despite the recent development of many molecular tests, DNA marker sequencing still remains as the only appropriate method for both haplotyping of the two pure fasciolid species, as well as for the detection of hybridization in intermediate forms. For such a purpose, the complete sequences of the two rDNA spacers ITS-2 and ITS-1 together with those of the complete mtDNA genes *cox*1 and *nad*1 have so far proved to be the markers of choice, and an exhaustive baseline and nomenclature for these four markers have already been provided (Mas-Coma et al. 2009a).

The main diagnostic tools continue to rely on stool and blood techniques and have been improved in the last two decades. Present availabilities for human diagnosis have recently been reviewed exhaustively, focusing on advantages and weaknesses,

sample management, egg differentiation, qualitative and quantitative diagnosis, antibody and antigen detection, post-treatment monitoring and post-control surveillance (Mas-Coma et al. 2014). Main conclusions referred to the pronounced difficulties of diagnosing fascioliasis given the different infection phases and parasite migration capacities, clinical heterogeneity, immunological complexity, different epidemiological situations and transmission patterns and finally lack of a diagnostic technique covering all needs and situations (Mas-Coma et al. 2014).

In livestock, it should be distinguished between (1) diagnosis of animal infection, (2) evaluation of drug efficacy and (3) evaluation of drug resistance. A number of tests are therefore available, including a few tests allowing for burden estimation by quantification by faecal egg counts, serological and coprological methods, egg hatch assays, molecular techniques and even histological methods (Fairweather et al. 2020).

6.7.1 Direct Techniques

Detection and identification of fasciolid eggs in stool sample, duodenal contents or bile continues to be the most appropriate diagnostic strategy for both detection of infection and estimation of intensity. This is even in spite of the recognized lower sensitivity of egg detection in faecal samples and its uselessness for the diagnosis of patients in the acute period, as well as the lack of an accurate relationship between egg counts per gram of faeces and the fluke burden (Valero et al. 2006a, 2009). Identifying fluke adults obtained during an endoscopy after surgical intervention either by microscopic morphometry (Periago et al. 2006) or molecular tools (Mas-Coma et al. 2009a) may also be performed nowadays, although such occasions are evidently not frequent at all. Moreover, the infrastructure for endoscopy or surgery is in general not available in rural endemic areas.

Techniques ranging from a simple direct smear to different concentration methods may be used. Egg concentration has been achieved by flotation and sedimentation techniques. The sedimentation techniques appear to be more accurate and sensitive than flotation techniques (Esteban et al. 1998; Mas-Coma et al. 1999c).

The size of the fluke eggs has always been used for human diagnosis. Basing on studies in livestock, the borderlines allowing differentiation between the two species were traditionally considered to be 150 μm in length and 90 μm in width, lower values representing *F. hepatica* and higher values *F. gigantica*. A study on morphometric traits of fasciolid eggs from different continents revealed that eggs shed by humans show traits different from eggs shed by animals. In humans, *F. hepatica* eggs are bigger and *F. gigantica* eggs are smaller than reported to date from livestock, and their measurements overlap when compared. Measurements of *F. hepatica* and *F. gigantica* eggs originating from humans and animals from sympatric areas overlap, and, therefore, they do not allow differential diagnosis when within this overlapping range (Valero et al. 2009; Mas-Coma et al. 2014). These new results should aid clinicians since the application of the classic egg size

range in human samples may lead to erroneous conclusions. Consequently, fasciolid egg size in human stool samples ought to be corrected in books and monographs as well as in guides of medical parasitology and tropical medicine.

Quantitative coprological analyses become important in epidemiological surveys as well as post-treatment monitoring. Egg burden is also crucial in the moment of deciding the appropriate treatment dose. The 400-epg threshold has been proposed for identifying high-intensity infections. To avoid risk of colic, a repeated, timely spaciated mid-dose is recommended in patients shedding more than 400 eggs (WHO 2007; Valero et al. 2012b). The second half of the regimen is administered 24 h later, once the absence of secondary effects is verified. The Kato-Katz technique appears to be appropriate, because of its simplicity, very low cost and reproducibility (Mas-Coma et al. 1999b). Its low sensitivity may be solved by repeated application.

Besides eggs in coprological analyses, adults and eggs may be also found elsewhere by means of other invasive techniques: obtaining duodenal fluid, duodenal and biliary aspirates; surgery (laparotomy, cholecystectomy, sphincterotomy); and histological examination of the liver and/or other organ biopsy materials (Mas-Coma et al. 1999b).

6.7.2 Indirect Techniques

Numerous serological, intradermal and stool antigen detection tests have been developed. Immunological techniques present the advantages of being applicable during all periods of the disease, but fundamentally during the invasive or acute period, as well as to the other situations in which coprological techniques may present problems. However, immunological techniques offer other types of problems related mainly to sensibility and specificity and are not able to differentiate between infection by *F. hepatica* and that by *F. gigantica*.

Efforts have been concentrated in obtaining purified excretory/secretory antigens and/or recombinant molecules to improve serological tests, owing to the problems of the parasitological diagnosis because of the delay in its usefulness in the acute period (coprological examination positive only after 3–4 months postinfection), intermittent egg output dynamics, very low or even absence of egg shedding in cases of only one or a few fluke adults and old chronic infections, ectopic infections, "false" fascioliasis related to eggs in transit after ingestion of infected liver from domestic animals or flukes unable to attain maturity in human subjects in non-human endemic areas (Esteban et al. 1998; Mas-Coma et al. 1999b).

Cysteine proteinases offer highly sensitive and specific markers for human fascioliasis serodiagnosis for *F. hepatica* (O'Neill et al. 1999; Strauss et al. 1999; Espinoza et al. 2007; Mezo et al. 2004) as well as for *F. gigantica* infection (Maleewong et al. 1999; Intapan et al. 1998; Tantrawatpan et al. 2005). *Fasciola hepatica* recombinant cysteine proteinases produced in yeast (O'Neill et al. 1999) or in *Escherichia coli* (Carnevale et al. 2001) have been used in ELISA methods for human infection diagnosis.

Studies in Bolivia and Peru have shown that the MM3 coproantigen-detection test allows for high sensitivity and specificity, fast large mass screening capacity, detection in the chronic period, early detection of treatment failure or reinfection in post-treated subjects and usefulness for surveillance programmes. However, this technique falls short when evaluating the fluke burden on its own (Valero et al. 2012b). The use of a new preservative/diluent CoproGuard™, developed for preservation of *Fasciola* coproantigens, proved to enhance coproantigen extraction and the antigenicity throughout the complete observation period (Ubeira et al. 2009).

The commercialized DRG *Fasciola hepatica* IgG (human) ELISA proved to be highly sensitive and specific, with a high negative predictive value but a low positive predictive value. No correlation with egg output was observed. This test may be used both as an individual serodiagnostic test when backed up by a compatible clinical history together with a second diagnostic technique for other cross-reactive helminth infections and in future large-scale epidemiological studies (Valero et al. 2012c).

A new lateral flow test (SeroFluke) for human diagnosis appears to be a useful step forward (Martinez-Sernandez et al. 2011). In comparison with an ELISA test (MM3-SERO), the SeroFluke test showed maximal specificity and sensitivity and the advantage of being applicable to both serum and whole blood samples. Its simplicity allows it to be used in major hospitals as well as in endemic/hyperendemic regions.

6.8 Treatment

Emetine and the better tolerated dehydroemetine were used widely and still continue to be used today, given intramuscularly or subcutaneously at doses of 1–10 mg/kg a day for 10 days. However, the use of emetine was progressively abandoned due to their toxic side effects involving the heart, liver and digestive tract (Mas-Coma et al. 2014).

Chloroquine improved the symptoms when applied in the acute phase. Bithionol was proposed as the drug of choice for fascioliasis treatment during the last three decades of the last century. It was usually applied at a dose of 30–50 mg/kg daily, divided into three oral doses on alternate days for 20–30 days. Occasionally, the patients required a second course to obtain a complete cure. The side effects were usually mild (Chen and Mott 1990; Esteban et al. 1998).

Regarding praziquantel, it is generally accepted today that *Fasciola* may be the only trematode genus that has practically no response to praziquantel. Metronidazole and albendazole and sporadically also mebendazole have been also applied for human fascioliasis treatment with more or less success.

Triclabendazole (Egaten®) has become the drug of choice for human fascioliasis caused by both *F. hepatica* and *F. gigantica* at present (Savioli et al. 1999; Gandhi et al. 2019). This drug is better adsorbed if administered after meals (Lecaillon et al. 1998). The recommended dosage is two separate regimens of 10 mg/kg. A cure rate of 79.2% when first used and 100% after a second round of therapy was found in

Chile (Apt et al. 1995) and 79.4% and 93.9%, respectively, in Egypt (El-Morshedy et al. 1999). Triclabendazole appears to keep its efficiency at standard regimes in human endemic areas after years (Talaie et al. 2004), although the need for a third dose has been reported in Cuba (Millan et al. 2000). This drug has moreover recently proved to be safe in the treatment of very small children (De et al. 2020).

Unfortunately, the risk of appearance of resistance to triclabendazole cannot be forgotten. Triclabendazole resistance was first described in Australia and later in European countries such as Ireland, Scotland, the Netherlands and Spain (see review in Mas-Coma et al. 2007). Very recently, it has also been found in southern Brazil (Oliveira et al. 2008) and Argentina (Olaechea et al. 2011). Up to that moment, triclabendazole resistance only concerned livestock in animal endemic areas, but unfortunately, it has very recently been also described (Ortiz et al. 2013) in a human highly endemic area such as Cajamarca, Peru (Gonzalez et al. 2011). Our understanding of the triclabendazole resistance mechanism remains far from complete, so that there is even a knowledge gap regarding its spreading capacity. A multigenic resistance origin has been suggested to underlie this resistance. Strategies to minimize the development of resistance include the use of synergistic drug combinations (Fairweather et al. 2020). Combinations of flukicides, anthelmintics and other drugs may be also useful. A number of existing flukicides for veterinary use are active against triclabendazole-resistant fasciolids, including albendazole, clorsulon, closantel, nitroxynil and oxyclozanide, although these alternatives do not act against migrating juveniles (Fairweather et al. 2020).

Nitazoxanide is a good alternative to triclabendazole, at least for the chronic stage of fascioliasis, mainly in those countries where Egaten® is still not registered but nitazoxanide is since several years. Nitazoxanide had demonstrated its efficacity against human fascioliasis in a few trials, in Egypt (Rossignol et al. 1998; Kabil et al. 2000) and Peru (Favennec et al. 2003). Its long 7-day treatment course may nevertheless become a problem. However, its usefulness for the treatment of human cases not responding to triclabendazole (Gargala et al. 2005) is of important additional value. A good nitazoxanide efficacy has recently been reported when applied to liver fluke-infected children in Mexico (Zumaquero-Ríos et al. 2013). However, differences in fasciolid susceptibility to nitazoxanide may exist depending on geographical strains. Thus, no response to nitazoxanide treatment was reported in 24 cases of liver fluke infection in Esmeralda, Camagüey, Cuba (Del Risco et al. 2001), and a triclabendazole-resistant *F. hepatica*-infected patient not responding to nitazoxanide treatment has recently been reported in the Netherlands (Winkelhagen et al. 2012).

A deep analysis of all the drugs presently available for the treatment of fascioliasis in both animals and humans has been recently published. Egg formation, production, development and viability are crucial in fascioliasis transmission and sensitive to drug action. A number of protocols or egg hatch tests have been developed to study drug action impact on the development and hatching of fasciolid eggs, but there is no standardized method (Fairweather et al. 2020).

6.9 Prognosis, Complications, Sequelae and Death Causes

The prognosis of fascioliasis depends on the promptness of the treatment. At the phase of hepatic invasion (acute phase), the treatment most usually gives rise to cure without sequelae. However, when treated late, the prognosis becomes subordinated to the importance of the affection of the biliary ducts.

With regard to cure criteria, from the clinical point of view, the improvement of the general condition of the patient with fever disappearance and weight recovery indicates a good prognosis, although one should be cautious as relapses are possible. The normalization of blood eosinophilia as best criterion in all cases, the disappearance of eggs in stools in cases of patients diagnosed in the biliary phase and the progressive disappearance of hepatic function test abnormalities are helpful indicators of treatment effectivity (Mas-Coma et al. 2014).

One complication may be bleeding. Subcapsular haematoma and severe haemobilia have been reported. Haematemesis and melaena were associated with obstructive jaundice, epigastric pain and severe anaemia. The sources of the bleeding were all identified from laparotomy, and other causes of upper gastrointestinal bleeding have been carefully ruled out. In one case, bleeding occurred due to an ulcer in the common bile duct, and in other cases, no single bleeding point was detected in the common bile ducts and/or in the gallbladders.

Biliary cirrhosis is another complication. During the course of the infection, inflammation, hyperplasia and hypertrophy of the bile duct epithelia may induce periductal fibrosis. Prolonged heavy infection rarely leads to biliary cirrhosis. The outstanding findings on physical examinations were firm untender hepatomegaly with or without jaundice and ascites. Splenomegaly was not prominent. Sclerosing cholangitis with biliary cirrhosis was detected by endoscopic retrograde cholangiopancreatography.

Another severe complication is the multiple extrahepatic venous thrombosis. In the post-mortem examination of one patient who died suddenly, multiple thrombosis of the ovaric, suprahepatic, mesenteric and myocardial veins, along with massive pulmonary embolism, was disclosed. During the invasion period, another patient developed a complete thrombosis of the superior vena cava (Mas-Coma et al. 1999b, 2000).

The severity of fascioliasis was emphasized in many cases, with regard to the progressive general deterioration of the patients with slimming, anorexia and physical and neuropsychic asthenia, as well as the fact that cure does not mean total recovery but that fascioliasis left them as handicapped and frail subjects. In several patients presenting neurological and ocular manifestations, given sequelae were noted to persist despite treatment. These sequelae were sometimes important, even making difficult or impeding the professional activity of the patient (Mas-Coma et al. 2014).

Several deaths related to fasciolosis have been reported in the recent literature. Post-mortem examination of a patient who died from an acute liver condition showed *F. hepatica* to be responsible for blockage of the bile duct. More than

40 flukes were detected from the liver of a patient at autopsy. Between 1 and 14 flukes were found in each liver at post-mortem in 81 inhabitants of the Samarkand region in 1968–1986, although deaths were not presumed to be due to fasciolosis. However, in human hyperendemic zones with depauperate socio-economic status, unhygienic conditions and high child morbidity and mortality, studies are still needed to ascertain whether fasciolosis may be related to death, above all in very young children (Mas-Coma and Bargues 1997; Mas-Coma et al. 2000). Many fatal cases have been reported among patients suffering from neurological disorders, whether due to direct or indirect affection of the central nervous system (Mas-Coma et al. 2013, 2014).

6.10 Prevention and Control

Studies on human endemic areas performed in the last two decades have shown that traditional epidemiological patterns of animal fascioliasis may not always explain the characteristics of human infection in a given area. Therefore, control measures for human fascioliasis should consider the results of the ecoepidemiological studies previously undertaken in the area concerned (Mas-Coma et al. 2009a). This is the reason why the World Health Organization (WHO) launched a worldwide initiative against this disease including different control strategies depending on the human endemic areas and countries. A recent complete analysis of individual and general preventive measures to avoid human infection has shown a scenario more complicated than that considered in the past (Mas-Coma et al. 2018).

6.10.1 Prevention of Human Infection

The prevention of human infection may be achieved by strict control of the human infection sources, mainly with regard to watercress and other aquatic plants for human consumption, especially in endemic zones. Unfortunately, potassium permanganate, which had been suggested to be the most effective preventive tool for killing metacercariae attached to leaves and vegetables used in salads, has been shown to have no effectivity on metacercarial viability, even at very high doses (Ashrafi et al. 2006).

Moreover, it should be considered that infection risks shall not be restricted to only ingestion of freshwater vegetables, as always mentioned. The different human infection sources may be taken into account, mainly in human endemic areas. Drinking of natural freshwater should be avoided in human endemic areas. In the Nile Delta region, persons living in houses where piped water is present showed to have a higher infection risk (Curtale et al. 2003).

The possibility of human infection in urban areas should not be neglected. Thanks to transport of vegetables (both aquatic and terrestrial) from rural endemic zones to

Fig. 6.4 Uncontrolled sale of vegetables involved in the transmission of human fascioliasis in a city market in Quy Nhon, Vietnam (Orig. S. Mas-Coma)

cities, plants carrying metacercariae can be sold in non-controlled city markets giving rise to urban infection (Fig. 6.4) (Mas-Coma 2004).

Education should always be included within general control measures to be applied in human endemic areas, mainly to let inhabitants know about the human infection sources. The community should be appropriately informed about the disease, its pathogenicity, its transmission and where to go for diagnosis if suggestive symptoms appear.

6.10.2 Control Measures at Community Level

The availability of triclabendazole prompted the WHO to launch a decisive step forward within its worldwide initiative against human fascioliasis (WHO 2007, 2008) in recent years. This initiative includes action in human fascioliasis endemic areas presenting different epidemiological situations and transmission patterns (Mas-Coma 2005; Mas-Coma et al. 2009a). Pilot schemes were designed to assess the best control strategies according to the different epidemiological situations and transmission patterns in the way to decrease morbidity, mainly in children. Selective patient treatment after passive detection in hospitals was the strategy applied in

Vietnam and infected subject treatment after active detection in surveys the one applied in Egypt. The Northern Altiplano in Bolivia was chosen as an example of the altiplanic pattern, while Cajamarca, Peru, was chosen as an example of the valley pattern. The pilot interventions in these two Andean areas demonstrated the absence of severe side effects in triclabendazole treatments of schoolchildren (Villegas et al. 2012), which subsequently allowed for the launching of mass treatments. Many other countries are nowadays receiving yearly triclabendazole donations through WHO for the treatment of their patients, in an expansion of the aforementioned WHO initiative.

In countries where watercress is included in food traditions, such as France, commercial growing of watercress should be carried out under completely controlled conditions, without access for ruminants and snail vectors to the watercress cultures.

In Egypt, the construction and utilization of the so-called washing units, in which the water was appropriately filtered, gave rise to a marked decrease of human infection in a locality of the Nile Delta region where a high prevalence in humans was initially found (Mas-Coma 2004).

The World Health Organization is recently launching an initiative to assess One Health control action to complement the preventive chemotherapy campaigns in human endemic areas, with the aim to decrease the infection and reinfection risk in between the yearly triclabendazole mono-dose mass treatments. This initiative comprises several multidisciplinary axes, including the environment, snail vectors, animal reservoirs and humans, all together considered from the dynamic point of view of the changes induced by climate change and global change factors (Bargues et al. 2020, 2021; Mas-Coma et al. 2020a, b, 2021).

6.10.3 Progress in Vaccinology

Initial research focused on the identification of molecules of fasciolid parasites that played critical functions at the host-parasite interface, by way of isolating and characterizing the molecules that were secreted by the flukes. ES products from adult worms showed two fractions: a $>$ 200 kDa fraction including several proteins and other molecules and a 40 kDa fraction consisting of cathepsin L cysteine peptidases subsequently fractioned into two subfractions presenting distinct enzymatic activities—cathepsin L1 (FhCL1) and cathepsin L2 (FhCL2) (Dalton et al. 1996). Cattle vaccination with FhCL1 induced protection levels of up to 69.5%, and combination vaccines reached a 72.4%. These vaccines also exhibited significant anti-fecundity effects of reduced egg output and lower egg viability in up to 98%. Thus, vaccines were showing for the first time that they could potentially block the transmission of the disease. Assays were further developed by using different adjuvants.

The development of high-level protective vaccines that can also impact on disease transmission may require combinations of various parasite molecules. Therefore, two new important vaccine candidates have been identified, peroxidase (FhPrx)

and helminth defence molecule (FhHDM), that also perform potent immunomodulatory functions (Robinson et al. 2013). Several other *Fasciola* molecules hold promise as components of combination vaccines, including glutathione S-transferase (FhGST), cathepsin B (FhCB1–10), fatty acid-binding protein (FhFABP) and leucine aminopeptidase (FhLAP) (Spithill et al. 2012).

In sheep, FhLAP was shown to induce protective responses both alone and in combination with FhCL1 and FhCL2 in native form (Piacenza et al. 1999). Moreover, impressive levels of protection have been observed in sheep (up to 87%) vaccinated with a recombinant FhLAP formulated in adjuvants that induce high-titre IgG1 and IgG2 (Maggioli et al. 2011).

Unfortunately, two challenges appear far from being solved at present. The first is the rapid and potent ability of *F. hepatica* to suppress the protective arm of the immune response, which explains why infected hosts do not develop immune resistance and provides a reasonable explanation for why efforts have been largely unsuccessful in developing efficacious vaccines against *Fasciola* in animals. Indeed, fluke-induced immunomodulation/immunosuppression is induced rapidly upon *Fasciola* invasion and maintained through the chronic infection. It is mediated by fluke molecules that alter the function of innate immune cells (dendritic cells, macrophages, mast cells) and the quality and magnitude of adaptive immune cell (T and B cells) responses (Dalton et al. 2013). The second challenge refers to the differences in cell- and/or antibody-mediated responses depending on host species, which means that even being successful in obtaining an effective vaccine against animal infections, this will not allow for a direct extrapolation to a vaccine for humans. However, the wide knowledge obtained on the immunological processes and molecules involved will undoubtedly facilitate the development of a vaccine for human use in the future.

6.11 Acknowledgements

Review of human fascioliasis carried out within the Health Research Project No. PI16/00520, Subprograma Estatal de Generación de Conocimiento de la Acción Estratégica en Salud (AES) y Fondos FEDER, Plan Estatal de Investigación Científica y Técnica y de Innovación, ISCIII-MINECO, Madrid, Spain; ISCIII-RETIC RD12/0018/0013, Red de Investigación de Centros de Enfermedades Tropicales (RICET), of the Program of Redes Temáticas de Investigación Cooperativa RETICS/FEDER, Ministry of Health and Consumption, Madrid, Spain; and PROMETEO/2016/099, of the Program of Ayudas para Grupos de Investigación de Excelencia, Generalitat Valenciana, Valencia, Spain

References

Acosta-Ferreira W, Vercelli-Retta J, Falconi LM (1979) Fasciola hepatica human infection. Histopathological study of sixteen cases. Virchows Arch A (Pathological Anatomy and Histology) 383:319–327

Afshan K, Fortes-Lima CA, Artigas P, Valero MA, Qayyum M, Mas-Coma S (2014) Impact of climate change and man-made irrigation systems on the transmission risk, long-term trend and seasonality of human and animal fascioliasis in Pakistan. Geospat Health 8:317–334

Apt W, Aguilera X, Vega F, Miranda C, Zulantay I, Perez C, Gabor M, Apt P (1995) Treatment of human chronic fascioliasis with triclabendazole: drug efficacy and serologic response. Am J Trop Med Hyg 52:532–535

Arjona R, Riancho JA, Aguado JM, Salesa R, Gonzalez-Macias J (1995) Fascioliasis in developed countries: a review of classic and aberrant forms of the disease. Medicine (Baltimore) 74:13–23

Artigas P, Bargues MD, Mera y Sierra R, Agramunt VH, Mas-Coma S (2011) Characterisation of fascioliasis lymnaeid intermediate hosts from Chile by DNA sequencing, with emphasis on *Lymnaea viator* and *Galba truncatula*. Acta Trop 120:245–257

Ashrafi K, Valero MA, Massoud J, Sobhani AR, Solaymani-Mohammadi S, Conde P, Khoubbane M, Bargues MD, Mas-Coma S (2006) Plant-borne human contamination by fascioliasis. Am J Trop Med Hyg 75:295–302

Bargues MD, Vigo M, Horak P, Dvorak J, Patzner RA, Pointier JP, Jackiewicz M, Meier-Brook C, Mas-Coma S (2001) European Lymnaeidae (Mollusca: Gastropoda), intermediate hosts of trematodiases, based on nuclear ribosomal DNA ITS-2 sequences. Infect Genet Evol 1(2): 85–107

Bargues MD, Artigas P, Mera y Sierra RL, Pointier JP, Mas-Coma S (2007) Characterisation of *Lymnaea cubensis*, *L. viatrix* and *L. neotropica* n. sp., the main vectors of *Fasciola hepatica* in Latin America, by analysis of their ribosomal and mitochondrial DNA. Ann Trop Med Parasit 101:621–641

Bargues MD, Artigas P, Khoubbane M, Flores R, Glöer P, Rojas-Garcia R, Ashrafi K, Falkner G, Mas-Coma S (2011a) Lymnaea schirazensis, an overlooked snail distorting fascioliasis data: genotype, phenotype, ecology, worldwide spread, susceptibility, applicability. PLoS One 6(9): e24567. (33 pp. + 3 Suppl. Tables + 5 Suppl. Figures)

Bargues MD, Artigas P, Khoubbane M, Mas-Coma S (2011b) DNA sequence characterisation and phylogeography of *Lymnaea cousini* and related species, vectors of fascioliasis in northern Andean countries, with description of *L. meridensis* n. sp. (Gastropoda: Lymnaeidae). Parasite Vect 4:132. (22 pp.)

Bargues MD, Gonzalez C, Artigas P, Mas-Coma S (2011c) A new baseline for fascioliasis in Venezuela: lymnaeid vectors ascertained by DNA sequencing and analysis of their relationships with human and animal infection. Parasite Vect 4:200. (18 pp.)

Bargues MD, Malandrini JB, Artigas P, Soria CC, Velasquez JN, Carnevale S, Mateo L, Khoubbane M, Mas-Coma S (2016) Human fascioliasis endemic areas in Argentina: multigene characterisation of the lymnaeid vectors and climatic-environmental assessment of the transmission pattern. Parasit Vectors 9:306

Bargues MD, Gayo V, Sanchis J, Artigas P, Khoubbane M, Birriel S, Mas-Coma S (2017) DNA multigene characterization of Fasciola hepatica and Lymnaea neotropica and its fascioliasis transmission capacity in Uruguay, with historical correlation, human report review and infection risk analysis. PLoS Negl Trop Dis 11:e0005352

Bargues MD, Artigas P, Angles R, Osca D, Duran P, Buchon P, Gonzales-Pomar RK, Pinto-Mendieta J, Mas-Coma S (2020) Genetic uniformity, geographical spread and anthropogenic habitat modifications of lymnaeid vectors found in a One Health initiative in the highest human fascioliasis hyperendemic of the Bolivian Altiplano. Parasit Vectors 13:171

Bargues MD, Angles R, Coello J, Artigas P, Funatsu IR, Cuervo PF, Buchon P, Mas-Coma S (2021) One Health initiative in the Bolivian Altiplano human fascioliasis hyperendemic area:

Lymnaeid biology, population dynamics, microecology and climatic factor influences. Braz J Vet Parasitol 30:e025620

Brady MT, O'Nneill SM, Dalton JP, Mills KH (1999) *Fasciola hepatica* suppresses a protective Th1 response against *Bordetella pertussis*. Infect Immun 67:5372–5378

Bergquist R, Luvall JC, Malone JB (2021) The changing risk of vector-borne diseases: global satellite remote sensing and geospatial surveillance at the forefront. Geospat Health 16:1047

Carnevale S, Rodriguez MI, Guarnera EA, Carmona C, Tanos T, Angel SO (2001) Immunodiagnosis of fasciolosis using recombinant procathepsin L cysteine proteinase. Diagn Microbiol Infect Dis 41:43–49

Chen MG, Mott KE (1990) Progress in assessment of morbidity due to Fasciola hepatica infection: a review of recent literature. Trop Dis Bull 87(4):R1–R38

Curtale F, Mas-Coma S, Hassanein YAE, Barduagni P, Pezzotti P, Savioli L (2003) Clinical signs and household characteristics associated with human fascioliasis among rural population in Egypt: a case-control study. Parassitologia 45:5–11

Cwiklinski K, Dalton JP, Dufresne PJ, La Course J, Williams DJL, Hodgkinson J, Paterson S (2015) The Fasciola hepatica genome: gene duplication and polymorphism reveals adaptation to the host environment and the capacity for rapid evolution. Genome Biol 16:71

Dalton JP, McConigle S, Rolph TP, Andrews SJ (1996) Induction of protective immunity in cattle against infection with *Fasciola hepatica* by vaccination with cathepsin L proteinases and with hemoglobin. Infect Immun 64:5066–5074

Dalton JP, Robinson MW, Mulcahy G, O'Neill SM, Donnelly S (2013) Immunomodulatory molecules of *Fasciola hepatica*: candidates for both vaccine and immunotherapeutic development. Vet Parasitol 195:272–285

Del Risco BU, Vazquez Drake CT, Garcia Gonzalez G, Sanchen Casa A (2001) Evaluación de la excreción de huevos de *Fasciola hepatica* por tres esquemas terapéuticos. Rev Electr Arch Méd Camagüey 5(4):4

De NV, Le TH, Agramunt VH, Mas-Coma S (2020) Early postnatal and preschool age infection by Fasciola spp.: report of five cases from Vietnam and worldwide review. Am J Trop Med Hyg 103:1578–1589

El-Morshedy H, Farghaly A, Sharaf S, Abou-Basha L, Barakat R (1999) Triclabendazole in the treatment of human fascioliasis: a community-based study. East Medit Health J 5:888–894

Espinoza JR, Maco V, Marcos L, Saez S, Neyra V, Terashima A, Samalvides F, Gotuzzo E, Chavarry E, Huaman C, Bargues MD, Valero MA, Mas-Coma S (2007) Evaluation of Fas2-ELISA for the serological detection of *Fasciola hepatica* infection in humans. Am J Trop Med Hyg 76:977–982

Espinoza JR, Terashima A, Herrera-Velit P, Marcos LA (2010) Fasciolosis humana yanimal en el Perú: Impacto en la economía de las zonas endémicas. Rev Peru Med Exp Salud Públ 27:604–612

Esteban JG, Flores A, Aguirre C, Strauss W, Angles R, Mas-Coma S (1997a) Presence of very high prevalence and intensity of infection with *Fasciola hepatica* among Aymara children from the northern Bolivian Altiplano. Acta Trop 66:1–14

Esteban JG, Flores A, Angles R, Strauss W, Aguirre C, Mas-Coma S (1997b) A population-based coprological study of human fascioliasis in a hyperendemic area of the Bolivian Altiplano. Trop Med Int Health 2:695–699

Esteban JG, Bargues MD, Mas-Coma S (1998) Geographical distribution, diagnosis and treatment of human fascioliasis: a review. Res Rev Parasitol 58:13–42

Esteban JG, Flores A, Angles R, Mas-Coma S (1999) High endemicity of human fascioliasis between Lake Titicaca and La Paz valley, Bolivia. Trans R Soc Trop Med Hyg 93:151–156

Esteban JG, Gonzalez C, Bargues MD, Angles R, Sanchez C, Naquira C, Mas-Coma S (2002) High fascioliasis infection in children linked to a man-made irrigation zone in Peru. Trop Med Int Health 7:339–348

Esteban JG, Gonzalez C, Curtale F, Muñoz-Antoli C, Valero MA, Bargues MD, El Sayed M, El Wakeel A, Abdel-Wahab Y, Montresor A, Engels D, Savioli L, Mas-Coma S (2003)

Hyperendemic fascioliasis associated with schistosomiasis in villages of the Nile Delta, Egypt. Am J Trop Med Hyg 69:429–437
Fairweather I, Brennan GP, Hanna REB, Robinson MW, Skuce PJ (2020) Drug resistance in liver flukes. Int J Parasitol Drugs Drug Resist 12:39–59
Favennec L, Jave Ortiz J, Gargala G, Lopez Chegne N, Ayoub A (2003) Double blind, randomized, placebo-controlled study of nitazoxanide in the treatment of fascioliasis in adults and children from northern Peru. Alim Pharmacol Ther 17:265–270
Gandhi P, Schmitt EK, Chen CW, Samantray S, Venishetty VK, Hughes D (2019) Triclabendazole in the treatment of human fascioliasis: a review. Trans R Soc Trop Med Hyg 113:797–804
Gargala G, Abboud P, Borsa-Lebas F, Courchay E, Koning E, Favennec L, Caron F (2005) Case report of successful treatment of triclabendazole resistant fascioliasis by nitazoxanide. In Medicine and health in the tropics (XVIth International Congress for tropical medicine and malaria, Marseille, France, 11–15 Sept. 2005), Abstract Book, P680: 283
Gil-Benito A, Ciolkovitch A, Mas-Coma S, Quilici M (1991) Enquête sur la distomatose à *Fasciola hepatica* en Corse. Méditerranée Médicale (Marseille) 403:21–25
Girones N, Valero MA, Garcia-Bodelon MA, Chico-Calero MI, Punzon C, Fresno M, Mas-Coma S (2007) Immune suppression in advanced chronic fascioliasis: an experimental study in a rat model. J Infect Dis 195:1504–1512
Gonzalez LC, Esteban JG, Bargues MD, Valero MA, Ortiz P, Naquira C, Mas-Coma S (2011) Hyperendemic human fascioliasis in Andean valleys: an altitudinal transect analysis in children of Cajamarca province, Peru. Acta Trop 120:119–129
Gonzalez-Miguel J, Valero MA, Reguera-Gomez M, Mas-Bargues C, Bargues MD, Simon-Martin F, Mas-Coma S (2019) Numerous Fasciola plasminogen-binding proteins may underlie blood-brain barrier leakage and explain neurological disorder complexity and heterogeneity in the acute and chronic phases of human fascioliasis. Parasitology 146:284–298
Hillyer GV (1999) Immunodiagnosis of human and animal fasciolosis. In: Dalton JP (ed) Fasciolosis. CAB International Publishing, Wallingford, pp 435–447
Intapan PM, Mallewong W, Wongkham C, Tomanakarn K, Ieamviteevanich K, Pipitgool V, Sukolapong V (1998) Excretory-secretory antigen components of adult *Fasciola gigantica* recognized by infected human sera. Southeast As J Trop Med Publ Health 29:579–583
Jaffar Z, Sivakuru T, Roberts K (2004) CD4(+)CD25(+) T cells regulate airway eosinophilic inflammation by modulating the Th2 cell phenotype. J Immunol 172:3842–3849
Kabil SM, El Ashry E, Ashraf NK (2000) An open-label clinical study of nitazoxanide in the treatment of human fascioliasis. Curr Ther Res 61:339–345
Lecaillon JB, Gobdillon J, Campestrini J (1998) Effect of food on bioavailability of triclabendazole in patients with fascioliasis. Br J Clin Pharmacol 45:601–604
Maggioli G, Acosta D, Silveira F, Rossi S, Giacaman S, Basika T, Gayo V, Rosadilla D, Roche L, Tort J, Carmona C (2011) The recombinant gut-associated M17 leucine aminopeptidase in combination with different adjuvants confers a high level of protection against *Fasciola hepatica* in sheep. Vaccine 29:9057–9063
Maleewong W, Wongkham C, Intapan PM, Pipitgol V (1999) *Fasciola gigantica*-specific antigens: purification by a continuous-elution method and its evaluation for the diagnosis of human fascioliasis. Am J Trop Med Hyg 61:648–651
Marcos Raymundo LA, Maco Flores V, Terashima Iwashita A, Samalvides Cuba F, Gotuzzo Herencia E (2002) Características clínicas de la infección crómica por *Fasciola hepatica* en niños. Rev Gastroenterol Peru 22:228–233
Marcos LA, Busalleu A, Terashima A, Espinoza JR (2009) Detection of antibodies against *Fasciola hepatica* in cirrhotic patients from Peru. J Helminthol 83:23–26
Martinez-Sernandez V, Muiño L, Perteguer MJ, Garate T, Mezo M, Gonzalez-Warleta M, Muro A, Correia da Costa JM, Romaris F, Ubeira FM (2011) Development and evaluation of a new lateral flow immunoassay for serodiagnosis of human fasciolosis. PLoS Negl Trop Dis 5(11): e1376

Mas-Coma S (2004) Human fascioliasis. In: Cotruvo JA, Dufour A, Rees G, Bartram J, Carr R, Cliver DO, Craun GF, Fayer R, Gannon VPJ (eds) World Health Organization (WHO), waterborne Zoonoses: identification, causes and control. IWA Publishing, London, pp 305–322

Mas-Coma S (2005) Epidemiology of fascioliasis in human endemic areas. J Helminthol 79:207–216

Mas-Coma S (2020) Human fascioliasis emergence risks in developed countries: from individual patients and small epidemics to climate and global change impacts. Enf Infec Microbiol Clín 38 (6):253–256

Mas-Coma S, Bargues MD (1997) Human liver flukes: a review. Res Rev Parasitol 57:145–218

Mas-Coma S, Angles R, Esteban JG, Bargues MD, Buchon P, Franken M, Strauss W (1999a) The northern Bolivian Altiplano: a region highly endemic for human fascioliasis. Trop Med Int Health 4:454–467

Mas-Coma S, Bargues MD, Esteban JG (1999b) Human Fasciolosis. In: Dalton JP (ed) Fasciolosis. CAB International Publishing, Wallingford, pp 411–434

Mas-Coma S, Esteban JG, Bargues MD (1999c) Epidemiology of human fascioliasis: a review and proposed new classification. Bull WHO 77:340–346

Mas-Coma S, Bargues MD, Marty AM, Neafie RC (2000) Hepatic Trematodiases. In: Meyers WM, Neafie RC, Marty AM, Wear DJ (eds) Pathology of infectious diseases, Vol. 1 helminthiases armed forces. Institute of Pathology and American Registry of Pathology, Washington DC, pp 69–92

Mas-Coma S, Bargues MD, Valero MA, Fuentes MV (2003) Adaptation capacities of Fasciola hepatica and their relationships with human fascioliasis: from below sea level up to the very high altitude. In: Combes C, Jourdane J (eds) Taxonomy, ecology and evolution of metazoan parasites, vol 2. Presses Universitaires de Perpignan, Perpignan, pp 81–123

Mas-Coma S, Bargues MD, Valero MA (2005) Fascioliasis and other plant-borne trematode zoonoses. Int J Parasitol 35:1255–1278

Mas-Coma S, Bargues MD, Valero MA (2007) Plant-borne trematode zoonoses: fascioliasis and fasciolopsiasis. In: Murrell D, Fried B (eds) World class parasites, Vol. 11. Food-borne parasites, fish and plant-borne parasites, vol 11. Springer Verlag, New York, pp 293–334

Mas-Coma S, Valero MA, Bargues MD (2009a) *Fasciola*, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. Adv Parasitol 69:41–146

Mas-Coma S, Valero MA, Bargues MD (2009b) Climate change effects on trematodiases, with emphasis on zoonotic fascioliasis and schistosomiasis. Vet Parasitol 163:264–280

Mas-Coma S, Agramunt VH, Valero MA (2013) Direct and indirect affection of the central nervous system by *Fasciola* infection. Handb Clin Neurol 114:297–310

Mas-Coma S, Agramunt VH, Valero MA (2014) Neurological and ocular fascioliasis in humans. Adv Parasitol 84:27–149

Mas-Coma S, Bargues MD, Valero MA (2018) Human fascioliasis infection sources, their diversity, incidence factors, analytical methods and prevention measures. Parasitology 145(13, Special Issue):1665–1699

Mas-Coma S, Buchon P, Funatsu IR, Angles R, Artigas P, Valero MA, Bargues MD (2020a) Sheep and cattle reservoirs in the highest human fascioliasis hyperendemic area: experimental transmission capacity, field epidemiology, and control within a One Health initiative in Bolivia. Front Vet Sci 7:583204

Mas-Coma S, Buchon P, Funatsu IR, Angles R, Mas-Bargues C, Artigas P, Valero MA, Bargues MD (2020b) Donkey fascioliasis within a One Health control action: transmission capacity, field epidemiology, and reservoir role in a human hyperendemic area. Front Vet Sci 7:591384

Mas-Coma S, Funatsu IR, Angles R, Buchon P, Mas-Bargues C, Artigas P, Valero MA, Bargues MD (2021) Domestic pig prioritized in one health action against fascioliasis in human endemic areas: experimental assessment of transmission capacity and epidemiological evaluation of reservoir role. One Health 13:100249

McNulty SN, Tort JF, Rinaldi G, Fischer K, Rosa BA, Smircich P, Fontenla S, Choi YJ, Tyagi R, Hallsworth-Pepin K, Mann VH, Kammili L, Latham PS, Dell'Oca N, Dominguez F, Carmona C, Fischer PU, Brindley PJ, Mitreva M (2017) Genomes of Fasciola hepatica from the Americas reveal colonization with Neorickettsia endobacteria related to the agents of Potomac horse and human Sennetsu fevers. PLoS Genet 13:e1006537

Mera y Sierra R, Neira G, Bargues MD, Cuervo PF, Artigas P, Logarzo L, Cortiñas G, Ibaceta DEJ, Lopez Garrido A, Bisutti IE, Mas-Coma S (2020) Equines as reservoirs of human fascioliasis: transmission capacity, epidemiology and pathogenicity in Fasciola hepatica infected mules. J Helminthol 94:e189

Mezo M, Gonzalez-Warleta M, Carro C, Ubeira FM (2004) An ultrasensitive capture ELISA for detection of *Fasciola hepatica* coproantigens in sheep and cattle using a new monoclonal antibody (MM3). J Parasitol 90:845–852

Millan JC, Mull R, Freise S, Richter J (2000) The efficacy and tolerability of triclabendazole in Cuban patients with latent and chronic *Fasciola hepatica* infection. Am J Trop Med Hyg 63: 264–269

Olaechea F, Lovera V, Larroza M, Raffo F, Cabrera R (2011) Resistance of *Fasciola hepatica* against Triclabendazole in cattle in Patagonia (Argentina). Vet Parasitol 178:364–366

Oliveira DR, Ferreira DM, Stival CC, Romero F, Cavagnolli F, Kloss A, Araujo F, Molento MB (2008) Triclabendazole resistance involving *Fasciola hepatica* in sheep and goats during an outbreak in Almirante Tamandare, Parana, Brazil. Rev Brasil Parasitol Vet 17(Supl. 1):149–153

O'Neill SM, Parkinson SM, Dowd AJ, Strauss W, Angles R, Dalton JP (1999) Immunodiagnosis of human fascioliasis using recombinant *Fasciola hepatica* cathepsin L1 cysteine proteinase. Am J Trop Med Hyg 60:749–751

O'Neill SM, Brady MT, Callanan JJ, Mulcahy G, Joyce P, Mills KH, Dalton JP (2000) *Fasciola hepatica* infection downregulates Th1 responses in mice. Parasite Immunol 22:147–155

Ortiz P, Scarcella S, Cerna C, Rosales C, Cabrera M, Guzman M, Lamenza P, Solana H (2013) Resistance of *Fasciola hepatica* against triclabendazole in cattle in Cajamarca (Peru): a clinical trial and in vive efficacy test in sheep. Vet Parasitol 91(1–2):153–158. https://doi.org/10.1016/j.vetpar.2013.01.001

Periago MV, Valero MA, Panova M, Mas-Coma S (2006) Phenotypic comparison of allopatric populations of *Fasciola hepatica* and *Fasciola gigantica* from European and African bovines using a computer image analysis system (CIAS). Parasitol Res 99:368–378

Piacenza L, Acosta D, Basmadjian L, Dalton JP, Carmona C (1999) Vaccination with cathepsin L proteinases and with leucine aminopeptidase induces high levels of protection against fascioliasis in sheep. Infect Immun 67:1954–1961

Robinson MW, Dalton JP, O'Brien BA, Donelly S (2013) *Fasciola hepatica*: the therapeutic potential of a worm secretome. Int. J. Parasitol 43:283–291

Rossignol JF, Abaza H, Friedman H (1998) Successful treatment of human fascioliasis with nitazoxanide. Trans R Soc Trop Med Hyg 92:103–104

Savioli L, Chistulo L, Montresor A (1999) New opportunities for the control of fascioliasis. Bull WHO 77:300

Spithill TW, Carmona C, Piedrafita D, Smooker PM (2012) Prospects for immunoprophylaxis against Fasciola hepatica (liver fluke). In: Caffrey CR, Selzer PM (eds) Parasitic helminths: targets, screens, drugs and vaccines. Wiley-VCH Verlag GmbH & Co, Weinheim

Strauss W, O'Neill SM, Parkinson M, Angles R, Dalton JP (1999) Diagnosis of human fascioliasis: detection of anti-cathepsin L antibodies in blood samples collected on filter paper. Am J Trop Med Hyg 60:746–748

Sunita K, Mas-Coma S, Bargues MD, Sadaf, Khan MA, Habib M, Mustafa S, Husain SA (2021) Buffalo infection by Fasciola gigantica transmitted by Radix acuminata in Uttar Pradesh, India: a molecular tool to improve snail vector epidemiology assessments and control surveillance. Acta Parasitol 66:1396–1405

Talaie H, Emami H, Yadegarinia D, Nava-Ocampo AA, Massoud J, Azmoudeh M, Mas-Coma S (2004) Randomized trial of a single, double and triple dose of 10 mg/kg of a human formulation of triclabendazole in patients with fascioliasis. Clin Exp Pharmacol Physiol 31:777–782

Tantrawatpan C, Maleewong W, Wongkham C, Wongkham S, Intapan PM, Nakashima K (2005) Serodiagnosis of human fascioliasis by cystatin capture enzyme-linked immunosorbent assay with recombinant *Fasciola gigantica* capthespsin L antigen. Am J Trop Med Hyg 72:82–86

Ubeira FM, Muiño L, Valero MA, Periago MV, Perez-Crespo I, Mezo M, Gonzalez-Warleta M, Romaris F, Paniagua E, Cortizo S, Llovo J, Mas-Coma S (2009) MM3-ELISA detection of *Fasciola hepatica* coproantigens in preserved human stool samples. Am J Trop Med Hyg 81: 156–162

Valero MA, Mas-Coma S (2000) Comparative infectivity of *Fasciola hepatica* metacercariae from isolates of the main and secondary reservoir animal host species in the Bolivian Altiplano high human endemic region. Folia Parasitol 47:17–22

Valero MA, Santana M, Morales M, Hernandez JL, Mas-Coma S (2003) Risk of gallstone disease in advanced chronic phase of fascioliasis: an experimental study in a rat model. J Infect Dis 188: 787–793

Valero MA, De Renzi M, Panova M, Garcia-Bodelon MA, Periago MV, Ordoñez D, Mas-Coma S (2006a) Crowding effect on adult growth, pre-patent period and egg shedding of *Fasciola hepatica*. Parasitology 133:453–463

Valero MA, Navarro M, Garcia-Bodelon MA, Marcilla A, Morales M, Garcia JE, Hernandez JL, Mas-Coma S (2006b) High risk of bacterobilia in advanced experimental chronic fasciolosis. Acta Trop 100:17–23

Valero MA, Girones N, Garcia-Bodelon MA, Periago MV, Chico-Calero I, Khoubbane M, Fresno M, Mas-Coma S (2008) Anaemia in advanced chronic fasciolosis. Acta Trop 108:35–43

Valero MA, Perez-Crespo I, Periago MV, Khoubbane M, Mas-Coma S (2009) Fluke egg characteristics for the diagnosis of human and animal fascioliasis by *Fasciola hepatica* and *F. gigantica*. Acta Trop 111:150–159

Valero MA, Perez-Crespo I, Khoubbane M, Artigas P, Panova M, Ortiz P, Maco V, Espinoza JR, Mas-Coma S (2012a) *Fasciola hepatica* phenotypic characterisation in Andean human endemic areas: valley versus altiplanic patterns analysed in liver flukes from sheep from Cajamarca and Mantaro, Peru. Infect Genet Evol 12:403–410

Valero MA, Periago MV, Perez-Crespo I, Angles R, Villegas F, Aguirre C, Strauss W, Espinoza JR, Herrera P, Terashima A, Tamayo H, Engels D, Gabrielli AF, Mas-Coma S (2012b) Field evaluation of a coproantigen detection test for fascioliasis diagnosis and surveillance in human hyperendemic areas of Andean countries. PLoS Negl Trop Dis 6(9):e1812. (11 pp.)

Valero MA, Periago MV, Perez-Crespo I, Rodriguez E, Perteguer MJ, Garate T, Gonzalez-Barbera EM, Mas-Coma S (2012c) Assessing the validity of an ELISA test for the serological diagnosis of human fascioliasis in different epidemiological situations. Trop Med Int Health 17:630–636

Valero MA, Bargues MD, Khoubbane M, Artigas P, Quesada C, Berinde L, Ubeira FM, Mezo M, Hernandez JL, Agramunt VH, Mas-Coma S (2016) Higher physiopathogenicity by Fasciola gigantica than by the genetically close F. hepatica: experimental long-term follow-up of biochemical markers. Trans Roy Soc Trop Med Hyg (Special Issue) 110(1):55–66

Valero MA, Perez-Crespo I, Chillon-Marinas C, Khoubbane M, Quesada C, Reguera-Gomez M, Mas-Coma S, Fresno M, Girones N (2017) Fasciola hepatica reinfection potentiates a mixed Th1/Th2/Th17/Treg response and correlates with the clinical phenotypes of anemia. PLoS One 12(3):e0173456. (23 pp)

Valero MA, Girones N, Reguera-Gomez M, Perez-Crespo I, Lopez-Garcia MP, Quesada C, Bargues MD, Fresno M, Mas-Coma S (2020) Impact of fascioliasis reinfection on Fasciola hepatica egg shedding: relationship with the immune-regulatory response. Acta Trop 209:105518

Villegas F, Angles R, Barrientos R, Barrios G, Valero MA, Hamed K, Grueningr H, Ault SK, Montresor A, Engels D, Mas-Coma S, Gabrielli AF (2012) Administration of triclabendazole is

safe and effective in controlling fascioliasis in an endemic community of the Bolivian Altiplano. PLoS Negl Trop Dis 6(8):e1720. (7 pp.)

Winkelhagen AJS, Mank T, de Vries PJ, Soetekouw R (2012) Apparent triclabendazole-resistant human *Fasciola hepatica* infection, the Netherlands. Emerg Infect Dis 18:1028–1029

World Health Organization (2007) Report of the WHO Informal Meeting on use of triclabendazole in fascioliasis control. World Health Organization, Headquarters Geneva, 17–18 October 2006: WHO/CDS/NTD/PCT/2007.1

World Health Organization (2008) Fact sheet on fascioliasis. In: Action against Worms, World Health Organization, Headquarters Geneva (December 2007), Newsletter, 10: 1–8

World Health Organization (2013) Sustaining the drive to overcome the global impact of neglected tropical diseases. World Health Organization, WHO Headquarters, Geneva, p 138

Zumaquero-Ríos JL, Sarracent-Pérez J, Rojas-García R, Rojas-Rivero L, Martínez-Tovilla Y, Valero MA, Mas-Coma S (2013) Fascioliasis and intestinal parasitoses affecting schoolchildren in Atlixco, Puebla State, Mexico: epidemiology and treatment with nitazoxanide. PLoS Negl Trop Dis 7(11):e2553

Chapter 7
Clonorchiasis and Opisthorchiasis

Edoardo Pozio and Maria Angeles Gomez Morales

Abstract Clonorchiasis and opisthorchiasis are helminthic diseases caused by the liver flukes *Clonorchis sinensis*, *Opisthorchis felineus* and *Opisthorchis viverrini*, respectively. Humans acquire these trematode infections by consuming raw or partially cooked freshwater fish infected with the larval stage metacercariae. More than 45 million people most of whom in Asia, have been estimated to be infected. These infections are prevalent in developing countries and are closely linked to poverty, pollution, and population growth, as well as to cultural food habits and tradition. However, people living in industrialised countries are not exempted to acquire these pathogens due to an increasing consumption of freshwater raw fish. Besides being the etiological agents of helminthic diseases, *C. sinensis* and *O. viverrini* have been classified as class I carcinogens, since they are the causative agents of cholangiocarcinoma in chronically infected people.

7.1 Introduction

Clonorchiasis and opisthorchiasis are helminthic diseases caused by the liver flukes *Clonorchis sinensis*, *Opisthorchis felineus*, and *Opisthorchis viverrini*, respectively (Keiser and Utzinger 2009). Humans acquire these trematode infections by consuming raw or partially cooked freshwater fish infected with the larval stage metacercariae. More than 45 million people have been estimated to be infected (Keiser and Utzinger 2009). These infections are prevalent in developing countries and are closely linked to poverty, pollution, and population growth, as well as to cultural food habits and tradition (Lim 2011). However, people living in industrialized countries are not exempted to acquire these pathogens due to an increasing consumption of raw fish (Pozio et al. 2013). Besides being the etiological agents of helminthic diseases, *C. sinensis* and *O. viverrini* have been classified as class I

E. Pozio (✉) · M. A. G. Morales
Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy
e-mail: edoardo.pozio@iss.it

F. Bruschi (ed.), *Helminth Infections and their Impact on Global Public Health*,
https://doi.org/10.1007/978-3-031-00303-5_7

carcinogens, since they are the causative agents of cholangiocarcinoma in chronically infected people (Bouvard et al. 2009).

7.2 History

An early documentation of clonorchiasis in the human beings dates back some 2000 years to the Ming and Western Han dynasties. In 1956, *C. sinensis* eggs were detected in desiccated fecal remains from a mummy of the Ming dynasty (about fifteenth century) in the Guangdong province of China. Again in 1975, *C. sinensis* eggs were detected in fecal remains from a corpse buried during the West Han dynasty (206 BC–23 AD) in the Hubei province (Lun et al. 2005). *Clonorchis sinensis* was first discovered in the bile ducts of a Chinese man in India in 1875, and the first autochthonous case was documented in China in 1908 (Lun et al. 2005). The first and second intermediate hosts of *C. sinensis* were discovered by two Japanese researchers, Masatomo Muto and Harujiro Kobayashi, in 1918 and in 1912, respectively (Yoshida 2012).

Opisthorchis felineus was described for the first time in cats and dogs in Pisa (Italy) and was referred to as *Distoma felineum* (Rivolta 1884). In 1891, this species was included in the new genus *Opisthorchis* (Blanchard 1895). The first human infections were described as caused by *Distomum sibericum* in the liver of eight persons from Siberia (Winogradoff 1892). The complete life cycle of *O. felineus* was described in Germany in 1934 (Vogel 1934; Schuster 2010).

The third species, *O. viverrini*, collected from the liver of a fishing cat (*Felis viverrus*, now *Prionailurus viverrinus*), was described at the end of the nineteenth century as *Distoma viverrini* (Poirier 1886), but the life cycle was fully described only in 1965 (Anonymous 2012). The first human case of *O. viverrini* infection was described in 1915 (Leiper 1915).

7.3 The Life Cycle

The natural life cycle is similar among the three liver fluke species. The adult hermaphrodite worms (*C. sinensis*, 10–25 × 3–5 mm; *O. felineus*, 7–12 × 1.5–2.5 mm; *O. viverrini*, 5.5–10 × 0.8–1.6 mm) are dorsoventrally flattened with an anterior oral sucker, a centrally located ventral sucker, and a uterine pore. These worms parasitize mainly the intrahepatic bile ducts and gallbladder, and less frequently the extrahepatic and pancreatic ducts of humans and other fish-eating mammals (Fig. 7.1). About 1 to 2 months after infection, operculate eggs (22–35 × 10–22 μm) containing the larval stage, or miracidium, are shed with feces (Fig. 7.1). An adult worm can produce from 1000 to 4000 eggs per day for at least 6 months, depending on the mammalian host species and worm burden. When the eggs reach a body of freshwater and are ingested by snails of the genus

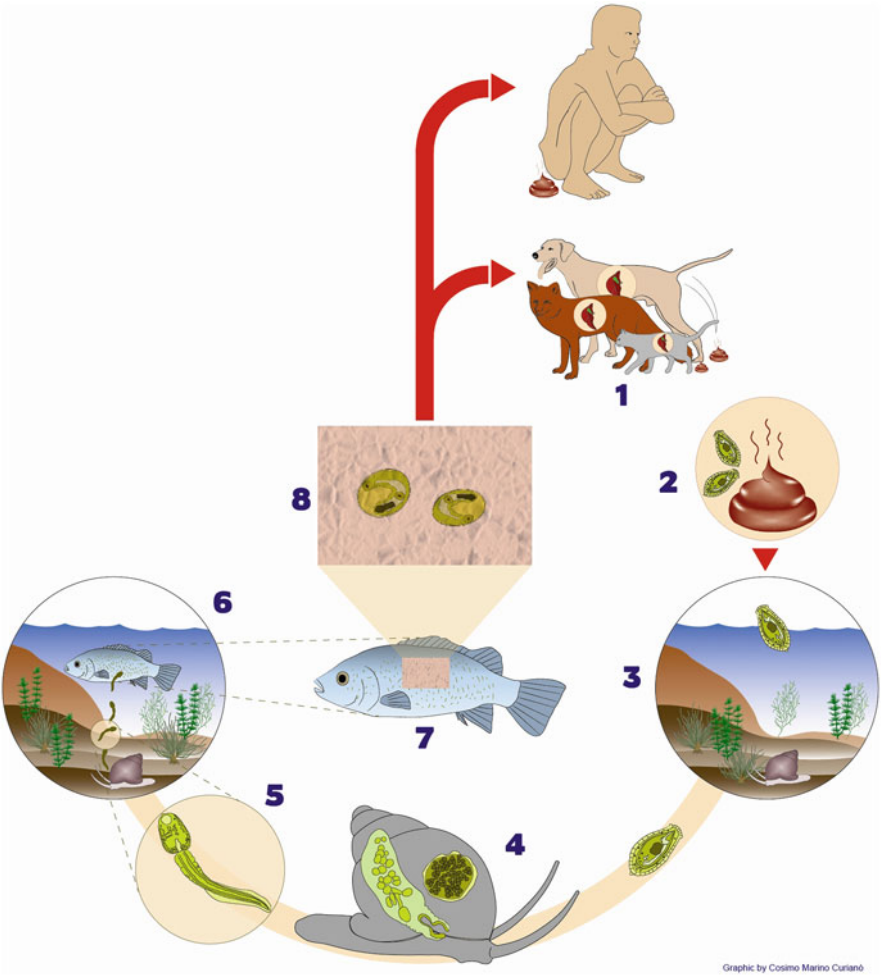

Fig. 7.1 The natural life cycle of *Clonorchis sinensis*, *Opisthorchis felineus*, and *O. viverrini*

Bithynia for *O. felineus* and *O. viverrini* (Pozio et al. 2013; Kiatsopit et al. 2013) or by snails belonging to five families for *C. sinensis* (Lun et al. 2005), they hatch in the gastrointestinal tract of the snail, and the miracidium develops into a sporocyst in the intestinal wall or in other organs to undergo asexual reproduction. The sporocyst produces rediae which mature in the hepatopancreas within about 17 days. The cercariae, about 5–50 per redia, leave the snail during the day, when it is warm and sunny, approximately 1–2 months after the snail is infected; however, the duration of development in the snail body is strongly influenced by the water temperature. The free-swimming cercariae, which are characterized by a positive photo- and geotropism, have a long tail with a long dorsal and some shorter ventral fins, a finely spined tegument, penetration and cystogenous glands, and a pair of eyespots (Fig. 7.1).

They shed their tail, penetrate fish tissue between the scales (mainly near the fins), and encyst as metacercariae under the skin or in the musculature approximately 3 weeks later. In doing so, the cercariae lose their eyespots and develop a sac-like excretory bladder filled with coarse, refractile granules. The metacercarial stage is usually ovoid (140 × 120 μm) with a thin wall (Fig. 7.1). Freshwater fish of the family Cyprinidae act as second intermediate hosts for all the three liver flukes; however, metacercariae of *C. sinensis* have been also detected in other fish families and in crustaceans even if their epidemiological importance in the natural cycle seems to be lower than that of the Cyprinidae fish (Lun et al. 2005; Chen et al. 2010). When infected fish are ingested by mammals, including humans, the metacercariae excyst in the duodenum, and the juvenile flukes migrate (within about 30 min) up through the ampulla of Vater and the common bile duct into the intrahepatic bile ducts where they attach to the bile duct epithelium using their suckers. Then, flukes develop into adults after at least 1 month. Adult flukes have been also detected in the duodenum and stomach. In humans, flukes can survive for 20–25 years (Kaewpitoon et al. 2008).

7.4 Taxonomy and Genetic Variability

According to the current taxonomy, *C. sinensis*, *O. felineus*, and *O. viverrini* belong to the phylum Platyhelminthes, class Trematoda, subclass Digenea, order Plagiorchiida, and family Opisthorchiidae (Mordvinov and Furman 2010). The genetic variability of the three liver flukes has been investigated at the inter- and intraspecies level with differences according to the target sequences (CO1, CO3, mDNA, ITS1, and ITS2) (Kang et al. 2008; Saijuntha et al. 2008; Liu et al. 2012; Brusentsov et al. 2013). Studies of the population genetic structure of these liver flukes have consistently uncovered substructure patterns that have been shown to be related to geographical isolates in defined catchment/wetland systems (Petney et al. 2018). For instance, molecular genetic investigations of *O. viverrini* in Southeast Asia showed that it is a species complex '*O. viverrini* sensu lato' containing two evolutionary lineages with many cryptic species (morphologically similar but genetically distinct species) occurring in and within different catchment (wetland) systems in Thailand and Lao PDR (Saijuntha et al. 2007, 2021; Sithithaworn et al. 2012a, 2012b).

7.5 Geographical Distribution

Large foci of *O. felineus* are present in the European Russia, Kazakhstan, and West Siberia (Mordvinov et al. 2012). In central, southern, and western Europe, *O. felineus* has been detected as isolated foci in Belarus, the Ukraine, and 13 countries of the European Union (EU) (Pozio et al. 2013) (Fig. 7.2, panel A).

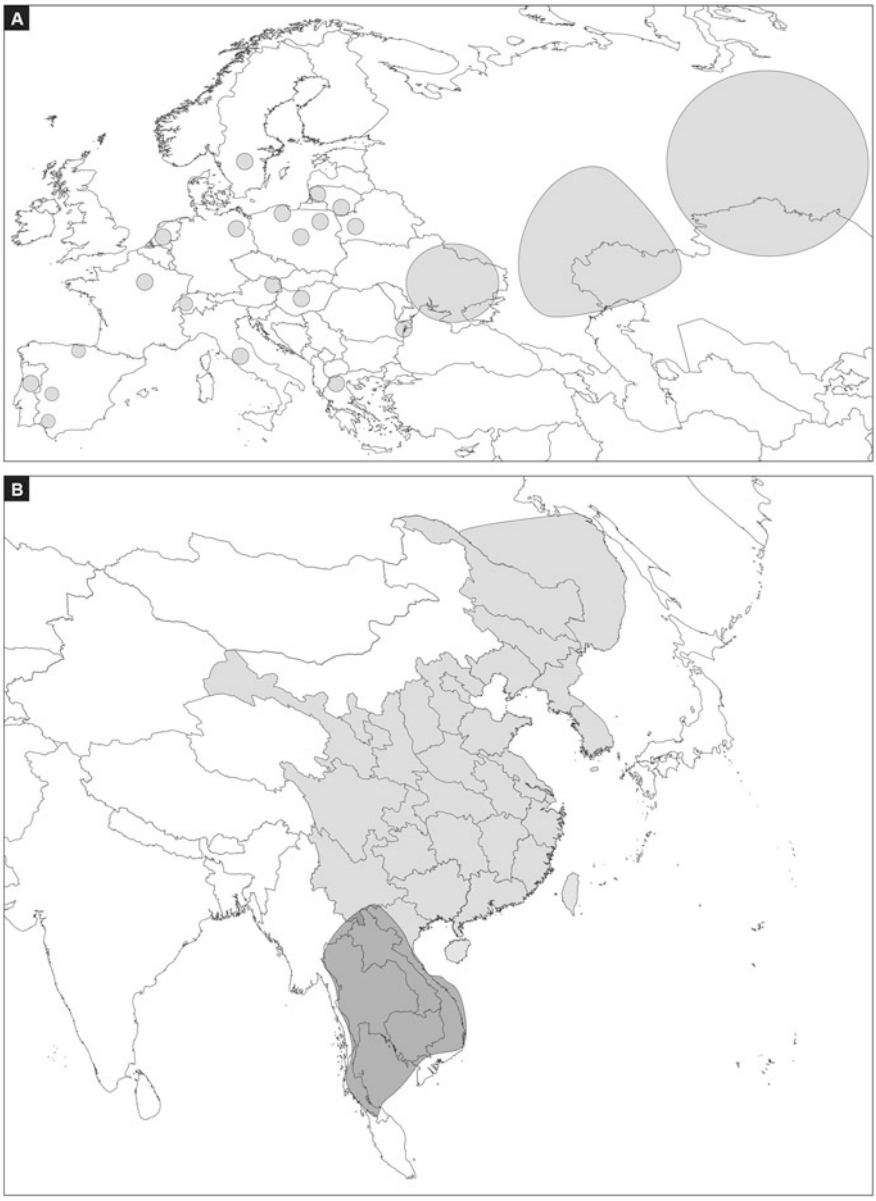

Fig. 7.2 Distribution areas of *Clonorchis sinensis*, *Opisthorchis felineus*, and *Opisthorchis viverrini*. Panel A, *O. felineus* distribution area. Panel B, *C. sinensis* distribution area (light gray); *O. viverrini* distribution area (dark gray)

C. sinensis is circulating in almost all of the Chinese provinces (excluding eight northern and western provinces or autonomous regions), North and South Korea, Taiwan, Northern Vietnam (Lun et al. 2005), the Amur basin in East Russia (Hong and Fang 2012), and central Thailand (Traub et al. 2009) (Fig. 7.2, panel B).

O. viverrini is endemic in Cambodia, Lao PDR (mainly southern areas, as revealed by hospital-based (Ayé Soukhathammavong et al. 2017) and community-based (Kim et al. 2018) studies), Thailand (mainly northeast areas), and Southern Vietnam (Andrews et al. 2008; Sohn et al. 2011, 2012; Yong et al. 2012) (Fig. 7.2, panel B).

7.6 Intermediate Hosts

The prevalence of infection of gastropoda molluscs with the larval stages of liver flukes (sporocyst and rediae) is, in general, low (<0.1%), even in highly endemic areas; however, a prevalence of up to 27% has been documented in some Chinese foci of *C. sinensis* (Lun et al. 2005). There is an increasing number of reports suggesting that the genetic diversity of the gastropoda molluscs that act as intermediate hosts of liver flukes is higher than expected (Lazuthina et al. 2009; Mordvinov et al. 2012). The snail populations show strong seasonality due to the temperature and rainfall variations (Brockelman et al. 1986).

A broad spectrum of mollusc species belonging to five families (Assimineidae, Bithyniidae, Hydrobiidae, Melaniidae, and Thiaridae) acts as first intermediate host for *C. sinensis* (Lun et al. 2005). Three mollusc species of the genus *Bithynia* (*B. inflata*, *B. leachii*, and *B. troscheli*) play the role of first intermediate hosts for *O. felineus* (Erhardt et al. 1962; Hering-Hagenbeck and Schuster 1996; Lazuthina et al. 2009; Mordvinov et al. 2012). Larval stages of *O. viverrini* have been detected in *Bithynia funiculata* and in two subspecies of *B. siamensis* (*B. siamensis siamensis* and *B. siamensis gomiomphalos*) (Petney et al. 2013; Sithithaworn et al. 2012a, 2012b).

The number of fish species as well as the prevalence and intensity of infection is much higher than that of the first intermediate hosts. Fish species acting as second intermediate host of *C. sinensis*, *O. felineus*, and *O. viverrini* belong prevalently to the family Cyprinidae. According to Lun et al. (2005), some additional 60 fish species not belonging to the family Cyprinidae can host *C. sinensis* metacercariae. A prevalence of *C. sinensis* infection of 80% and 95% has been detected in *Parabramis pekinensis* and in *Abbottina sinensis*, respectively (Lun et al. 2005). In the Mekong area, *O. viverrini* metacercariae have been isolated from 40 fish species of 18 genera of the family Cyprinidae (Sithithaworn et al. 2007). In Europe, *O. felineus* metacercariae have been detected in *Alburnus alburnus*, *Abramis brama*, *A. ballerus*, *Blicca bjoerkna*, *Idus idus*, *Rutilus rutilus*, *Scardinius erythrophthalmus*, and *Tinca tinca* with prevalences up to 95% (De Liberato et al. 2011; Pozio et al. 2013). The most important second intermediate hosts of *O. viverrini* are cyprinoid fish of the genera *Cyclocheilichthys*, *Hampala*, and *Puntius* (Wykoff et al. 1965).

The number of metacercariae in fish varies by season, species, and physical and biological parameters of the water bodies (Sithithaworn et al. 2007). The metacercarial burden of *C. sinensis*, *O. felineus*, and *O. viverrini* peaks in spring and

summer, summer and autumn, and winter, respectively (Sithithaworn et al. 2007; De Liberato et al. 2011). The number of metacercariae per fish ranges from 1 to hundreds, but over 30,000 parasites have been detected per fish with more than 6000 metacercariae/g depending on species, biological, ecological, and epidemiological circumstances (Chen et al. 1994). However, a fish can harbor metacercariae of several zoonotic and non-zoonotic fluke species (both liver and intestinal flukes); therefore, metacercariae should be carefully identified by morphology or molecular methods.

7.7 Final Hosts

All fish-eating mammals, including humans, can act as final hosts of *C. sinensis*, *O. felineus*, and *O. viverrini* (Table 7.1), but their role as reservoir hosts is strongly influenced by biological, ecological, and epidemiological factors, including the human impact on fishing (Ashford 2003). Humans are the main final hosts for *C. sinensis*, *O. viverrini*, and *O. felineus* in Siberian foci (Keiser and Utzinger 2009; Mordvinov and Furman 2010; Mordvinov et al. 2012), whereas domestic and wild carnivore animals are the main final hosts of *O. felineus* in European foci (Pozio et al. 2013). However, in *C. sinensis*, *O. viverrini*, and *O. felineus* foci, where humans play the most important role of reservoir, the role of animals should be considered. In fact, if fecal contamination from humans is stopped by mass treatment and proper sanitation, animals may maintain the natural cycle at a hypo-endemic level.

7.8 Epidemiology

Over 9% of world inhabitants, i.e., 700 million people, were estimated to be at risk of foodborne trematodiases. These infections persist as a significant neglected tropical disease risk within East and Southeast Asia, including Siberia, as well as some European countries (Sithithaworn et al. 2012a, 2012b; Sripa et al. 2010). According to Qian et al. (2016), eight million of *C. sinensis* infections occur in China, Northern Vietnam, South Korea, and Eastern Russia. Ten million human *O. viverrini* infections have been estimated in the Lower Mekong Basin (Thailand, Lao PDR, Cambodia, Myanmar, and Southern Vietnam) (Aung et al. 2017; Sithithaworn et al. 2012a, 2012b; Suwannatrai et al. 2018). The number of *O. felineus* infections in humans has been estimated to be 1.6 million in Western Siberia and other parts of the Russian Federation (Fedorova et al. 2018). In addition, some hundreds of cases have been documented in Europe (Pozio et al. 2013).

The transmission patterns of the three liver flukes are substantially different. In most of *C. sinensis* foci, both humans and domestic and wild fish-eating mammals play the role of final hosts, i.e., a zoonotic and an anthroponotic cycle occur

Table 7.1 Epidemiological pattern of the etiological agents of opisthorchiasis and clonorchiasis

Etiological agent	Distribution	Main host species	References
Clonorchis sinensis	China, Korea, Taiwan, Vietnam, Thailand	I. Intermediate hosts Gasteropods of the families Assimineidae, Bithyniidae, Hydrobiidae, Melaniidae, Thiaridae	Lun et al. (2005), Traub et al. (2009), Petney et al. (2013)
		II. Intermediate hosts 132 fish species	Lun et al. (2005)
		Final hosts Human, dog, cat, pig, rat	Lun et al. (2005), Petney et al. (2013)
Opisthorchis felineus	Belarus, Russia, West Siberia, Kazakhstan, the Ukraine, Austria, Croatia, France, Germany, Greece, Hungary, Italy, Lithuania, the Netherlands, Poland, Portugal, Romania, Spain, Scandinavia, Switzerland	I. Intermediate hosts *Bithynia inflata*, *B. leachii*, *B. troscheli* II. Intermediate hosts *Alburnus alburnus*, *Abramis brama*, *A. ballerus*, *Blicca bjoerkna*, *Idus idus*, *Rutilus rutilus*, *Scardinius erythrophthalmus*, *Tinca tinca* Final hosts Human, dog, cat, fox (3 species), seal (3 species), wolf, mustelids (11 species), raccoon dog, raccoon, wild boar, rodent (4 species)	Erhardt et al. (1962), Hering-Hagenbeck and Schuster (1996), Lazuthina et al. (2009), Mordvinov and Furman (2010), Mordvinov et al. (2012), Petney et al. (2013) Pozio et al. (2013)
Opisthorchis viverrini	Cambodia, Lao PDR, Myanmar, Thailand, Vietnam	I. Intermediate hosts *Bithynia funiculate*, *B. siamensis siamensis*, *B. siamensis gomiomphalos* II. Intermediate hosts *Cyclocheilichthys*, *Hampala*, *Puntius* Final hosts Human, dog, cat	Andrews et al. (2008), Aung et al. (2017), Petney and Sithithaworn (2012), Sayasone et al. (2007), Sithithaworn et al. (2012a, 2012b), Wykoff et al. (1965), Petney et al. (2013)

concurrently. In China, the prevalence of human clonorchiasis reaches 16.4% and 9.8% in the Guangdong and Guangxi provinces, respectively (Hong and Fang 2012). *C. sinensis* has been detected in cats, dogs, and pigs with a prevalence of up to 100%, 100%, and 25%, respectively (Lun et al. 2005). There are also some reports on *C. sinensis* in cattle and rats (up to 14%) (Lun et al. 2005). This liver fluke was present in three localities of southern Taiwan (Rim 2005); however, no recent

information is available on its prevalence in the human population of Taiwan. In Korea, a prevalence rate of 2.9% of *C. sinensis* in the general population was recorded, with an estimated prevalence of 1.3 million people with clonorchiasis (Kim et al. 2009). The Amur River basin in Eastern Russia is also an endemic area for *C. sinensis* where one million people are estimated to be infected (Figurnov et al. 2002). In North Vietnam, one million people were estimated to be infected (Dang et al. 2008). Globally, 1.5–two million people infected with *C. sinensis* are symptomatic, and 10% of them are heavily infected with complications (Hong and Fang 2012).

In *O. viverrini* foci, humans play an important role as final host, and the biomass of parasites present in fish-eating mammals was considered to be low when compared to that in humans living in the same areas. However, a report shows that cats are the most important animal reservoir of human opisthorchiasis in Northeastern Thailand with a prevalence of infection of 35.51% (Aunpromma and Tangkawattana 2012). In this region, the prevalence of human opisthorchiasis (16.6%) is the highest in the world, and it is coincident with the highest incidence of cholangiocarcinoma (CCA) (Sithithaworn et al. 2012a, 2012b; Sripa et al. 2012). Traditional raw fish consumption and food-sharing practice among villagers played an important role in liver fluke infection and transmission dynamics as a risk factor (Phimpraphai et al. 2018). Most victims of CCA are small-scale farmers. Mortality occurs most commonly in males between the ages of 40 and 65 (Khuntikeo et al. 2018).

Opisthorchis felineus shows two well-distinct transmission patterns in the EU, Eastern Europe (Byelorussia, Russia, the Ukraine), and Siberia. In the EU, the cycle is typically zoonotic with domestic (cats and dogs) and wild fish-eating mammals (red foxes) playing the most important role of definitive hosts. Human infections are sporadic (5 cases in Germany and 2 cases in Greece in the last 50 years) with few exceptions related to a change in food behavior as observed in Italy in the last 10 years, where more than 200 cases were documented (Pozio et al. 2013). Some of the people who acquired opisthorchiasis in Italy were tourists who developed the disease when they returned home in Austria and the Netherlands (Pozio et al. 2013). Cases of opisthorchiasis caused by *O. felineus* are regularly recorded in several regions of Central Russia (Volga-Kama basin), the Ukraine, and Kazakhstan. However, the highest *O. felineus* prevalence is observed in Western Siberia (Pakharukova and Mordvinov 2016). In these foci, both humans and domestic animals (cats and dogs) play the role as final hosts (Mordvinov et al. 2012). The official statistics for average opisthorchiasis infection rate in Russia is 20 cases per 100,000 inhabitants; however, this rate increases up to 1000 per 100, 000 inhabitants in some counties of the Ob-Irtysh basin (Ob, Irtysh, Tura, and Tom Rivers). Women and older people had a higher risk of infection. The consumption, stock, smoked, frozen, and raw fish and fishing activities increased the risk of infection. Persons with a higher socioeconomic status had a lower risk of infection (Fedorova et al. 2020). In Siberia, the prevalence of CCA is higher among people with *O. felineus* infection than in the noninfected people although there is a lack of accurate studies on the relationship between infection with this parasite and the development of CCA (Kovshirina et al. 2019). Fish-borne trematodes are an important cause of morbidity in Kazakhstan.

The number of human cases of opisthorchiidosis reached a peak of 2521 recorded cases (17 cases per 100,000 inhabitants) in 2002 with a gradual decline to 1225 cases (7.4 cases per 100,000 inhabitants) in 2011. Most human cases are found in the north and northeast part of Kazakhstan in areas drained by the Irtysh River and its tributaries. A further focus is found in the northwest in the Ural River basin in the European part of Kazakhstan (Sultanov et al. 2014).

7.9 Animal Reservoir

In the habitat, where humans live, the most important reservoirs of *O. viverrini*, *O. felineus*, and *C. sinensis* are domestic and stray cats and dogs because they have ready access to the remains of raw or undercooked fish in household and restaurant waste. Furthermore, a vicious circle is established in that infected cats and dogs shed the parasite eggs in the environment close to rivers and pools, where humans fish. The infection rates in cats were much higher than those in dogs. In addition, reservoir host can get either single or repeated infections without or with minimal clinical symptoms (Tangkawattana and Tangkawattana 2018). Epidemiological investigations suggest that wild animals could sustain the life cycle of the liver flukes in the absence of domestic cycle (Murrell and Pozio 2017). So far at least four families (Felidae, Herpestidae, Mustelidae, and Viverridae) of fish-eating mammals were indicated as possible reservoir hosts of *O. viverrini* in the Mekong region (Petney et al. 2013). These include fishing cat (*Prionailurus viverrinus*), leopard cat (*Prionailurus bengalensis*), crab-eating mongoose (*Herpestes urva*), Eurasian otter (*Lutra lutra*), oriental small-clawed otter (*Aonyx cinerea*), smooth otter (*Lutrogale perspicillata*), civet or large Indian civet (*Viverra zibetha*), and binturong (*Arctictis binturong*).

O. viverrini infects not only fish-eating mammals but also some laboratory animals such as hamsters, gerbils, guinea pigs, rabbits, mice, and rats by orally feeding certain number of metacercariae. However, *O. viverrini* can maturate in hamsters, gerbils, rabbits, and guinea pigs, whereas it cannot reach maturity in mice and rats.

In addition to cats and dogs, *O. felineus* has been detected also in pigs. Although the number of investigations on the role of wild animals as reservoir hosts of *O. felineus* is limited, this fluke has been documented in the wild boar (*Sus scrofa*), red fox (*Vulpes vulpes*), corsac fox (*Vulpes corsac*), arctic fox (*Alopex lagopus*), wolf (*Canis lupus*), ermine (*Mustela erminea*), weasel (*Mustela nivalis*), Siberian weasel (*Mustela sibirica*), steppe polecat (*Mustela eresmani*), American mink (*Mustela vison*), European mink (*Mustela lutriola*), sable (*Martes zibellina*), European polecat (*Mustela putorius*), otter (*Lutra lutra*), wolverine (*Gulo gulo*), Eurasian badger (*Meles meles*), raccoon dog (*Nyctereutes procyonoides*), raccoon (*Procyon lotor*), gray seal (*Halichoerus grypus*), Caspian seal (*Phoca caspia*), bearded seal (*Erignathus barbatus*), chipmunk (*Eutamias sibiricus*), beaver (*Castor fiber*), European water vole (*Arvicola amphibius*), brown rat (*Rattus norvegicus*),

and European rabbit (*Oryctolagus cuniculus*) (Petney et al. 2013; Pozio et al. 2013; Hermosilla et al. 2017).

In addition to cats and dogs, *C. sinensis* has been detected in pigs and cattle, red foxes, muskrats (*Ondatra zibethicus*), and rats of unknown species (Petney et al. 2013).

7.10 Pathology and Pathogenesis

The magnitude of the pathology caused by these parasites depends on their number, duration of the infection, and susceptibility of the host (Sripa 2003). Pathological features of liver fluke infections have been reported both in humans and in experimental animal models.

7.10.1 Clonorchis sinensis

The *C. sinensis* juvenile flukes migrate up to and clog biliary branches and release excretory/secretory products (ESPs), which are taken up by the biliary epithelial cells. The ESPs stimulate proliferation of the biliary epithelium and provoke inflammation along the biliary tree. At late stages of infections, *C. sinensis* adults block the biliary passages and bile flow. The congested bile increases hydrostatic pressure, presses on the ductal wall, and causes dilatation (Na et al. 2020). In light *C. sinensis* human infections, the liver appears normal, whereas in massive infections, a localized dilation of the thickened peripheral bile ducts can be seen on the surface beneath the Glisson's capsule (Rim 2005). The intraductal flukes cause a mechanical injury by their feeding and migrating activities, contributing to the biliary damage (Fig. 7.3, panel A). Both oral and ventral suckers of the fluke hook up the biliary epithelium, resulting in tissue damage, which starts by the formation of an edema followed by tissue desquamation mainly in the areas close to the flukes. At later infection stages, the epithelial hyperplasia advances to glandular proliferation and to adenomatous hyperplasia in the biliary epithelium with a high number of goblet cells (Kim et al. 2008). In chronic infections, epithelial cells proliferate with metaplasias of the biliary epithelial cells into mucin-producing cells. Goblet cells can proliferate to produce many small gland-like structures in the mucosa (adenomatous hyperplasia), leading to an excessively high mucus content in the bile which, in combination with the presence of eggs and worm fragments, causes cholestasis and serves as support for bacterial superinfection and intrahepatic stone formation (cholelithiasis) (Lim 2011). Secondary bacterial infections, mainly of enteric origin, may occur, and *Escherichia coli* is the pathogen most frequently identified. These alterations may progress to a pyogenic cholangitis, liver abscess, and hepatitis (Rim 2005). Persistent infections result in varying degrees of periductal fibrosis. In some chronic cases, hepatobiliary fibrosis progresses to biliary cirrhosis (Na et al. 2020). Cholecystitis

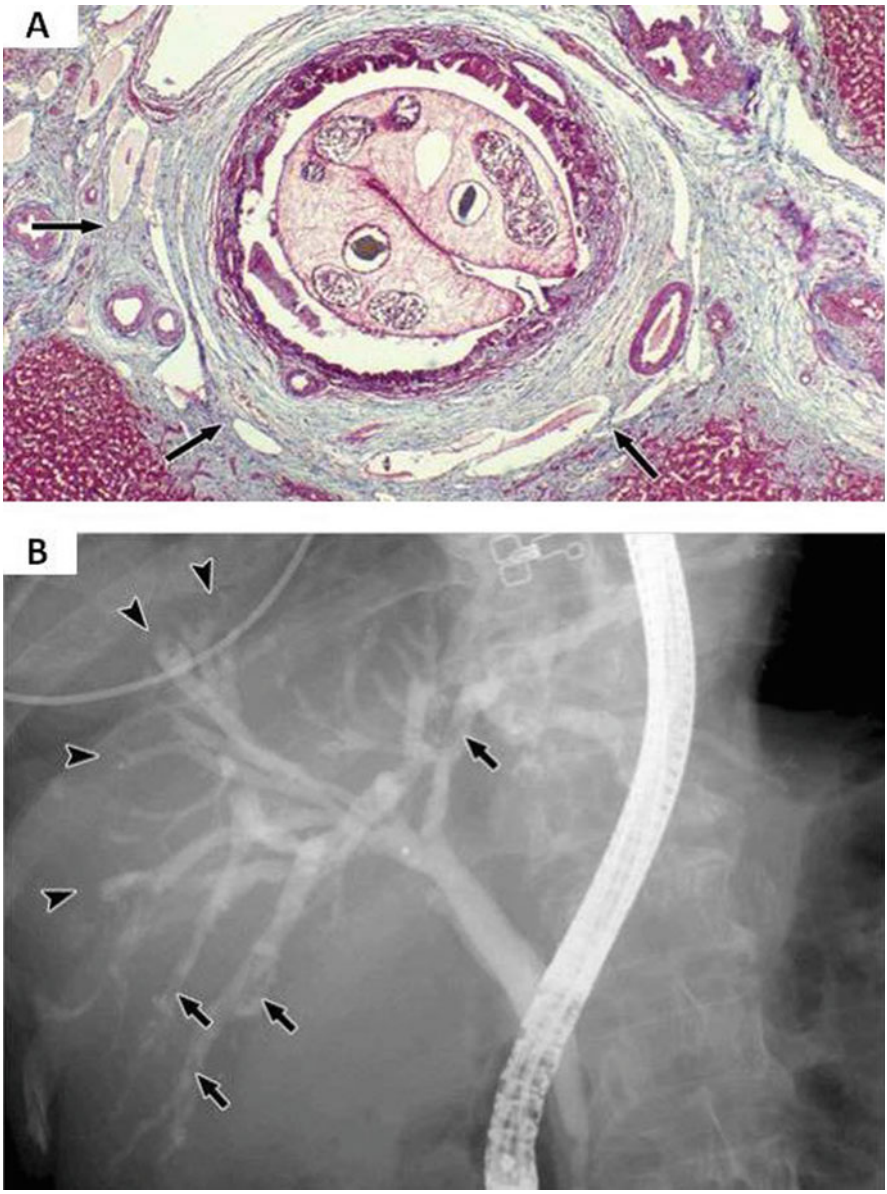

Fig. 7.3 (**a**) Photomicrograph of a pathologic specimen shows an adult fluke of *Clonorchis sinensis* in an intrahepatic bile duct. Note adenomatous hyperplasia of mucosa and severe fibrous thickening of bile duct wall (arrows). Masson's trichrome stain X 40. (**b**) Endoscopic retrograde cholangiogram shows innumerable elongated or elliptic small filling defects, indicating adult *C. sinensis* (arrows) in peripheral small branches of bile ducts. Many peripheral bile ducts are occluded by flukes (arrowheads) (From Lim et al. 2007, con permission)

may appear associated with *C. sinensis* infection and consists of fibrosis, infiltration of mast cells and eosinophils, and mucosal hyperplasia of the gallbladder wall (Sripa et al. 2010). Consequently, a poor function of the gallbladder causes precipitation of bilirubinate, calcium carbonate crystals, and mucin on the eggs, which causes stone formation in the gallbladder (Kim 1999; Qiao et al. 2014). As the fibrosis proceeds, the epithelia proliferation appears milder and fecal egg production drops markedly. The cholelithiasis is one of the most serious complications that can lead to the biliary obstruction (Sithithaworn et al. 2007).

7.10.2 Opisthorchis viverrini

In the early stage of light infections caused by *O. viverrini*, no detectable changes in biliary epithelium and periductal areas of the liver can be found. However, in community-based studies in Northeast Thailand, using ultrasonography and cholecystography, an increase in the frequency and severity of the gallbladder disease has been demonstrated, specifically wall irregularity, enlargement, and bile sludge, among apparently healthy individuals with a moderate *O. viverrini* infection (Elkins et al. 1996; Mairiang et al. 1992). In heavy *O. viverrini* infections, the liver may be enlarged and its weight may be more than double the normal (3000–3500 g or more). In the liver, the predominant changes are desquamation of the biliary epithelium, epithelial hyperplasia, bile duct hyperplasia, and periductal fibrosis (Riganti et al. 1989; Sripa et al. 2012). The adult flukes may be seen in the gallbladder, common bile duct, and pancreatic duct. In the large- and medium-sized bile ducts, the flukes can cause chronic cholecystitis. In case of a superimposed bacterial infection, empyema of the gallbladder may exist. Cholelithiasis is not particularly frequent in *O. viverrini* infections; however, biliary sludge is often seen in heavy infections. The enlargement of the gallbladder is commonly found in autopsy (Riganti et al. 1989) than ultrasonographic studies (Mairiang et al. 1992, 2012). Following anthelmintic treatment, many of the gallbladder abnormalities can be eliminated, as indicated by the reduction of the length and regained contractility (Mairiang et al. 1993). As liver flukes have a long life span for more than 10 years (Attwood and Chou 1978), human infection generally establishes chronic infection (see the review of Sripa 2003). In this stage, periductal fibrosis developed along the infected bile ducts is the most prominent pathological feature, and it is implicated, in its advanced form, i.e., as advanced periductal fibrosis (APF), as one of the risk factors for CCA development (Sripa et al. 2012). Granulomatous inflammation around the parasite eggs is occasionally seen in the gallbladder wall during *O. viverrini* infections (Riganti et al. 1989).

O. viverrini infection modulates the microbiota of the biliary tract. Significant differences in specific enteric microbes (Bifidobacteriaceae, Enterobacteriaceae, and Enterococcaceae) between adjacent normal tissues and tumors from *O. viverrini*-infected people have not been reported. However, there is a documented increased

microbial diversity in *O. viverrini*-associated tissues versus non-*O. viverrini*-associated tissues, whether tumoral or normal (Chng et al. 2016).

7.10.3 Opisthorchis felineus

In *O. felineus* human infections, pathological changes seem to be similar to those induced by the other liver flukes; however, the information is scarce. In patients with heavy or prolonged infections or superinvasion, 84% develop duodenal hypertension, 94% gastric hypertension, and 75% duodenogastric reflux with formation of chronic gastritis (Suvorov et al. 2004). Reflux of gastric contents into the esophagus causes chronic esophagitis. Moreover, regurgitation of intestinal contents into the pancreatic duct is a cause of chronic indurative pancreatitis of the head of the gland. In cases of duodenal hypertension, the rates of pancreatic *O. felineus* invasion are as high as 93.7% (Suvorov et al. 2004). Sonographic studies carried out in *O. felineus*-infected people have evidenced disturbances in the gallbladder ranging from dyskinesia to cholestatic syndrome; the most profound abnormalities in the indices were seen in the early phase of the disease (Bronshteĭn et al. 1989). Unspecific findings by an abdominal ultrasonography scan and multiple hypodense nodules with hyperenhancement in the arterial phase by a computed tomography have been reported in some patients. In a patient who underwent liver biopsy, acute inflammatory signs with dilatation of portal spaces and eosinophilic infiltration with lymphocytes and monocytes were found (Traverso et al. 2012). *O. felineus* infection may cause complications, such as cholangitis, cholecystitis, cholangiofibrosis, hepatic cysts, hepatic abscesses, and pancreatitis. In studies conducted in a hepatology center from Western Siberia (Russia), out of 4756 people chronically infected with *O. felineus*, 1170 (24.6%) required surgical intervention for bile duct strictures (730, 62.4%), pancreatitis (188, 16.0%), hepatic cysts (37, 3.1%), sclerosing cholangitis (27, 2.3%), hepatic abscesses (31, 2.6%), and liver cirrhosis (43 3.6%) (cited by Pakharukova and Mordvinov 2016). Analyses of microbial communities did not reveal differences in bacterial richness between participants infected with *O. felineus* and noninfected individuals (Saltykova et al. 2016).

7.10.4 Associated Pathologies

Renal alterations have been described in human infected with *O. viverrini* (Boonpucknavig and Soontornniyomkij 2003) and *O. felineus* (Lapteva 1990). From a series of 113 cases of nephropathy that coincided with chronic opisthorchiasis caused by *O. felineus*, Lapteva (1990) detected signs of renal lesions: nephritis, pyelonephritis, dyskinesia of the urinary system, a tendency to right-sided lesion, involvement of interstitium, and chronic renal failure. An acute renal failure in obstructive jaundice due to CCA, which is associated with opisthorchiasis caused

by *O. viverrini* in Thailand, was observed in nearly all patients (Mairiang et al. 1992). Conversely, *O. felineus* chronic infection was found to be associated with lower serum total cholesterol levels and a significant attenuation of atherosclerosis (Magen et al. 2013).

Developmental retardation has been reported in *C. sinensis*-infected children with heavy infection. These children often present with inappetence, diarrhea, malnutrition, anemia, and hepatomegaly (Zhu et al. 1983).

The lesions induced by *C. sinensis* and *O. viverrini* enhance the susceptibility of DNA to carcinogens. The association between CCA and liver flukes has been observed since approximately 60 years (Viranuvatti et al. 1955). The International Agency for Research on Cancer (IARC) has considered *O. viverrini* and *C. sinensis* as Group 1 carcinogen agents and *O. felineus* as Group 3 (IARC 1994; Bouvard et al. 2009). Chronic *O. viverrini* infection and CCA have been considered to be the strongest association between a parasitic infection and cancer based on the epidemiological data collected from South Asia (Aunpromma and Tangkawattana 2012; Sripa et al. 2012).

7.10.5 Carcinogenesis Induced by Liver Flukes

Infection in hamsters with *O. viverrini* closely mimics the carcinogenic processes in humans. This process starts with Phase 1 which is characterized by edema and desquamation of the bile duct epithelium, followed by epithelial hyperplasia, pseudostratification of the biliary epithelium, and mucin-secreting cell metaplasia. During Phase 2, metaplastic squamous cells appear in conjunction with glandular proliferation and periductal infiltrates composed of plasma cells, lymphocytes, and other mononuclear cell types, producing high levels of pro-inflammatory cytokines. In Phase 3, the final phase (>12 weeks), the now chronically inflamed biliary tree shows APF along its length. APF is considered the precursor event to CCA, and, similar to human opisthorchiasis, progression of infection to CCA is accelerated by the inclusion of dietary nitrosamines (Sripa et al. 2007, 2012), coinfections with other carcinogenic microbes, local and systemic chronic inflammation, and secretion of mitogens and other mediators by the parasite (Brindley et al. 2015). Nonfibrotic presentations, such as gallbladder morphology and function, recover after removal of parasites by drug treatment; however, APF appears to be permanent (Mairiang et al. 1993).

Epidemiological studies revealed that current or past *C. sinensis* infections are the major risk factor of intrahepatic CCA (Honjo et al. 2005; Choi et al. 2006). Moreover, the highest risk factor is the elevated serological positivity associated to the host genetic polymorphism of glutathione S-transferase mu 1 (GSTM1) gene. Additional risk factors were the area of residence, alcohol consumption, age (older than 60), sex (male), smoking, and consumption of fermented raw fish (Fried et al. 2011).

There are two premalignant lesions in cholangiocarcinogenesis: biliary intraepithelial neoplasia (BilIN) and intraductal papillary neoplasm of the bile duct (Zen et al. 2006). Even if an understanding of the mechanisms leading from liver fluke infection to CCA is not complete, the general mechanisms proposed to contribute to CCA through chronic infection are mainly mechanical damages (see Pathology and Pathogenesis section) to the biliary epithelia, toxic effects of parasite excretory/secretory (ES) products, and the immunopathology due to infection-related inflammation (Sripa et al. 2012).

7.10.6 Toxic Effects of Parasite Excretory/Secretory (ES) Products

The fluke secretes or excretes several metabolic products from the tegument and excretory opening into the bile (or in a culture medium), some of which are highly immunogenic (Sripa and Kaewkes 2000; Wongratanacheewin et al. 2003). Apart from inducing host immune response, the metabolic ES products may be toxic to or may interact with the biliary epithelium. Experimental studies clearly indicate that ES products of *O. viverrini* are internalized preferentially by biliary cell lines and most efficiently by the H69 cholangiocytes but not by Caco-2 cells from the colon epithelia (Chaiyadet et al. 2015). In addition, they can induce cell proliferation of biliary epithelial cells but not intestinal cell lines, which corresponds to the hyperplasia of biliary epithelial cells present in opisthorchiasis (Sripa and Kaewkes 2000). Chaiyadet et al. (2015) described for the first time the release of extracellular vesicles (EVs) with the *O. viverrini* ES products as for communication with neighboring host cells, highlighting the role of fluke EVs in promoting an inflammation yet simultaneously acting as modulator. EVs are highly enriched in tetraspanins (TSPs), proteins that interact with transmembrane and cytosolic signaling proteins (Hemler 2005). Antibodies to a TSP located on the surface of *O. viverrini* EVs blocked the ability of fluke EVs to be internalized by cholangiocytes and suppress their proliferation and secretion of IL-6 (Chaiyadet et al. 2015). To understand the cellular response to *O. viverrini* ES products, gene expression analysis of NIH-3 T3 noncontact co-cultured fibroblasts with *O. viverrini* adults was compared with that without *O. viverrini* ES product treatment. Among all genes, 885 genes showed upregulation of twofold or more after stimulation by *O. viverrini* ES products. Among these genes, 239 had cell proliferation-related functions. The TGF-β and EGF signal transduction pathways have been indicated as the possible pathways of *O. viverrini*-driven cell proliferation (Thuwajit et al. 2006). The expression of a protein kinase implicated in coordination of membrane cytoskeleton events, which can control the reattachment, migration, and invasion of the CCA cells, has been demonstrated (Techasen et al. 2012).

O. viverrini ES products comprise a complex mixture of proteins, some of which have homologs in the human host that are associated with cancer, including

proteases, protease inhibitors, orthologs of mammalian growth factors, and anti-apoptotic proteins. A protein (Ov-GRN-1) with a sequence similar to that of the mammalian growth factor, granulin, has been identified in ES products from *O. viverrini* (Smout et al. 2009, 2011) and can bind to biliary epithelial cells of *O. viverrini*-infected hamsters (Sripa et al. 2012). The human pro-GRN (PGRN) is overexpressed in many human tumors and stimulates angiogenesis, suppresses apoptosis, and promotes tumor invasion and anchorage independence, all of which support tumor expansion in an unfavorable interstitial environment (Monami et al. 2006; Frampton et al. 2012). ES products from *C. sinensis*, as well as from *O. viverrini*, induce the proliferation of human embryonic kidney cells via regulation of the transcription factor E2F1 (Kim et al. 2008). Recent studies show that *C. sinensis* ES enhance the activation of hepatic stellate cells (HSCs) by a cross-talk of TLR4 and TGF-β/Smads signaling pathway, which promotes secretion of TGF-β1 from activated HSCs and other cells. TGF-β1 is an essential mediator of fibrogenesis (Li et al. 2020).

7.10.7 Immunopathology due to Infection-Related Inflammation

During liver fluke infections, macrophages, mast cells, eosinophils, epithelial cells, and neutrophils infiltrate the sites of inflammation activated by the parasite. Recently, Salao et al. (2020) reported that circulating neutrophils had enhanced functional responses following *O. viverrini* infection and even higher responses in infected individuals with liver disease. Moreover, these cells if sensitized to *O. viverrini* ES in vivo enhanced their functional capacity and potential to cause tissue damage and generate pro-inflammatory molecules (Salao et al. 2020). These new data are in line with the increased macrophage function in *O. viverrini*-infected individuals with APF (Salao et al. 2019), implicating a broad activation of innate immune systems in this disease.

On the other hand, specific T cells synthesize nitric oxide (NO) (Haswell-Elkins et al. 1994); furthermore, NO reacts with superoxide anion (O_2^-) to form peroxynitrite, a highly reactive species that causes nitrative and oxidative DNA damage to the cells. In fact, an increased level of urinary nitrates and salivary nitrites in *O. viverrini*-infected subjects in Northeast Thailand has been reported; the concentration of these substances decreased following treatment with praziquantel (Haswell-Elkins et al. 1994). Moreover, a tenfold greater potential for endogenous nitrosation among people living in endemic areas with positive antibody titers for *O. viverrini* as compared to uninfected controls has been demonstrated (Pinlaor et al. 2005, 2006). Nitrosamines play a very important role in inflammation-associated carcinogenesis, especially if they are generated in situ and their production is both chronic and located in close proximity to cells containing P450 enzymes which can metabolize the nitrosamine to DNA methylating agents. The nearby biliary

epithelium may be highly susceptible to malignant transformation due to chronic proliferation, which is another pathologic response to infection. This combination of events could explain the very high risk of CCA associated with liver fluke infection (Satarung et al. 1998).

Increased levels of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-alpha and oxidative stress response enzymes such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), encoded by genes activated by the transcription factor NF-kappaB (NF-κB), are also involved in the inflammatory processes (Hussain et al. 2003; Karin and Greten 2005).

Mediators produced from the inflammatory cells, such as reactive oxygen species, are also toxic to the fluke. Despite this potent oxidative assault, *O. viverrini* can survive in infected hosts for many years. The thioredoxin peroxidase (Ov-TPx-1), an antioxidant enzyme, has been identified and characterized from *O. viverrini* and suggested to be the main enzyme that protects the parasite from reactive oxygen species produced by host effectors' cells (Suttiprapa et al. 2008, 2012). Additional factors including carriage of *Helicobacter* and other microbiome changes within the biliary tract might participate in the inflammatory process (Brindley et al. 2015; Deenonpoe et al. 2017).

Even if no association has been reported for *O. felineus* and CCA, epidemiological and clinical data in humans and animals suggest that *O. felineus* can be the cause of neoplasia (Pozio et al. 2013). In Russia, the highest incidence of bile duct cancer in humans was documented in the same area (i.e., Tyumen oblast) with the highest prevalence of *O. felineus* infection in humans (Mordvinov et al. 2012). In Italy, pre- or cancerous lesions have been found during autopsy in *O. felineus* naturally infected animals, in particular cats and dogs (Pozio et al. 2013).

7.11 Immune Response

Neither mucosal nor tissue immune responses appear to cause parasite death or protect against newly established flukes, as evidenced by the persistence of infection for decades in the body and rapid reinfection following treatment. Experimental studies in *O. viverrini* suggest that specific immune suppressive mechanisms may promote parasite persistence, therefore allowing continued secretion of parasite products that damage the biliary epithelium, both directly through mechanical damage and mitogenicity and through innate and adaptive immune responses (Sripa et al. 2018).

7.11.1 O. viverrini

O. viverrini induces a strong antibody response to somatic and ES antigens in the human host, mainly constituted by specific IgG, IgA, and IgE, with IgG being the

predominant subclass followed by IgA and IgE. The specific immune response is detectable in serum and bile, and it comes along with a marked increase of total IgE in serum (Wongratanacheewin et al. 1988). There is a significant positive correlation of the IgG antibody titer and the severity of disease (Haswell-Elkins et al. 1991; Pinlaor et al. 2012). In infected individuals, the level of serum antibodies decreased slowly but remained elevated for several months after the praziquantel treatment. This can be due to a long-lasting immunological memory or to new antigenic stimulations by reinfections with the same or other parasites (cross-reacting) (Ruangkunaporn et al. 1994).

The fact that many individuals from endemic areas can harbor many parasites (Haswell-Elkins et al. 1994) suggests than reinfection occurs, in spite of the strong cellular and humoral immune response elicited by the parasites during the primary infection, as it has been demonstrated in hamsters (Wongratanacheewin et al. 1991). The role of T cells and cytokines in immunity and pathogenesis of opisthorchiasis is not well known. The involvement of T cells in pathogenesis is supported by the fact that T-cell-deprived *O. viverrini*-infected hamsters show less severe damage of the bile duct tract (Flavell and Flavell 1986). In experimentally infected hamsters, the parasite stimulates the expression of the Th1-inducing cytokine, IL-12, in the early stage of infection (2 weeks postinfection), whereas the expression of the Th2-inducing cytokine, IL-4, and the regulatory cytokines, TGF-β and IL-10, is significantly increased in chronic and/or heavy infections (Jittimanee et al. 2007). It has been hypothesized that the high level of expression of these regulatory cytokines following *O. viverrini* infection may play an important role in the disease process by inhibiting T-cell proliferation that in turn leads to prolonged worm survival (Maizels and Yazdanbakhsh 2003).

The antigenic recognition molecules of T and B cells have been found to be different (Wongratanacheewin et al. 2003). Reinfection with metacercariae elicits high levels of IgG.

Resistance to *O. viverrini* challenge in hamsters can be induced only in animals harboring a low number of worms (Flavell 1982). The lack of a protective immune response can be related to the suppression caused by the parasite, which can be abolished by anthelmintic treatment (Wongratanacheewin et al. 1987).

7.11.2 C. sinensis

Humans are susceptible to infection, reinfection, and superinfection by *C. sinensis* (Hong and Fang 2012). However, rats, mice, and rabbits resist against reinfection and superinfection of *C. sinensis* (Sohn et al. 2006). Only a few worms survive as small and immature forms in the bile duct of re-infected or super-infected rats; however, the rat resistance was not observed in immune-suppressed or nude rats (Zhang et al. 2008a, 2008b). Specific antibodies to *C. sinensis* are produced in serum and bile, mainly IgE in serum and IgA in bile, and their levels correlated with resistance in rats (Zhang et al. 2008a, 2008b). The immune response to *C. sinensis* is

of the Th2 type as confirmed by the significant production of IgG1, IL-4, and IgE in rats (Wang et al. 2009). It has been demonstrated that *C. sinensis*-derived total proteins can suppress the development of allergen-specific asthma by induction of an increase in the number of CD4 + CD25 + Foxp3+ Treg cells, which maintain immune response homeostasis, and interfere with the priming of naïve T cells by airway dendritic cells (Jeong et al. 2011).

Zhang and colleagues reported an association between increased hepatic Th2 and Treg cell subsets and biliary fibrosis in *C. sinensis*-infected mice (Zhang et al. 2017).

7.11.3 O. felineus

As other liver flukes, *O. felineus* elicits a humoral immune response already detectable from the third week after infection (Armignacco et al. 2008, 2013; Traverso et al. 2012). The main antigens recognized by human antibodies have been associated to the tegument, muscles, uterus, gonads, intestine, and eggs of the liver fluke, as showed by immune electron microscopy. These findings have led to the conclusion that the surface structures of liver flukes stimulate a low B-cell immune response, whereas the structures linked to the ES system of the parasite and their products contain main antigens able to induce B-cell immune response in man (Kotelkin et al. 2001).

7.12 Clinical Manifestations

The types of clinical diseases caused by liver fluke infections seem to vary. Most of the reported *O. viverrini* and *C. sinensis* infections are dormant, and the infected people are asymptomatic, except for the patients with very heavy infections and for those presenting complications. On the contrary, there are many reports detailing specific signs and symptoms accompanying well-defined clinical stages of the *O. felineus* infection, in which acute infections are frequently reported (Bronshteĭn et al. 1989; Lim 2011).

7.12.1 Opisthorchis viverrini

In chronic infections, an increased frequency of hepatomegaly, as revealed by community studies based on physical examination, can be found (Mairiang et al. 2012). Hematological and liver function tests are generally unremarkable, regardless of infection intensity; instead, ultrasound examinations have shown high frequencies of left lobe liver and gallbladder enlargement, sludge and stones in the gallbladder, and poor hepatobiliary function. In patients with a severe infection, the clinical signs

and symptoms include lassitude, hepatomegaly, and nonspecific abdominal complaints such as anorexia, nausea, vomiting, abdominal discomfort, diarrhea, indigestion, weight loss, ascites, and edema (Furst et al. 2012). Jaundice is due to the mechanical obstruction caused by large number of flukes in the bile ducts in patients with a heavy infection, or it is due to bile duct obstruction caused by stone, cholangitis, or CCA as a late complication of chronic infection (Lim 2011).

There is no report of acute *O. viverrini* infections. Most subjects with opisthorchiasis have nonspecific symptoms or no symptoms at all. Mild hepatomegaly occurs in 14% of the heavily infected persons (egg counts >10,000/g). Enlargement of the gallbladder is only detected by ultrasonography and is reversed after elimination of flukes by praziquantel (Mairiang et al. 1993). Intrahepatic duct stones and recurrent suppurative cholangitis are not a common manifestation of opisthorchiasis caused by *O. viverrini* but can be present. Since jaundice is the main clinical manifestation of the CCA, whenever jaundice and ascending cholangitis are detected in endemic areas, the fluke-related CCA is suggested (Uttaravichien et al. 1999). Obstructive jaundice can be presented alone, with fever, or with acute abdominal complications, such as cholangitis, acalculous cholecystitis, and generalized bile peritonitis (Uttaravichien et al. 1999). Non-jaundiced patients may present dyspeptic pain, anorexia, weight loss, and right upper abdominal mass (Chunlertrith et al. 1992).

7.12.2 Clonorchis sinensis

In chronic infections, an increased frequency of hepatomegaly, as revealed by community studies based on physical examination, can be found (Choi et al. 2005). The clinical manifestations of clonorchiasis tend to reflect the worm burden. Most patients with mild infections, i.e., with fewer than 100 flukes, have few symptoms. Early symptoms may include general malaise, abdominal discomfort, and diarrhea. In 10–40% of patients, peripheral eosinophilia accompanies a fluctuating jaundice that is usually obstructive. Moderate infection (generally fewer than 1000 flukes) presents with fever and chills, as well as fatigue, anorexia, diarrhea, weight loss, discomfort, and abdominal distension. Up to 20,000 flukes may be present in patients with severe disease, who present with acute right upper quadrant pain, often superimposed on the signs and symptoms seen in moderate infections. In the late stage of severe cases, jaundice, diarrhea, portal hypertension, hepatosplenomegaly, ascites, and edema can occur. Pyogenic cholangitis, cholelithiasis, chronic cholecystitis, pancreatitis, and CCA have been described as potential long-term complications of clonorchiasis. Many hepatic and biliary diseases can mimic clonorchiasis in their clinical presentation. Differential diagnoses of clonorchiasis include acute or chronic hepatitis, cancer along the bile ducts, hepatocholedocholithiasis with recurrent pyogenic cholangitis, sclerosing cholangitis, Caroli's disease, and *Fasciola hepatica* infection (Choi et al. 2006; Keiser and Utzinger 2009).

7.12.3 Opisthorchis felineus

The clinical manifestations caused by *O. felineus* during the acute stages of the infection in humans are characterized by fever, abdominal pain, headache, asthenia, arthralgia, lymphadenopathy, skin rash, diarrhea, nausea, hepatitis-like symptoms, eosinophilia, and increased liver enzymes (Mairiang and Mairiang 2003; Mordvinov and Furman 2010; Traverso et al. 2012; Pozio et al. 2013). Acute opisthorchiasis occurs early in infection and may be associated with primary exposure to a large dose of metacercariae (Furst et al. 2012). These clinical features may lead to misdiagnosis as acute viral hepatitis (Belova et al. 1981) and rheumatic disease (Gordon et al. 1984). In endemic regions such as Ukraine, Russia, and Siberia, where people frequently consume raw fish, the number of worms in the bile ducts can be very high, inducing chronic infection, which is characterized by anorexia, dyspepsia, dryness and bitter taste in the mouth, fatigue, intolerability to greasy foods, nausea, and pain in the right hypochondrium (Mordvinov and Furman 2010). Other people frequently reported cholecystitis, duodenitis, and pancreatitis. In persons with a high worm burden, chronic infection can become severe, being characterized by acute pancreatitis, bile peritonitis, hepatic abscesses, obstruction of bile ducts with jaundice, and recurrent cholangitis (Mordvinov and Furman 2010). In non-endemic areas, such as EU countries, most infected persons show pauci-symptomatic or asymptomatic forms, and in some cases, clinical disease and the seroconversion can develop up to 2 months after infection. In Italy, about 1/3 of infections was asymptomatic (Pozio et al. 2013). In symptomatic persons, during the acute stage, the more frequently observed signs and symptoms were asthenia, headache, abdominal pain, and fever, which started about 2–3 weeks after the infection (Armignacco et al. 2008; Traverso et al. 2012; Pozio et al. 2013). Jaundice was not observed. The main laboratory findings were leukocytosis; eosinophilia; increased transaminases, e.g., aspartate aminotransferase and alanine aminotransferase; and gamma-glutamyl transpeptidase (Armignacco et al. 2008; Traverso et al. 2012). In an outbreak in Italy, in persons not diagnosed during the acute phase and then not treated, there was a spontaneous remission of the clinical symptoms within 2–3 months of infection, although the liver flukes were still present and produced eggs (Armignacco et al. 2013).

7.13 Diagnosis

In non-endemic areas, liver fluke infections are very difficult to diagnose because of the lack of pathognomonic signs and symptoms and the decreasing number of professionals able to identify opisthorchiid eggs in stool samples (Yossepowitch et al. 2004; Pozio et al. 2013). Moreover, in some cases the clinical disease and the seroconversion can develop up to 2 months after infection (Pozio et al. 2013). In endemic areas, the presence of signs revealing injury of the bile ducts by ultrasound

or other imaging techniques is suggestive of infection (Sithithaworn et al. 2007) (Fig. 7.3, panel B). However, in any case, the clinical diagnosis of liver fluke infections should be confirmed by the detection of eggs in stools (Saijuntha et al. 2018).

7.13.1 Parasitological Diagnosis

Fecal examinations by the Kato-Katz (KK), the formalin-ether concentration technique (FECT), and the Stoll's dilution egg count methods have been frequently used for diagnosis of liver fluke infections. However, FECT seems more sensitive than the other methods, especially for the diagnosis of extremely low burden infections and for the follow-up examination after treatment (Sithithaworn et al. 2007). The KK and FECT methods are commonly used for mass screening in endemic areas for *O. viverrini* and *C. sinensis*, and both are considered to have a comparable sensitivity and reliability (Hong and Fang 2012; Sithithaworn et al. 2007)). The Stoll's dilution egg count method presents a detection rate slightly inferior to KK but is believed to be suitable for the measurement of the intensity of *O. viverrini* infection (Viyanant et al. 1983). The sensitivity of stool examination for *O. viverrini* eggs by formalin-ether concentration and Stoll's dilution methods has been assessed in autopsied subjects grouped according to the number of worm recovered from their liver. At a worm burden >20, the rate of egg detection by both methods was comparable with the worm recovery (sensitivity 100%), but at a worm burden of 10–19 worms, false negative samples are detected (Sithithaworn et al. 1991). The strength of these methods is the possibility of determining the infection intensity, as expressed by the number of parasite eggs per gram of feces, which allows quantifying treatment outcomes both in terms of cure rate and egg reduction rate (Wood et al. 1995). However, the sensitivity of these direct diagnostic tests, in particular for low-intensity infections, is frequently insufficient. Hence, multiple stool sampling or the combination of different diagnostic tests should be considered to enhance diagnostic accuracy (Bergquist et al. 2009; Johansen et al. 2010). Promising results have been obtained with a new multivalent flotation method (FLOTAC), which allows considerably larger amounts of feces to be examined and showed a considerably higher sensitivity than the classical methods (Cringoli et al. 2010). It is necessary to take into account that there are several species of foodborne trematodes which have similar egg morphology (as Opisthorchiidae, Heterophyidae, and Lecithodendriidae families); consequently, recognition of the egg morphology is essential for a correct diagnosis (Chai and Lee 2002).

7.13.2 Detection of Parasite Antigens in Stools

ELISAs for antigen detection in stools from infected persons have been developed using monoclonal antibodies (MAb) to different antigenic proteins of *O. viverrini* (Chaicumpa et al. 1992; Sirisinha et al. 1995). A sandwich ELISA using recombinant *O. viverrini* cathepsin F chicken IgY in combination with rabbit IgG antibody to the somatic *O. viverrini* antigens for coproantigen detection was applied for the diagnosis, exhibiting sensitivity and specificity of 93.3% and 76.7%, respectively (Teimoori et al. 2017). A monoclonal antibody-ELISA was developed for the quantitative diagnosis of opisthorchiasis in urine, showing 81% sensitivity and 70% specificity when compared with the current gold standard diagnostic method (Worasith et al. 2015).

7.13.3 Detection of Parasite DNA in Stools

The detection of parasite DNA by PCR and sequencing or by real-time PCR constitutes an alternative to parasitological diagnosis and is a very sensitive and specific way to identify cryptic infections. Since there are species-specific PCR tests to identify *O. viverrini* (Ando et al. 2001; Wongratanacheewin et al. 2002), *O. viverrini* and *C. sinensis* (Le et al. 2006; Traub et al. 2009; Sato et al. 2009; Kim et al. 2009; Cai et al. 2010, 2012; Huang et al. 2012; Arimatsu et al. 2012), and *O. felineus* (Pauly et al. 2003; Müller et al. 2007) from various parasite stages (eggs, metacercariae, and adult worms), these methods have been largely applied for the diagnosis. The sensitivity can vary and reaches 100% in moderate to severe infections (eggs per gram >1000), whereas in light infections (eggs per gram <200), the sensitivity drops to 68.2% (Wongratanacheewin et al. 2002). The presence of PCR inhibitors in human fecal specimens can strongly reduce PCR sensitivity (Wongratanacheewin et al. 2003; Sithithaworn et al. 2007).

7.13.4 Serological Diagnosis

The detection of anti-liver fluke antibodies is widely used since the sensitivity and specificity of the serological tests have greatly improved. Moreover, studies in humans have shown a close relationship between parasite-specific IgG (in serum and saliva), salivary parasite-specific IgA, and intensity of *O. viverrini* infection (Elkins et al. 1991; Haswell-Elkins et al. 1991; Sawangsoda et al. 2012). Furthermore, in *O. viverrini* infections, the level of parasite-specific IgG is correlated to the severity of the clinical disease rather than to the egg count in stools (Haswell-Elkins et al. 1991; Tesana et al. 2007). Since the time between infection and the detection of antibodies in serum ranges from 3 to 8 weeks for *O. felineus* infections (Armignacco

et al. 2008, 2013; Traverso et al. 2012; Pozio et al. 2013), and it is reported from 2 to 4 weeks for *O. viverrini* infection in hamsters (Sripa and Kaewkes 2000), the detection of specific antibodies, mainly IgG, has been considered as a complementary tool to establish a definitive diagnosis of the infection (Sripa and Kaewkes 2000; Upatham and Viyanant 2003).

The serodiagnosis of liver fluke infections caused by *O. viverrini* and *C. sinensis* has been attempted using crude adult extracts, metabolic products, and egg antigens together with different immunodiagnostic methods, producing results of varying degrees of sensitivity and specificity (Wongratanacheewin et al. 1988; Haswell-Elkins et al. 1991; Sawangsoda et al. 2012; Sakolvaree et al. 1997; Pinlaor et al. 2012). A main problem in the serological diagnosis of parasitic infections, and especially for those caused by helminths, is the cross-reactivity, in particular when parasite crude extracts (CE) are used. In fact, using CE from adults, metacercariae and eggs, and ES products from adults, some authors have reported that the specificity in the detection of circulating antibodies to *O. viverrini* is limited by the cross-reactive nature of the antigens (Wongratanacheewin et al. 1988; Sirisinha et al. 1995; Wongsaroj et al. 2001; Sawangsoda et al. 2012). Serum titers of anti-*O. viverrini* antibodies have been found to be higher in cases of CCA than in patients with cholangitis caused by the liver fluke. In infected patients, the detection of IgG and IgG4 levels in serum yielded good sensitivities (99.2% and 93%, respectively) but poor specificity (23.1% and 29.6%, respectively), whereas the detection of IgG and IgG4 levels in urine had much lower sensitivity (43% and 45.9%, respectively) but better specificity (64.5% and 67.2%, respectively) (Tesana et al. 2007).

ELISA is widely used in Korea for *C. sinensis* infections (Choi et al. 2003; Lee et al. 2010; Kim et al. 2009) and has a 93.1% sensitivity when ESPs are used as antigens and 87.8% when CE is used (Choi et al. 2003). Several recombinant proteins from *C. sinensis* have been produced and identified (Kim et al. 2009; Shen et al. 2009; Ju et al. 2009; Chen et al. 2011; Nagano et al. 2004; Na et al. 2008) and shown to be sensitive and specific for serodiagnosis of clonorchiasis, but not enough to replace CE (Hong and Fang 2012).

The indirect hemagglutination test, intradermal test, and ELISA have been developed using *O. felineus* CE from adult worms as antigens (Wongratanacheewin et al. 2003). According to Meniavtseva et al. (1996), the ELISA shows the best performance among all serological tests. An ELISA based on ES products has been validated for *O. felineus* infection in humans from low endemic areas (Gómez-Morales et al. 2013).

7.14 Human Treatment

Currently, the drug of choice to treat people with clonorchiasis or opisthorchiasis is praziquantel (2-(cyclohexanecarbonyl)-3,6,7,11b-tetrahydro-1H-pyrazino[2,1-a] isoquinolin-4-one). The commercial preparation contained a racemic mixture of equal portion of levo- R(−) and dextro- S(−) isomers, but only the R(−) enantiomer

has antihelminthic activity (Mordvinov and Furman 2010). Since 2004, the commercial preparation contains only the R(−) enantiomer. This drug is safe for pregnant and lactating women (Olds 2003). It is recommended to use praziquantel only for children older than 4 years of age. Praziquantel induces a muscle contraction and a vacuolization of the tegumental syncytium of the flukes. This drug is rapidly absorbed, and peak serum levels occur 1–3 h after administration; then, it is excreted with bile and urine within 24 h. It follows that the praziquantel treatment has little or no effect on subsequent exposure to infection. According to WHO, the recommended dosage of praziquantel is 25 mg/kg of body weight, three times a day on two consecutive days, or 40 mg per kg of body weight in a single administration with a repeat interval of 6 or 12 months (WHO 2011). This treatment gives 100% and 80–85% cure rate for *O. viverrini* and *C. sinensis* infection, respectively (WHO 1995). However, in endemic areas, the praziquantel treatment alone is not sufficient when people get reinfected by continuously consuming raw fish (Hong et al. 2001). Quite frequently (up to 90% of treated people), praziquantel can cause side-effect reactions such as abdominal pain, nausea, headache, dizziness, drowsiness, and urticaria. Drug resistance issues for praziquantel have not been reported in liver fluke; however, there is a report of low cure rate (29%) for the drug for *C. sinensis* in Vietnam (Tinga et al. 1999).

The second drugs of choice are the benzimidazole derivatives mebendazole and albendazole. However, these two drugs are effective only when given over a long period or at high doses (Jaroonvesama et al. 1981; Pungpak et al. 1984; Armignacco et al. 2008). The treatment of *O. felineus* infections with albendazole (10 mg/kg body weight daily in two doses for 7 days) failed to eradicate all the flukes from one patient involved in an outbreak in Italy (Armignacco et al. 2013). Indeed, 2 years later, the patient still shed *O. felineus* eggs in her feces in the absence of reinfection (Armignacco et al. 2013). In this patient, the albendazole treatment probably induced the flukes to stop the egg production, suggesting a false recovery. Tribendimidine is another candidate for liver fluke infection treatment. It is an L-subtype nicotinic acetylcholine receptor antagonist (Hu et al. 2009; Xiao et al. 2013). Clinical studies of tribendimidine conducted in Lao PDR showed efficacy similar to that of praziquantel, since the egg reduction rates of tribendimidine and praziquantel are 99.3% and 98.4%, respectively (Sayasone et al. 2016, 2018; Soukhathammavong et al. 2011). A dosage of 600 mg/kg shows high therapeutic efficacy in adults and adolescents, while the effective dose for children (8 e 14 years) is 100 mg/kg.

7.15 Prevention and Control

Prevention and control measures should be proportionate to the epidemiological situation. The snail control by molluscicides was approached to reduce the first intermediate host populations but failed to reduce the prevalence of the infection in addition to cause considerable ecological damages (Mordvinov and Furman 2010; Tesana and Thapsripair 2012). In high endemic areas, morbidity can be prevented or

controlled by treatment, health education, improved sanitary conditions, and implementation of food safety measures (WHO 1995; Sithithaworn et al. 2007). The ultimate aim is to change human behavior, because the consumption of raw or undercooked freshwater fish is the key risk factor for acquiring clonorchiasis and opisthorchiasis. Vaccines are not available yet for the prevention of these zoonoses. The use of chemotherapy with or without health education failed to eradicate or control these infections in humans. Successful results were obtained only when a mass praziquantel treatment was combined to an extensive improvement of the healthcare system and to socioeconomic development (Jongsuksuntigul and Imsomboon 2003).

To prevent clonorchiasis and opisthorchiasis, freshwater fish should be cooked until the core reaches 65 °C for at least 1 min (EFSA 2010). *O. felineus* metacercariae in fish fillets can be killed by freezing at −28 °C for 20 h, at −35 °C for 8 h, and at −40 °C for 2 h (Fattakhov 1989). According to Lloyd and Soulsby (1998), metacercariae may be killed by freezing at −10 °C for 5–70 days, depending on the size of the fish. Metacercariae of *O. felineus* present in tench muscles were devitalized at −18 °C for 96 h (Pozio et al. 2013); however, only freezers marked with three or four stars reach a temperature of −18 °C. Metacercariae of *O. felineus* can survive in smoked fish causing human infections (Yossepowitch et al. 2004). Marinating does not kill *O. felineus* metacercariae present in tench muscles (Armignacco et al. 2008, 2013; Traverso et al. 2012). Metacercariae of *O. viverrini* are killed in fish at 13.6% NaCl after 24 h (Kruatrachue et al. 1982).

References

Ando K, Sithithaworn P, Nuchjungreed C et al (2001) Nucleotide sequence of mitochondrial CO I and ribosomal ITS II genes of *Opisthorchis viverrini* in northeast Thailand. Southeast Asian J Trop Med Publ Health 32:17–22

Andrews RH, Sithithaworn P, Petney TN (2008) *Opisthorchis viverrini*: an underestimated parasite in world health. Trends Parasitol 24:497–501

Anonymous (2012) Discovery of human opisthorchiasis: a mysterious history. Parasitol Int 61:3–4

Arimatsu Y, Kaewkes S, Laha T et al (2012) Rapid detection of *Opisthorchis viverrini* copro-DNA using loop-mediated isothermal amplification (LAMP). Parasitol Int 61:178–182

Armignacco O, Caterini L, Marucci G et al (2008) Human illnesses caused by *Opisthorchis felineus* flukes, Italy. Emerg Infect Dis 14:1902–1905

Armignacco O, Ferri F, Gomez Morales MA et al (2013) Cryptic and asymptomatic *Opisthorchis felineus* infections. Am J Trop Med Hyg 88:364–366

Ashford RW (2003) When is a reservoir not a reservoir? Emerg Infect Dis 9:1495–1496

Attwood HD, Chou ST (1978) The longevity of *Clonorchis sinensis*. Pathology 10:153–156

Aung WPP, Htoon TT, Tin HH et al (2017) First report and molecular identification of *Opisthorchis viverrini* infection in human communities from Lower Myanmar. PLoS One 12:e0177130

Aunpromma S, Tangkawattana P (2012) High prevalence of *Opisthorchis viverrini* infection in reservoir hosts in four districts of Khon Kaen Province, an opisthorchiasis endemic area of Thailand. Parasitol Int 61:60–64

Ayé Soukhathammavong P, Vonghachack Y, Hatz C et al (2017) Suspected cases of cholangiocarcinoma seen in reference hospitals in Lao People's Democratic Republic. Parasitol Int 66:510–514

Belova VG, Paturina NG, Gurevich EN (1981) Early differential diagnosis of icteric variants of viral hepatitis, acute opisthorchiasis and sporadic pseudotuberculosis. Klin Med (Mosk) 59:73–76

Bergquist R, Johansen MV, Utzinger J (2009) Diagnostic dilemmas in helminthology: what tools to use and when? Trends Parasitol 25:151–156

Blanchard R (1895) Animaux parasites (Notice preliminaire). Bull Soc Zool France 20:217–225

Boonpucknavig V, Soontornniyomkij V (2003) Pathology of renal diseases in the tropics. Semin Nephol 23:88–106

Bouvard V, Baan R, Straif K et al (2009) A review of human carcinogens-Part B: biological agents. Lancet Oncol 10:321–322

Brindley PJ, Correia da Costa JM, Sripa B (2015) Why does infection with some helminths cause cancer? Trends in Cancer 1:174–182

Brockelman WY, Upatham ES, Viyanant V et al (1986) Field studies on the transmission of the human liver fluke, *Opisthorchis viverrini*, in northeast Thailand: population changes of the snail intermediate host. Int J Parasitol 16:545–552

Bronshteĭn AM, Mironov SP, Silaev AV et al (1989) Radionuclide and sonographic diagnosis of lesions of the hepatobiliary system in opisthorchiasis. Med Parazitol 5:13–17

Brusentsov II, Katokhin AV, Brusentsova IV et al (2013) Low genetic diversity in wide-spread Eurasian liver fluke *Opisthorchis felineus* suggests special demographic history of this trematode species. PLoS One 8:e62453

Cai XQ, Xu MJ, Wang YH et al (2010) Sensitive and rapid detection of *Clonorchis sinensis* infection in fish by loop-mediated isothermal amplification (LAMP). Parasitol Res 106:1379–1383

Cai XQ, Yu HQ, Bai JS et al (2012) Development of a TaqMan based real-time PCR assay for detection of *Clonorchis sinensis* DNA in human stool samples and fishes. Parasitol Int 61:183–186

Chai JY, Lee SH (2002) Food-borne intestinal trematode infections in the Republic of Korea. Parasitol Int 51:129–154

Chaicumpa W, Ybanex L, Kitikoon V et al (1992) Detection of Opisthorchis viverrini antigens in stools using specific monoclonal antibody. Int J Parasitol 22:527–531

Chaiyadet S, Sotillo J, Smout M et al (2015) Carcinogenic liver fluke secretes extracellular vesicles that promote cholangiocytes to adopt a tumorigenic phenotype. J Infect Dis 212:1636–1645

Chen M, Lu Y et al (1994) Progress is assessment of morbidity due to *Clonorchis sinensis* infection: a review of recent literature. Trop Dis Bull 91:R7–R65

Chen DX, Chen JY et al (2010) Epidemiological investigation of *Clonorchis sinensis* infection in freshwater fishes in the Pearl River Delta. Parasitol Res 107:835–839

Chen J, Xu H, Zhang Z et al (2011) Cloning and expression of 21.1-kDa tegumental protein of *Clonorchis sinensis* and human antibody response to it as a trematode-nematode pan-specific serodiagnosis antigen. Parasitol Res 108:161–168

Chng KR, Chan SH, Qi Ng AH et al (2016) Tissue microbiome profiling identifies an enrichment of specific enteric bacteria in *Opisthorchis viverrini* associated cholangiocarcinoma. EBioMedicine 8:195–202

Choi MH, Park IC, Li S et al (2003) Excretory–secretory antigen is better than crude antigen for the serodiagnosis of clonorchiasis by ELISA. Korean J Parasitol 41:35–39

Choi MS, Choi D, Choi MH et al (2005) Correlation between sonographic findings and infection intensity in clonorchiasis. Am J Trop Med Hyg 73:1139–1144

Choi D, Lim JH, Lee KT et al (2006) Cholangiocarcinoma and *Clonorchis sinensis* infection: a case control in Korea. J Hepatol 44:1066–1073

Chunlertrith K, Sukeepaisarnjaroen W, Mairiang E et al (1992) The study of discriminant values of dyspeptic symptoms for identifying the etiology of dyspepsia. J Med Assoc Thail 75:341–349

Cringoli G, Rinaldi L, Maurelli MP et al (2010) FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat Protoc 5:503–515

Dang TC, Yajima A et al (2008) Prevalence, intensity and risk factors for clonorchiasis and possible use of questionnaires to detect individuals at risk in northern Vietnam. Trans R Soc Trop Med Hyg 102:1263–1268

De Liberato C, Scaramozzino P et al (2011) Investigation on *Opisthorchis felineus* occurrence and life cycle in Italy. Vet Parasitol 177:67–71

Deenonpoe R, Mairiang E, Mairiang P et al (2017) Elevated prevalence of *Helicobacter* species and virulence factors in opisthorchiasis and associated hepatobiliary disease. Sci Rep 7:42744

Elkins DB, Sithithaworn P, Haswell-Elkins M et al (1991) *Opisthorchis viverrini*: relationships between egg counts, worms recovered and antibody levels within an endemic community in northeast Thailand. Parasitology 102:283–288

Elkins DB, Mairiang E, Sithithaworn P et al (1996) Cross-sectional patterns of hepatobiliary abnormalities and possible precursor conditions of cholangiocarcinoma associated with *Opisthorchis viverrini* infection in humans. Am J Trop Med Hyg 55:295–301

Erhardt A, Germer WD, Hörning B et al (1962) Die Opisthorchiasis, hervorgerufen durch den Katzenleberegel Opisthorchis felineus (Riv.). Parasitologische Schriftenreihe, vol 15. Veb Gustav Fischer Verlag, Jena

European Food Safety Authority (2010) Scientific Opinion on risk assessment of parasites in fishery products. EFSA J 8:1543

Fattakhov RG (1989) Low-temperature regimens for the decontamination of fish of the larvae of *Opisthorchis*. Med Parazitol 5:63–64

Fedorova OS, Fedotova MM, Sokolova TS et al (2018) *Opisthorchis felineus* infection prevalence in Western Siberia: a review of Russian literature. Acta Trop 178:196–204

Fedorova OS, Fedotova MM, Zvonareva OI et al (2020) *Opisthorchis felineus* infection, risks, and morbidity in rural Western Siberia, Russian Federation. PLoS Negl Trop Dis 14:e0008421

Figurnov VA, Chertov AD, Romanenko NA et al (2002) Clonorchiasis in the Upper Amur region: biology, epidemiology, clinical presentation. Med Parazitol 4:20–23

Flavell DJ (1982) Acquired resistance to *Opisthorchis viverrini* in the hamster. Trans R Soc Trop Med Hyg 76:800–802

Flavell DJ, Flavell SU (1986) *Opisthorchis viverrini*: pathogenesis of infection in immunodeprived hamsters. Parasite Immunol 8:455–466

Frampton G, Invernizzi P, Bernuzzi F et al (2012) Interleukin-6-driven progranulin expression increases cholangiocarcinoma growth by an Akt-dependent mechanism. Gut 61:268–277

Fried B, Reddy A, Mayer D (2011) Helminths in human carcinogenesis. Cancer Lett 305:239–249

Furst T, Duthaler U, Sripa B et al (2012) Liver and lung flukes. Infect Dis Clin N Am 26:399–419

Gómez-Morales MA, Ludovisi A, Amati M et al (2013) Validation of an Excretory/Secretory Antigen Based-Elisa for the Diagnosis of *Opisthorchis felineus* Infection in Humans from Low Trematode Endemic Areas. PLoS One 8:e62267

Gordon IB, Razumov VV, Baranova MN et al (1984) Clinical disguises of opisthorchiasis. Klin Med 62:108–111

Haswell-Elkins MR, Sithithaworn P, Mairiang E et al (1991) Immune responsiveness and parasite-specific antibody levels in human hepatobiliary disease associated with *Opisthorchis viverrini* infection. Clin Exp Immunol 84:213–218

Haswell-Elkins MR, Satarug S, Tsuda M et al (1994) Liver fluke infection and cholangiocarcinoma: model of endogenous nitric oxide and extragastric nitrosation in human carcinogenesis. Mutat Res 305:241–252

Hemler ME (2005) Tetraspanin functions and associated microdomains. Nat Rev Mol Cell Biol 6: 801–811

Hering-Hagenbeck S, Schuster R (1996) A focus of opisthorchiidosis in Germany. Appl Parasitol 37:260–265

Hermosilla C, Kleinertz S, Silva LMR et al (2017) Protozoan and helminth parasite fauna of free-living Croatian wild wolves (*Canis lupus*) analyzed by scat collection. Vet Parasitol 233:14–19
Hong TH, Fang Y (2012) *Clonorchis sinensis* and clonorchiasis, an update. Parasit Intern 61:17–24
Hong ST, Rim HJ, Min DY et al (2001) Control of clonorchiasis by repeated treatments with praziquantel. Korean J Parasitol 39:285–292
Honjo S, Srivatanakul P, Sriplung H et al (2005) Genetic and environmental determinants of risk of cholangiocarcinoma via *Opisthorchis viverrini* in a densely infected area in Nakhon Phanom, northeast Thailand. Int J Cancer 117:854–860
Hu Y, Xiao SH, Aroian RV (2009) The new anthelmintic tribendimidine is an L-type (levamisole and pyrantel) nicotinic acetylcholine receptor agonist. PLoS Negl Trop Dis 3:e499
Huang S, Zhao GH, Fu BQ et al (2012) Genomics and molecular genetics of *Clonorchis sinensis*: current status and perspectives. Parasitol Int 61:71–76
Hussain SP, Hofseth LJ, Harris CC (2003) Radical causes of cancer. Nat Rev Cancer 3:276–285
IARC (1994) Infection with liver flukes (*Opisthorchis viverrini, Opisthorchis felineus* and *Clonorchis sinensis*). IARC Monogr Eval Carcinog Risks Hum 61:121–175
Jaroonvesama N, Charoenlarp K, Cros JH (1981) Treatment of *Opisthorchis viverrini* with mebendazole. Southeast Asian J Trop Med Public Health 12:595–597
Jeong YI, Kim SH, Ju JW et al (2011) Clonorchis sinensis-derived total protein attenuates airway inflammation in murine asthma model by inducing regulatory T cells and modulating dendritic cell functions. Biochem Biophys Res Commun 407:793–800
Jittimanee J, Sermswan RW, Puapairoj A et al (2007) Cytokine expression in hamsters experimentally infected with *Opisthorchis viverrini*. Parasite Immunol 29:159–167
Johansen MV, Sithithaworn P, Bergquist R et al (2010) Towards improved diagnosis of zoonotic trematode infections in Southeast Asia. Adv Parasitol 73:171–195
Jongsuksuntigul P, Imsomboon T (2003) Opisthorchiasis control in Thailand. Acta Trop 88:229–232
Ju JW, Joo HN, Lee MR et al (2009) Identification of a serodiagnostic antigen, legumain, by immunoproteomic analysis of excretory– secretory products of *Clonorchis sinensis* adult worms. Proteomics 9:3066–3078
Kaewpitoon N, Kaewpitoon SJ, Pengsaa P et al (2008) *Opisthorchis viverrini*: the carcinogenic human liver fluke. World J Gastroenterol 14:666–674
Kang S, Sultana T et al (2008) Molecular identification and phylogenetic analysis of nuclear rDNA sequences among three opisthorchid liver fluke species (Opisthorchiidae: Trematoda). Parasitol Int 57:191–197
Karin M, Greten FR (2005) NF-kappaB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol 5:749–759
Keiser J, UtzingerJ (2009) Food-Borne Trematodiases. Clin Microbiol 22:466–483
Khuntikeo N, Thinkhamrop B, Bundhamcharoen K et al (2018) The socioeconomic burden of cholangiocarcinoma associated with *Opisthorchis viverrini* sensu lato infection in northeast Thailand: a preliminary analysis. Adv Parasitol 102:141–163
Kiatsopit N, Sithithaworn P, Saijuntha W et al (2013) *Opisthorchis viverrini*: implications of the systematics of first intermediate hosts, *Bithynia* snail species in Thailand and Lao PDR. Infect Genet Evol 14:313–319
Kim YH (1999) Pancreatitis in association with *Clonorchis sinensis* infestation: CT evaluation. Am J Roentgenol 172:1293–1296
Kim YJ, Choi MH, Hong ST et al (2008) Proliferative effects of excretory/secretory products from *Clonorchis sinensis* on the human epithelial cell line HEK293 via regulation of the transcription factor E2F1. Parasitol Res 102:411–417
Kim TS, Cho SH, Huh S et al (2009) A nationwide survey on the prevalence of intestinal parasitic infections in the Republic of Korea, 2004. Korean J Parasitol 47:37–47
Kim JY, Yong TS, Rim HJ et al (2018) Ultrasonographic investigation of cholangiocarcinoma in Lao PDR. Acta Trop 182:128–134

Kotelkin AT, Kolesnikova LV, Riabchikova EI et al (2001) Localization of immunodominant antigens of the liver fluke *Opisthorchis felineus* by immunoelectron microscopy. Vestn Ross Akad Med Nauk 3:34–39

Kovshirina YV, Fedorova OS, Vtorushin SV et al (2019) Case report: two cases of cholangiocarcinoma in patients with *Opisthorchis felineus* infection in Western Siberia, Russian Federation. Am J Trop Med Hyg 100:599–603

Kruatrachue M, Chitramvong YP, Upathan ES et al (1982) Effects of physico-chemical factors on the infection of hamsters by metacercariae of *Opisthorchis viverrini*. Southeast Asian J Trop Med Public Health 13:614–617

Lapteva GF (1990) Opisthorchiasis-related nephropathy. Vrach Delo 2:67–69

Lazuthina EA, Andreyev NI et al (2009) On the taxonomic state of *Bithynia troschelii* var. *sibirica* Westerlund, 1886, a Siberian endemic bithyniid snail (Gastropoda: Bithyniidae). Mollusca 27: 113–122

Le TH, Van De N, Blair D et al (2006) *Clonorchis sinensis* and *Opisthorchis viverrini*: development of a mitochondrial-based multiplex PCR for their identification and discrimination. Exp Parasitol 112:109–114

Lee MK, Hong SJ, Kim HR (2010) Seroprevalence of tissue invading parasitic infections diagnosed by ELISA in Korea. J Korean Med Sci 25:1272–1276

Leiper RT (1915) Notes of the occurrence of parasites presumably rare in man. J R Army Med Corps 24:569–575

Li B, Yan C, Wu J et al (2020) *Clonorchis sinensis* ESPs enhance the activation of hepatic stellate cells by a cross-talk of TLR4 and TGF-β/Smads signaling pathway. Acta Trop 205:105307

Lim JH (2011) Liver flukes: the malady neglected. Korean J Radiol 12:269–279

Lim JH, Kim SY, Park CM (2007) Parasitic diseases of the biliary tract. AJR Am J Roentgenol 188: 1596–1603

Liu GH, Li B et al (2012) Genetic variation among Clonorchis sinensis isolates from different geographic regions in China revealed by sequence analyses of four mitochondrial genes. J Helminthol 86:479–484

Lloyd S, Soulsby EJL (1998) Other trematodes infections. In: Palmer SR, Soulsby L, Simpson DI (eds) Zoonoses: biology, clinical practice, and public health control. Oxford University Press, Oxford, pp 789–802

Lun ZR, Gasser RB et al (2005) Clonorchiasis: a key foodborne zoonosis in China. Lancet Infect Dis 5:31–41

Magen E, Bychkov V, Ginovker A et al (2013) Chronic *Opisthorchis felineus* infection attenuates atherosclerosis—an autopsy study. Int J Parasitol 43(10):819–824

Mairiang E, Mairiang P (2003) Clinical manifestations of opisthorchiasis and treatment. Acta Trop 88:221–227

Mairiang E, Elkins DB, Mairiang P et al (1992) Relationship between intensity of *Opisthorchis viverrini* infection and hepatobiliary disease detected by ultrasonography. J Gastroenterol Hepatol 7:17–21

Mairiang E, Haswell-Elkins MR, Mairiang P et al (1993) Reversal of biliary tract abnormalities associated with *Opisthorchis viverrini* infection following praziquantel treatment. Trans R Soc Trop Med Hyg 87:194–197

Mairiang E, Laha T, Bethony JM et al (2012) Ultrasonography assessment of hepatobiliary abnormalities in 3359 subjects with *Opisthorchis viverrini* infection in endemic areas of Thailand. Parasitol Int 61:208–211

Maizels RM, Yazdanbakhsh M (2003) Immune regulation by helminth parasites: cellular and molecular mechanisms. Nat Rev Immunol 3:733–744

Meniavtseva TA, Ratner GM, Struchkova SV et al (1996) Immunoenzyme analysis in the diagnosis of opisthorchiasis. I. The development of an immunoenzyme method for determining IgM antibodies to the *Opisthorchis* antigen. Med Parazitol 65:41–43

Monami G, Gonzalez EM, HelLman M et al (2006) Proepithelin promotes migration and invasion of 5637 bladder cancer cells through the activation of ERK1/2 and the formation of a paxillin/FAK/ERK complex. Cancer Res 66:7103–7110

Mordvinov VA, Furman DP (2010) The Digenea parasite *Opisthorchis felineus*: a target for the discovery and development of novel drugs. Infect Disord Drug Targets 10:385–401

Mordvinov VA, Yurlova NI et al (2012) *Opisthorchis felineus* and *Metorchis bilis* are the main agents of liver fluke infection of humans in Russia. Parasitol Int 61:25–31

Müller B, Schmidt J, Mehlhorn H (2007) PCR diagnosis of infections with different species of Opisthorchiidae using a rapid clean-up procedure for stool samples and specific primers. Parasitol Res 100:905–909

Murrell KD, Pozio E (2017) The liver flukes: Clonorchis sinensis, Opisthorchis spp. and Metorchis spp. In: Rose JB, Jiménez-Cisneros B (eds) Global water pathogens project. Michigan State University, UNESCO, E. Lansing, MI, p 17. http://www.waterpathogens.org/book/liver-flukes

Na BK, Kang JM, Sohn WM (2008) CsCF-6, a novel cathepsin F-like cysteine protease for nutrient uptake of *Clonorchis sinensis*. Int J Parasitol 38:493–502

Na BK, Pak JH, Hong SJ (2020) *Clonorchis sinensis* and clonorchiasis. Acta Trop 203:105309

Nagano I, Pei F, Wu Z et al (2004) Molecular expression of a cysteine proteinase of *Clonorchis sinensis* and its application to an enzyme-linked immunosorbent assay for immunodiagnosis of clonorchiasis. Clin Diagn Lab Immunol 11:411–416

Olds GR (2003) Administration of praziquantel to pregnant and lactating women. Acta Trop 86:185–195

Pakharukova MY, Mordvinov VA (2016) The liver fluke *Opisthorchis felineus*: biology, epidemiology and carcinogenic potential. Trans R Soc Trop Med Hyg 110:28–36

Pauly A, Schuster R, Steuber S (2003) Molecular characterization and differentiation of opisthorchiid trematodes of the species *Opisthorchis felineus* (Rivolta, 1884) and *Metorchis bilis* (Braun, 1790) using polymerase chain reaction. Parasitol Res 90:409–414

Petney T, Sithithaworn P (2012) The ecology of the *Bithynia* first intermediate hosts of *Opisthorchis viverrini*. Parasitol Int 61:38–45

Petney TN, Andrews RH, Saijuntha W et al (2013) The zoonotic, fish-borne liver flukes *Clonorchis sinensis*, *Opisthorchis felineus* and *Opisthorchis viverrini*. Int J Parasitol 43:1031–1046

Petney TN, Andrews RH, Saijuntha W et al (2018) Taxonomy, ecology and population genetics of Opisthorchis viverrini and its intermediate hosts. Adv Parasitol 101:1–39

Phimpraphai W, Tangkawattana S, Kasemsuwan S et al (2018) Social influence in liver fluke transmission: application of social network analysis of food sharing in Thai Isaan culture. Adv Parasitol 101:97–124

Pinlaor S, Sripa B, Ma N et al (2005) Nitrative and oxidative DNA damage in intrahepatic cholangiocarcinoma patients in relation to tumor invasion. World J Gastroenterol 30:4644–4649

Pinlaor S, Hiraku Y, Yongvanit P et al (2006) iNOS-dependent DNA damage via NF-kB expression in hamsters with *Opisthorchis viverrini* and its suppression by the antihelminthic drug praziquantel. Int J Cancer 119:1067–1072

Pinlaor P, Pongsamart P, Hongsrichan N et al (2012) Specific serum IgG, but not IgA, antibody against purified *Opisthorchis viverrini* antigen associated with hepatobiliary disease and cholangiocarcinoma. Parasitol Int 61:212–216

Poirier J (1886) Trematodes nouveaux on peu connus. Bull Soc Philomath Paris 7:20–40

Pozio E, Armignacco O, Ferri F et al (2013) *Opisthorchis felineus*, an emerging infection in Italy and its implication for the European Union. Acta Trop 126:54–62

Pungpak S, Bunnag D, Harinasuta T (1984) Albendazole in the treatment of opisthorchiasis and concomitant intestinal helminthic infections. Southeast Asian J Trop Med Public Health 15:4–50

Qian MB, Utzinger J, Keiser J et al (2016) Clonorchiasis. Lancet 387:800–810

Qiao T, Ma RH, Luo ZL et al (2014) *Clonorchis sinensis* eggs are associated with calcium carbonate gallbladder stones. Acta Trop 138:28–37

Riganti M, Pungpak S, Punpoowong B et al (1989) Human pathology of *Opisthorchis viverrini* infection: a comparison of adults and children. Southeast Asian J Trop Med Public Health 20: 95–100

Rim HJ (2005) Clonorchiasis: an update. J Helmintho 79:269–281

Rivolta S (1884) Sopra una specie di Distoma nel gatto e nel cane. Giornale di Anatomia Fisiologica e Patologia degli Animali 16:20–28

Ruangkunaporn Y, Akai PS, Chongsa-Nguan M et al (1994) Changes in serum antibodies to *Opisthorchis viverrini* in humans and hamsters following treatment of opisthorchiasis. Asian Pac J Allergy Immunol 12:83–84

Saijuntha W, Sithithaworn P, Wongkham S et al (2007) Evidence of a species complex within the food-borne trematode *Opisthorchis viverrini* and possible co-evolution with their first intermediate hosts. Int J Parasitol 37:695–703

Saijuntha W, Sithithaworn P, Wongkham S et al (2008) Mitochondrial DNA sequence variation among geographical isolates of *Opisthorchis viverrini* in Thailand and Lao PDR, and phylogenetic relationships with other trematodes. Parasitology 135:1479–1486

Saijuntha W, Duenngai K, Tangkawattana S et al (2018) Recent advances in the diagnosis and detection of *Opisthorchis viverrini* Sensu Lato in human and intermediate hosts for use in control and elimination programs. Adv Parasitol 101:177–214

Saijuntha W, Sithithaworn P, Petney TN et al (2021) Foodborne zoonotic parasites of the family Opisthorchiidae. Res Vet Sci 135:404–411

Sakolvaree Y, Ybanez L, Chaicumpa W (1997) Parasites elicited cross-reacting antibodies to *Opisthorchis viverrini*. Asian Pac J Allergy Immunol 2:115–122

Salao K, Watakulsin K, Mairiang E et al (2019) High macrophage activities are associated with advanced periductal fibrosis in chronic *Opisthorchis viverrini* infection. Parasite Immunol 41: e12603

Salao K, Spofford EM, Price C et al (2020) Enhanced neutrophil functions during *Opisthorchis viverrini* infections and correlation with advanced periductal fibrosis. Int J Parasitol 50:145–152

Saltykova IV, Petrov VA, Logacheva MD et al (2016) Biliary microbiota, gallstone disease and infection with *Opisthorchis felineus*. PLoS Negl Trop Dis 10:e0004809

Satarung S, Haswel-Eltkins M, Sithithaworn P et al (1998) Relationships between the synthesis of N-nitrosodimethylamine and immune responses to chronic infection with the carcinogenic parasite, *Opisthorchis viverrini*, in men. Carcinogenesis 19:485–491

Sato M, Thaenkham U, Dekumyoy P et al (2009) Discrimination of *O. viverrini, C. sinensis*, *H. pumilio* and *H. taichui* using nuclear DNA-based PCR targeting ribosomal DNA ITS regions. Acta Trop 2009109:81–83

Sawangsoda P, Sithithaworn J, Tesana S et al (2012) Diagnostic values of parasite-specific antibody detections in saliva and urine in comparison with serum in opisthorchiasis. Parasitol Int 61:196–202

Sayasone S, Odermatt P, Phoumindr N et al (2007) Epidemiology of *Opisthorchis viverrini* in a rural district of southern Lao PDR. Trans R Soc Trop Med Hyg 101:40–47

Sayasone S, Odermatt P, Vonghachack Y et al (2016) Efficacy and safety of tribendimidine against *Opisthorchis viverrini*: two randomized, parallel-group, single-blind, dose-ranging, phase 2 trials. Lancet Infect Dis 16:e1145–e1153

Sayasone S, Odermatt P, Vonghachack Y et al (2018) Efficacy and safety of tribendimidine versus praziquantel against *Opisthorchis viverrini* in Laos: an open-label, randomised, non-inferiority, phase 2 trial. Lancet Infect Dis 18:155–161

Schuster RK (2010) Opisthorchiidosis—a review. Infect Disord Drug Targets 10:402–415

Shen C, Lee JA, Allam SR et al (2009) Serodiagnostic applicability of recombinant antigens of *Clonorchis sinensis* expressed by wheat germ cell-free protein synthesis system. Diagn Microbiol Infect Dis 64:334–229

Sirisinha S, Chawengkirttikul R, Haswell-Elkins MR et al (1995) Evaluation of a monoclonal antibody based enzyme-linked immunosorbent assay for the diagnosis of *Opisthorchis viverrini* infection in an endemic area. Am J Trop Med Hyg 52:521–524

Sithithaworn P, Tesana S, Pipitgool V et al (1991) Quantitative post-mortem study of *Opisthorchis viverrini* in man in north-east Thailand. Trans R Soc Trop Med Hyg 85:765–768

Sithithaworn P, Yongvanit P, Tesana S et al (2007) Liver flukes. In: Murrell KD, Fried B (eds) World class parasites, vol 11. Springer, Dordrecht, pp 3–52

Sithithaworn P, Andrews RH, Nguyen VD et al (2012a) The current status of opisthorchiasis and clonorchiasis in the Mekong Basin. Parasitol Int 61:10–16

Sithithaworn P, Andrews RH, Petney TN et al (2012b) The systematics and population genetics of *Opisthorchis viverrini* sensu lato: implications in parasite epidemiology and bile duct cancer. Parasitol Int 61:32–37

Smout MJ, Laha T, Mulvenna J et al (2009) A granulin-like growth factor secreted by the carcinogenic liver fluke, *Opisthorchis viverrini*, promotes proliferation of host cells. PLoS Pathog 5:e1000611

Smout MJ, Mulvenna JP, Jones MK et al (2011) Expression, refolding and purification of Ov-GRN-1, a granulin-like growth factor from the carcinogenic liver fluke, that causes proliferation of mammalian host cells. Protein Expr Purif 79:263–270

Sohn WM, Zhang H, Choi MH et al (2006) Susceptibility of experimental animals to reinfection with *Clonorchis sinensis*. Korean J Parasitol 44:163–166

Sohn WM, Shin EH, Yong TS et al (2011) Adult *Opisthorchis viverrini* flukes in humans, Takeo, Cambodia. Emerg Infect Dis 17:1302–1304

Sohn WM, Yong TS, Eom KS et al (2012) Prevalence of *Opisthorchis viverrini* infection in humans and fish in Kratie Province, Cambodia. Acta Trop 124(3):215–220

Soukhathammavong P, Odermatt P, Sayason, et al. (2011) Efficacy and safety of mefloquine, artesunate, mefloquine-artesunate, tribendimidine, and praziquantel in patients with *Opisthorchis viverrini*: a randomised, exploratory, open-label, phase 2 trial. Lancet Infect Dis 11:110–118

Sripa B (2003) Pathobiology of opisthorchiasis: one overview. Acta Trop 88:209–220

Sripa B, Kaewkes S (2000) Relationship between parasite specific antibody responses and intensity of *Opisthorchis viverrini* infection in hamsters. Parasite Immunol 22:139–145

Sripa B, Kaewkes S, Sithithaworn P et al (2007) Liver flukes induces cholangiocarcinoma. PLoS Med 7:201e

Sripa B, Kaewkes S, Intapan PM et al (2010) Food-borne trematodiases in Southeast Asia epidemiology, pathology, clinical manifestation and control. Adv Parasitol 72:305–350

Sripa B, Brindley PJ, Mulvenna J et al (2012) The tumorigenic liver fluke *Opisthorchis viverrini*-multiple pathways to cancer. Trends Parasitol 10:395–407

Sripa B, Jumnainsong A, Tangkawattana S et al (2018) Immune Response to *Opisthorchis viverrini* infection and its role in pathology. Adv Parasitol 102:73–95

Sultanov A, Abdybekova A, Abdibaeva A et al (2014) Epidemiology of fishborne trematodiasis in Kazakhstan. Acta Trop 138:60–66

Suttiprapa S, Loukas A, Laha T et al (2008) Characterization of the antioxidant enzyme, thioredoxin peroxidase, from the carcinogenic human liver fluke, *Opisthorchis viverrini*. Mol Biochem Parasitol 160:116–122

Suttiprapa S, Matchimakul P, Loukas A et al (2012) Molecular expression and enzymatic characterization of thioredoxin from the carcinogenic human liver fluke *Opisthorchis viverrini*. Parasitol Int 61:101–106

Suvorov AI, Krylov GG, Bychkov VG (2004) Duodenogastroesophageal reflux disease as a complication of superinvasion opisthorchiasis. Med Parazitol 3:30–32

Suwannatrai A, Saichua P, Haswell M (2018) Epidemiology of *Opisthorchis viverrini* infection. Adv Parasitol 101:41–67

Tangkawattana S, Tangkawattana P (2018) Reservoir animals and their roles in transmission of *Opisthorchis viverrini*. Adv Parasitol 101:69–95

Techasen A, Loilome W, Namwat N et al (2012) *Opisthorchis viverrini*-antigen induces expression of MARCKS during inflammation-associated cholangiocarcinogenesis. Parasitol Int 61:140–144

Teimoori S, Arimatsu Y, Laha T et al (2017) Chicken IgY-based coproantigen capture ELISA for diagnosis of human opisthorchiasis. Parasitol Int 66:443–447
Tesana S, Thapsripair P (2012) Effects of Bayluscide on *Bithynia siamensis goniomphalos*, the first intermediate host of the human liver fluke, *Opisthorchis viverrini*, in laboratory and field trials. Parasitol Int 61:52–55
Tesana S, Srisawangwong T, Sithithaworn P et al (2007) The ELISA-based detection of anti-*Opisthorchis viverrini* IgG and IgG4 in samples of human urine and serum from an endemic area of north-eastern Thailand. Ann Trop Med Parasitol 101:585–591
Thuwajit C, Thuwajit P, Uchida K et al (2006) Gene expression profiling defined pathways correlated with fibroblast cell proliferation induced by *Opisthorchis viverrini* excretory/secretory product. World J Gastroenterol 12:3585–3592
Tinga N, De N, Vien HV et al (1999) Little effect of praziquantel or artemisinin on clonorchiasis in Northern Vietnam. A pilot study. Tropical Med Int Health 4:814–818
Traub RJ, Macaranas J, Mungthin M et al (2009) A new PCR-based approach indicates the range of *Clonorchis sinensis* now extends to Central Thailand. PLoS Negl Trop Dis 3:e367
Traverso A, Repetto E, Magnani S et al (2012) A large outbreak of *Opisthorchis felineus* in Italy suggests that opisthorchiasis develops as a febrile eosinophilic syndrome with cholestasis rather than a hepatitis-like syndrome. Eur J Clin Microbiol Infect Dis 31:1089–1093
Upatham ES, Viyanant V (2003) *Opisthorchis viverrini* and opisthorchiasis: a historical review and future perspective. Acta Trop 88:171–176
Uttaravichien T, Bhudhisawasdi V, Pairojkul C et al (1999) Intrahepatic cholangiocarcinoma in Thailand. J Hepato-Biliary-Pancreat Surg 6:128–135
Viranuvatti V, Kshemsant D, Bhamarapravati N (1955) Retention cyst of liver caused by opisthorchiasis associated with carcinoma; case report. Am J Gastroenterol 23:442–446
Viyanant V, Brockelman WY, Lee P et al (1983) A comparison of a modified quick-Kato technique and the Stoll dilution method for field examination for *Opisthorchis viverrini* eggs. J Helminthol 57:191–195
Vogel H (1934) The development cycle of *Opisthorchis felineus* (Riv.) together with remarks on systematic and epidemiology. Zoologica, Stuttgart 86:1–103
Wang X, Liang C, Chen W et al (2009) Experimental model in rats for study on transmission dynamics and evaluation of *Clonorchis sinensis* infection immunologically, morphologically, and pathologically. Parasitol Res 106:15–21
WHO (1995) Control of foodborne trematodes infection. WHO Technical Report Series 849
WHO (2011) Report of the WHO Expert Consultation on Foodborne Trematode Infections and Taeniasis/Cysticercosis. World Health Organization, Geneva, 59 pp
Winogradoff K (1892) On a new species of distomum (*Distomum sibiricum*) in the human liver. Cenralbl Allg F Pathol U Pathol Anatomie 3:910–911
Wongratanacheewin S, Rattanasiriwilai W, Priwan R et al (1987) Immunodepression in hamsters experimentally infected with *Opisthorchis viverrini*. J Helminthol 61:151–156
Wongratanacheewin S, Bunnag D, Vaeusorn N (1988) Characterization of humoral immune response in the serum and bile of patients with opisthorchiasis and its application in immunodiagnosis. Am J Trop Med Hyg 38:356–362
Wongratanacheewin S, Good MF, Sithithaworn P et al (1991) Molecular analysis of T and B cell repertoires in mice immunized with *Opisthorchis viverrini* antigens. Int J Parasitol 21:719–721
Wongratanacheewin S, Pumidonming W, Sermswan RW (2002) Detection of *Opisthorchis viverrini* in human stool specimens by PCR. J Clin Microbiol 40:3879–3880
Wongratanacheewin S, Sermswan RW, Sirisinha S (2003) Immunology and molecular biology of *Opisthorchis viverrini* infection. Acta Trop 88:195–207
Wongsaroj T, Sakolvaree Y, Chaicumpa W et al (2001) Affinity purified oval antigen for diagnosis of *Opisthorchiasis viverrini*. Asian Pac J Allergy Immunol 19:245–258
Wood IB, Amaral NK, Bairden K et al (1995) World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). Vet Parasitol 58:181–213

Worasith C, Kamamia C, Yakovleva A et al (2015) Advances in the diagnosis of human opisthorchiasis: development of *Opisthorchis viverrini* antigen detection in urine. PLoS Negl Trop Dis 9:e0004157

Wykoff DE, Harinasuta C, Juttijudata P (1965) *Opisthorchis viverrini* in Thailand—the life cycle and comparison with *O. felineus*. J Parasitol 51:207–214

Xiao SH, Utzinger J, Tanner M et al (2013) Advances with the Chinese anthelminthic drug tribendimidine in clinical trials and laboratory investigations. Acta Trop 126:115–126

Yong TS, Shin EH, Chai JY et al (2012) High prevalence of Opisthorchis viverrini infection in a riparian population in Takeo Province, Cambodia. Korean J Parasitol 50:173–176

Yoshida Y (2012) Clonorchiasis—a historical review of contributions of Japanese parasitologists. Parasitol Int 61:5–9

Yossepowitch O, Gotesman T, Assous M et al (2004) Opisthorchiasis from imported raw fish. Emerg Infect Dis 10:2122–2126

Zen Y, Sasaki M, Fujii T et al (2006) Different expression patterns of mucin core proteins and cytokeratins during intrahepatic cholangiocarcinogenesis from biliary intraepithelial neoplasia and intraductal papillary neoplasm of the bile duct--an immunohistochemical study of 110 cases of hepatolithiasis. J Hepatol 44:350–358

Zhang H, Chung BS, Li S et al (2008a) Factors in the resistance of rats to reinfection and super-infection by *Clonorchis sinensis*. Parasitol Res 102:1111–1117

Zhang H, Chung BS, Li S et al (2008b) Changing patterns of serum and bile antibodies in re-infected rats with *Clonorchis sinensis*. Korean J Parasitol 46:17–22

Zhang BB, Yan C, Fang F et al (2017) Increased hepatic Th2 and Treg subsets are associated with biliary fibrosis in different strains of mice caused by *Clonorchis sinensis*. PLoS One 12: e0171005

Zhu SH, Zhong XS, Luo ZY (1983) Clonorchiasis and developmental disorder in the children (dwarfism). Xin Yi Xue 14:71–72

Chapter 8
Echinococcosis

Francesca Tamarozzi, Tommaso Manciulli, Enrico Brunetti, and Dominique A. Vuitton

Abstract Cystic echinococcosis (CE) and alveolar echinococcosis (AE) are zoonoses of great medical and veterinary importance, caused by *Echinococcus granulosus* species complex and *E. multilocularis*, respectively. The life cycle of these parasites develops between the dog and other canids, which harbor the adult tapeworm in the intestine, and mammal intermediate hosts (including humans as dead-end occasional hosts) where the larval form, the metacestode, develops in different organs.

The impact of CE and AE on human health is important, with an estimated 1.2 million people affected and 3.6 million DALYs lost globally for CE and 666,434 DALYs for AE.

We describe epidemiology, host's immune response to parasite, clinical manifestations, diagnosis, and treatment of both CE and AE.

F. Tamarozzi
Department of Infectious Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Verona, Italy

T. Manciulli
WHO Collaborating Centre on Clinical Management of Cystic Echinococcosis, Pavia, Italy

Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

Largo Giovanni Alessandro Brambilla, Florence, Italy

E. Brunetti (✉)
Infectious Diseases and Immunology Unit, WHO Collaborating Centre on Clinical Management of Cystic Echinococcosis, IRCCS San Matteo Hospital Foundation, University of Pavia, Pavia, Italy
e-mail: enrico.brunetti@unipv.it

D. A. Vuitton
French National Reference Centre for Echinococcoses, University Bourgogne Franche-Comté and University Hospital, Besançon, France

F. Bruschi (ed.), *Helminth Infections and their Impact on Global Public Health*,
https://doi.org/10.1007/978-3-031-00303-5_8

8.1 Introduction

Cystic echinococcosis (CE) and alveolar echinococcosis (AE) are zoonoses of great medical and veterinary importance, caused by *Echinococcus granulosus* sensu lato and *E. multilocularis*, respectively. The life cycle of these parasites develops between the dog and other canids, which harbor the adult tapeworm in the intestine, and mammal intermediate hosts (including humans as dead-end occasional hosts) where the larval form, the metacestode, develops in different organs.

The impact of CE and AE on human health is important, with an estimated 1.2 million people affected and 3.6 million DALYs lost globally for CE (Craig et al. 2007a; Budke 2006) and 666,434 DALYs for AE (Torgerson et al. 2010). Unlike AE, CE has also a major economic impact with an estimated annual livestock production loss of up to 2190 million US$ (Budke 2006). Despite these figures, these infections are still neglected (Budke 2006).

CE has been defined as a chronic, complex, and neglected disease (Brunetti et al. 2011; Craig et al. 2007a) for the following reasons: (1) *E. granulosus* s.l. is a zoonotic parasite with a life cycle that is difficult to interrupt without sustained, long-lasting, and expensive programs; (2) control of infection in humans does not affect its transmission, unlike control in animals, but CE is not perceived as an important animal health problem, which does not stimulate veterinary control measures; (3) its burden is difficult to quantify because of its geographical dispersal in vast rural areas, absence of specific symptoms, and lack of effective reporting systems; and (4) it affects mostly poor pastoral communities, with a low fatality rate and with difficult and expensive diagnosis and treatment.

AE is also a chronic, complex, and neglected disease for the following reasons, some of them shared with CE, some others specific to AE: (1) *E. multilocularis* wild life cycle is very difficult to interrupt; (2) AE is a rare disease with a long asymptomatic period, and its diagnosis is difficult and generally made at an advanced stage; (3) its burden is difficult to quantify; (4) it affects mostly rural communities; and (5) treatment is difficult and expensive, and it requires a multidisciplinary approach, and is not available in many countries (Brunetti et al. 2010; Agudelo et al 2016).

"Neotropical" echinococcoses caused by *E. vogeli* and *E. oligarthrus* in South America are rare in humans (Grenouillet et al. 2013) (D'Alessandro and Rausch 2008). These species will not be described here, but their impact is likely to increase in the future, given the major changes induced by human activities in the Amazonian areas.

Recently, a consensus document on terminology to be used in the field of echinococcoses has been released, with the aim of standardizing the complex and often on fusing terminology used in this field (Vuitton et al. 2020). This terminology as well as the currently approved nomenclature for *Echinococcus* spp. will be used throughout this chapter.

8.2 The Agent

8.2.1 Life Cycle

Echinococcus spp. are cestodes (tapeworms) of the family Taeniidae, with a predator-prey life cycle and a complex biology (Thompson 2017; Romig et al. 2017).

The adult worm is 2–7 mm long and resides in the intestine of dogs, foxes, and other canids (Thompson 2017). It is composed of a scolex with 4 suckers and a rostellum with a double ring of 25–50 hooks and a strobilus composed of 2–6 proglottids. These mature progressively from the scolex end, and the last one, when it is gravid with hundreds of oncosphere-containing eggs, is released every 1–2 weeks. Eggs are 25–30 μm, round, with a striated embryophore which contains the exacanth larva, indistinguishable from eggs of *Taenia* spp. that infect dogs. Eggs are dispersed in the environment, where they can survive up to 2 years in optimal conditions of moisture and shade due to an envelope, which allows them to resist very low temperatures (−40 °C).

Intermediate hosts are ruminants, horses, pigs, and other mammals in case of *E. granulosus* s.l., usually wild rodents of various species, and the lagomorph *Ochotona curzoniae*, on the Tibetan plateau of China, and, less commonly, other carnivores including raccoon dogs, coyotes, and cats (Torgerson et al. 2011), in case of *E. multilocularis*. Humans are occasional dead-end intermediate hosts. They get infected via the fecal-oral route by ingestion of eggs. These hatch in the duodenum, and the hexacanth larva penetrates the gut wall. The metacestode then develops in different organs, mainly the liver and the lungs. The metacestode of *E. granulosus* s.l. or echinococcal cyst (often commonly referred to as hydatid cyst) is a fluid-filled bladder that grows centrifugally and can survive decades in the intermediate host. Fertility, i.e., development of protoscoleces (PSC) from its inner surface, varies with the host-parasite strain combination. The growth rate of cysts varies greatly between species (Barnes et al. 2007; Gemmell et al. 1986). AE lesions consist of a mass of small vesicles ("microcysts") that grow by infiltration invading also tissues close to the liver or disseminating in microthrombi via the bloodstream.

The life cycle is completed when the definitive host eats organs of the intermediate host that contain fertile metacestodes. The PSC develop into adult worms in the small intestine with a prepatent period of 4–7 weeks.

8.2.2 The Echinococcal Cyst and Its Natural History

The echinococcal cyst is the anatomical lesion that develops in humans after infection with eggs of the *E. granulosus* s.l. species complex; this currently encompasses several genetically defined species: *E. granulosus* sensu stricto, *E. canadensis*, *E. ortleppi*, *E. felidis*, and *E. equinus*; only the first three of them

are of clinical relevance in humans Agudelo et al 2016 (Wen et al. 2019). It has a complex structure encompassing a parasitic part and a host-derived adventitia. From the outer to the inner surface, there are (1) the host-derived fibrous or poorly cellular adventitial layer (the term "pericyst," used in the past sometimes to refer to this structure, is no longer accepted and should be avoided), which may be absent in serosal cavities or bones; (2) the parasite-derived inner layers composed of an outer acellular laminated layer (LL) and an inner syncytial germinal layer (again the term "endocyst," used in the past to indicate these structures, is no longer accepted and should be avoided); and (3) a liquid content or hydatid cyst fluid (HCF), where both parasite- and host-derived molecules are found (Monteiro et al. 2010; da Silva 2011). The LL is an acellular multi-laminated structure synthesized by the hexacanth embryo first and then by the germinal layer. It is formed by a mesh of highly glycosylated glycoproteins (Diaz et al. 2011a, b) and has a pivotal role in immune evasion (Diaz et al. 2011a, b). From the germinal layer form brood capsules containing PSC. Structures distinct from the brood capsules, which generally appear after aggression or disruption of the cyst layers, are called "daughter cysts" (Rogan et al. 2006; Galindo et al. 2002). The various structures released in the HCF have been referred to as "hydatid sand." Each viable PSC may develop into an adult worm if ingested by a definitive host or into a new cyst if disseminated in tissues of an intermediate host as the consequence of cyst rupture (secondary echinococcosis). Around intact cysts there is a remarkably scarce inflammatory reaction (Breijo et al. 2008; Coltorti and Varela-Diaz 1974; Marco et al. 2006; Sakamoto and Cabrera 2003).

The growth rate of cysts in humans is highly variable (1–160 mm/year). On average cysts grow 1.5–15 mm/year reaching sizes of 1–15 cm (Romig et al. 1986; Moro et al. 1999), but growth rate is not constant. Fertility is acquired in no less than 10 weeks (Romig et al. 1986). In humans, CE cysts develop predominantly in the liver (60–70%), followed by lungs (20–30%), but all organs and tissues can be affected. In up to 10% of cases, CE cysts may affect the kidneys, spleen, bones (most frequently the spine), and central nervous system, but rarer localizations have been described; in about 40% of cases, there are multiple localizations (Pedrosa et al. 2000; WHO/OIE and Echinococcosis 2001).

The natural history of the cyst is not completely known. Moreover, cysts with morphological aspects indicating viability may be not viable in biological terms and vice versa (Hosch et al. 2008b, c; Stojkovic et al. 2009).

CE cysts have been classified by the WHO Informal Working Group on Echinococcosis (Macpherson et al, 2003a; Brunetti et al. 2010) in five groups: CE1 (unilocular), CE2 (with daughter cysts), CE3 (with detached parasitic layers—CE3a—or with daughter cysts in a solid matrix, CE3b), CE4 (folded parasitic layers in a solid matrix), and CE5 (solid with calcifications). CL (cystic lesion) allocates cysts, the parasitic nature of which is unclear based on ultrasound only, and that deserves further evaluation (Fig. 8.1). CE1 and CE2 cysts are classified as active; CE3 as transitional; and CE4 and CE5 as inactive. Presence of calcification does not *per se* indicate the inactivity of a cyst (Hosch et al. 2007). Based on histology and metabolic studies, and as indicated by their different response to therapy, CE3 cysts

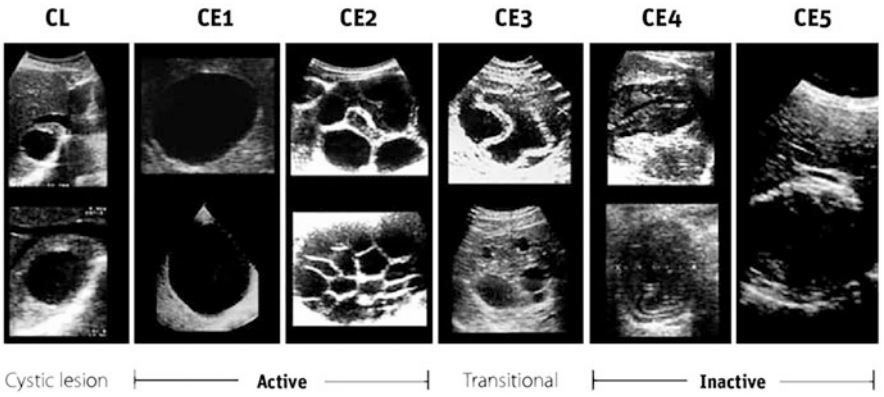

Fig. 8.1 The original WHO Informal Working Group Classification of Echinococcal Cysts. Cysts are grouped according to their activity. CE1 (unilocular) and CE2 (with daughter vesicles) are active, CE3 (with detached parasitic layers or with daughter cysts in a solid matrix) is transitional, and CE4 (folded parasitic layers in a solid matrix) and CE5 (solid with calcifications) are inactive. CE3 cysts have been later divided into CE3a (with detached parasitic layers—top) and CE3b (with daughter cysts in a solid matrix—bottom); this distinction must be always indicated due to the different biological and clinical characteristics of these two stages. CL (cystic lesion) is a cyst whose parasitic nature is uncertain and that requires further investigation

are further classified into CE3a (with detached parasitic layers) and CE3b (with daughter cysts in a solid matrix), the latter being active and the former being equally likely to be active or inactive (Junghanss et al. 2008; Brunetti et al. 2010; Hosch et al. 2008b, c; Golemanov et al. 2011) .

If not modified by treatment, CE cysts may pass spontaneously through several stages, from active to inactive (Romig et al. 1986; Keshmiri et al. 1999; Rogan et al. 2006) (Solomon et al. 2017a, 2018), but generally tend to be stable over time (Frider et al. 1999; Tamarozzi et al. 2018) (Fig. 8.2).

Up to 50% of infected individuals show evidence of spontaneous involution (to CE4–CE5 stages) of cysts (Li et al. 2011a; Larrieu et al. 2004; Wen et al. 1994; Wang et al. 2006; Keshmiri et al. 2001; Solomon et al. 2017a, 2018).

8.2.3 Alveolar Echinococcosis Lesions and Their Natural History

The metacestode of *E. multilocularis* appears as an aggregation of small cysts ("microcysts"), each one lined by the germinal layer and the LL (Vuitton and Gottstein 2010). The germinal layer forms "buds" and then fluid-filled, multiple, aggregated, 1–10-mm-wide "microcysts" (Eckert and Deplazes 2004). Fertility is common in susceptible hosts, where it is reached within 2–4 months (Liance et al. 1984). It is far rarer (<20%) in resistant hosts, such as humans, or most domestic

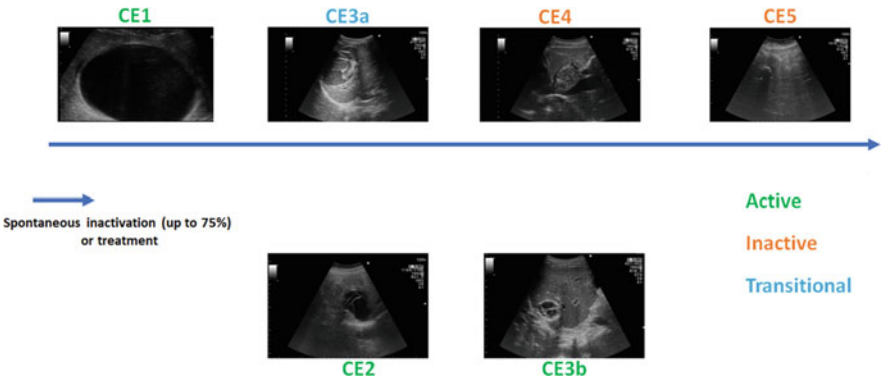

Fig. 8.2 Sequence of cyst stages as seen from early diagnosis to successful nonsurgical treatment (from *L* to *R upper row*). This is also thought to be the same course followed by spontaneous involution, which can be observed in up to 75% of untreated cases detected during population screening. The *red lines* show the formation of daughter cysts, CE2 developing likely from CE1 or CE3a stages and CE3b from the reactivation of a CE4 stage

animals. The LL is surrounded by epithelium-like macrophages (epithelioid cells) and then by concentric layers of immune cells (macrophages, lymphocytes, eosinophils, and giant cells), cells involved in fibrosis (fibroblasts and myofibroblasts), and collagen bundles and extracellular matrix (Bresson-Hadni et al. 2007). This "granulomatous" periparasitic infiltrate is usually bordered by T lymphocytes. The extent of the periparasitic infiltrate and the presence of PSC in the parasite vesicles depend on the susceptibility of the host (Vuitton and Gottstein 2010; Bresson-Hadni et al. 2007; Vuitton et al. 2003). The periparasitic infiltrate may undergo necrosis, especially after years of evolution in humans, sometimes giving a "pseudocystic" appearance to AE lesions (Bresson-Hadni et al. 2006a, b) (Fig. 8.3). Such "pseudocysts" are completely different, regarding their structure and origin, from the hydatid cyst observed in CE.

The growth of *E. multilocularis* metacestode is slow. In humans, symptoms present 5–15 years, or even more, after infection (Kern et al. 2003). The latent period may be shorter in case of immune suppression as in patients with liver transplantation for AE, where recurrence is observed less than 2 years after transplantation (Koch et al. 2003). Parasite growth is also faster in patients with immune suppression-associated conditions such as cancer, chronic inflammatory diseases, kidney or heart transplantation, or hematological disorders, sometimes with unexpected "acute" clinical presentation (Chauchet et al. 2013). Conversely, population-based mass screening in endemic areas revealed that infection may never be followed by disease in most cases (Bartholomot et al. 2002; Yang et al. 2006a; Bresson-Hadni et al. 1994).

AE lesions are located mostly (over 98% of cases) in the liver and look like a malignant tumor, and most complications are due to the invasion of bile ducts or portal/hepatic vessels (Bresson-Hadni et al. 2007). The "PNM" WHO-IWGE classification/staging of AE is modeled after the "tumor, lymph node, metastasis" or

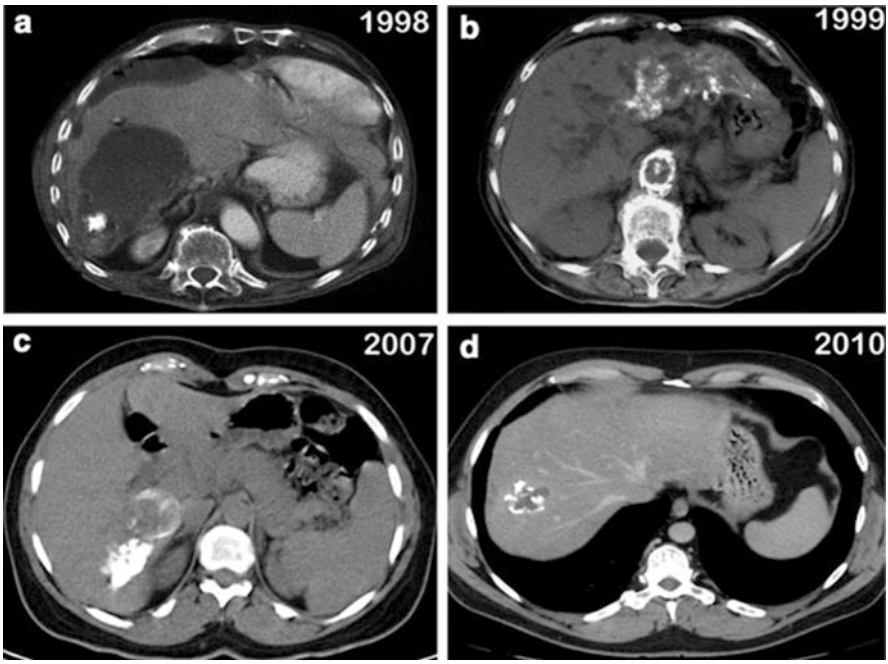

Fig. 8.3 Alveolar echinococcosis. CT scan. (**a**, **b**) Typical images of AE lesions in symptomatic patients. (**a**) A huge AE lesion with central necrosis in the right liver of a 75-year-old man is diagnosed in 1998; (**b**) 1 year later, a very advanced AE invading both lobes of the liver, with portal and biliary obstruction, is diagnosed in his 72-year-old sister presenting with jaundice. (**c**, **d**) Typical images of AE lesions in asymptomatic patients. (**c**) Fortuitous discovery of a middle-sized totally calcified presumably aborted AE lesion, in the right liver of a 47-year-old woman in 2007; ultrasound screening is recommended to first-degree relatives; (**d**) through this screening, 3 years later, a small-sized mixed progressing/calcified AE lesion is discovered in the right liver of her 46-year-old brother (Courtesy Prof. Solange Bresson-Hadni, WHO Collaborating Centre for Prevention and Treatment of Human Echinococcosis, Besanc¸on, France).

"TNM" classification/staging of cancer (Kern et al. 2006). "P" indicates the extent and location of parasitic lesions, "N" the invasion of neighboring organs, and "M" the presence of metastases (Brunetti et al. 2010).

8.3 Epidemiology of Infection

8.3.1 Geographical Distribution and Burden of Infection

CE is reported in all continents with the exception of Antarctica, while very few islands are free from the infection (Deplazes et al. 2017). It is especially prevalent in regions where sheep and livestock are raised. The synanthropic cycle with high zoonotic potential is maintained between the domestic dog and livestock in pastoral

communities. The most endemic regions are South America, the Mediterranean, Eastern Europe, North and East Africa, the Middle East, Central Asia, the Indian subcontinent, China, Mongolia, and Australia (Deplazes et al. 2017). In endemic countries CE can also occur in urban centers where transmission occurs in unlicensed and unsupervised abattoirs (Reyes et al. 2012).

The exact identification of endemic areas and quantification of CE burden is difficult due to lack of data and significant underreporting of both human and animal cases. Moreover, hospital records may not reflect accurately the real prevalence of infection, as CE is often asymptomatic and affects communities with limited access to health facilities (Yang et al. 2006b).

Figures obtained in the twenty-first century (that are likely underestimates) indicate that 1.2 million people are infected, with a global annual loss of an estimated 3.6 million DALYs, a higher figure than that given for dengue or Chagas disease (Budke et al. 2006; Craig et al. 2007a). Prevalence and incidence of human infection vary greatly between areas and reports, reaching peaks of 12% prevalence and annual incidence of 80/100,000 in certain communities of Xinjiang (China), where up to 99% of sheep are infected (Craig et al. 2007a) (WHO/OIE and Echinococcosis 2001). Annual economic losses due to diagnosis and treatment costs in humans have been estimated at over 763 million US$, while global annual livestock-associated losses due to liver condemnation and decreased productions by infected animals were calculated at over 2190 million US$ (Budke 2006).

AE is only observed in the northern hemisphere, in geographical areas where *E. multilocularis* sylvatic life cycle can occur (Fig. 8.4). AE is a rare disease in most endemic regions where the cycle is maintained in the wildlife only (<10/100,000 in regions with 70% of foxes infected) (Piarroux et al. 2013, 2011) due to both the rare encounter of humans with infected fox feces and their natural resistance as an intermediate host (Vuitton and Gottstein 2010; Bresson-Hadni et al. 2007; Vuitton et al. 2003). It is far more frequent where the cycle involves dogs such as in areas of western China, which is now considered the region with the highest number of human AE (Vuitton et al. 2003; Craig 2006), up to 100 times higher than that in endemic areas of Europe. In China, Turkey, and Central Asia, CE and AE coexist in some communities and sometimes in the same patient (Wen et al. 1992; Zhang et al. 2006). In the last two decades, endemic areas have expanded in Europe, with foxes infected with *E. multilocularis* being reported in all countries, except the UK, Spain, and Portugal (Romig 2009). Lithuania is now considered a major endemic area (Bruzinskaite et al. 2007). In Japan and Europe, fox urbanization is posing new issues for the prevention of AE (Deplazes et al. 2004). Although the autochthonous wildlife cycle of *E. multilocularis* is well known in Northern America, including the USA and Canada, human cases of AE were extremely rare all over the twentieth century. An emergence of cases during the last decade of the twenty-first century in several Canadian areas, especially in young and immunocompromised patients, is a subject of concern; infection of intermediate and definitive animal hosts close to urban communities, as is observed in Calgary, Alberta, and a possible importation of European strains of *E. multilocularis*, may explain this increased incidence of AE in Canada (Houston et al. 2021).

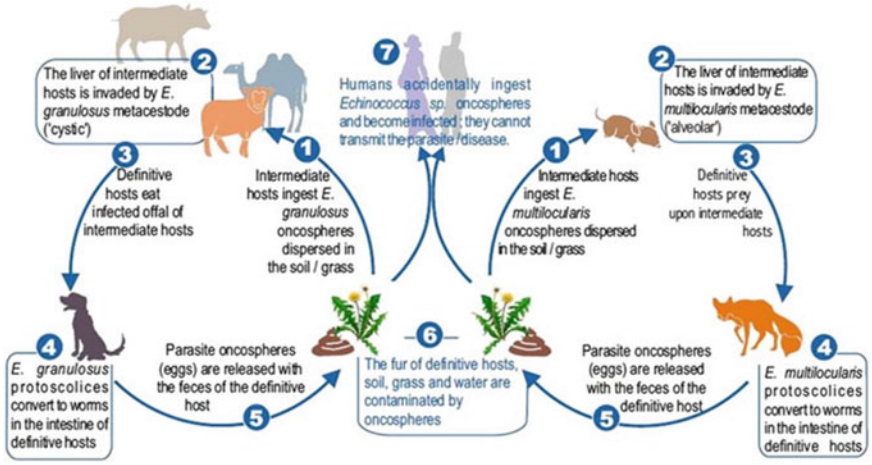

Fig. 8.4 Parasite cycle of *Echinococcus granulosus* (*L*) and of *Echinococcus multilocularis* (*R*). The adult stage, present in the intestine of various species of carnivores, is only killed by praziquantel, which, however, is unable to kill the oncospheres (eggs) released in the feces with the last segment of the worm. The larval stage (metacestode), which may be observed in humans, is partially sensitive to mebendazole and albendazole; protoscoleces of *Echinococcus granulosus* (metacestode-derived fertile stage which transforms into adult worms in the intestine of carnivores but may also disseminate the metacestode in intermediate hosts) may be killed by praziquantel (*Picture designed and kindly provided by Sophie Muraccioli and Lydie Belpois; Communication Unit of the University Hospital, Besanc¸on, France*)

Compared to CE, the burden of human infection is lower, because of its lower prevalence; however, AE is a very severe disease similar to liver cancer and requires complex and expensive treatments, often lifelong. This considerably increases the cost of the disease, evaluated in Europe at an average of 108,762€ per patient (Torgerson et al. 2008). Annual loss due to AE has been estimated at 666,434 DALYs (Torgerson et al. 2010). Due to its prevalently wild cycle, there is no special veterinary or economic impact from the infection of the intermediate hosts. However, symptoms close to those observed in humans are often recognized in a number of domestic animals, such as horses, pigs, boars, chinchillas, or even dogs, which appear to also serve occasionally as intermediate hosts (Vuitton 2003; Scharf et al. 2004; Bottcher et al. 2013; Ueno et al. 2012), and in zoo animals (Rehmann et al. 2005).

8.3.2 Transmission and Risk Factors for Human Infection

Humans get infected by ingesting *Echinococcus* spp. eggs; however the main routes of infection may differ depending on the specific conditions and habits of populations in different endemic areas (Tamarozzi et al. 2020a). The parasite eggs

may be dispersed over >20 km and are extremely resistant in moist and cold environments (16 months in water at 4 °C) and to detergents, while they are sensitive to desiccation and heat (5 min at 60 °C) (WHO/OIE and Echinococcosis 2001). Insects may have a role as mechanical vectors of eggs (Lawson and Gemmell 1990). Taeniid eggs can also remain attached to the dog's coat, and direct contact with an infected dogs may be a source of infection (Matoff and Kolev 1964; Larrieu et al. 2002; Yang et al. 2008; Campos-Bueno et al. 2000). In reality, dog ownership was generally not associated with CE infection in community studies (Possenti et al. 2016). Female sex and not washing hands before eating have been generally associated with CE infection, while other potential risk factors such as livestock ownership and home slaughter, eating raw vegetables, or use of unsafe water source were inconsistently or not associated with infection (Possenti et al. 2016; Tamarozzi et al. 2019; Uchiumi et al. 2021).

Environmental factors such as altitude, climate (low temperatures or high annual precipitations), landscape characteristics and use, and predator-prey relationship (availability and predation level on potential intermediate hosts) play a critical role in *E. multilocularis* infection in foxes (Vuitton et al. 2003; Giraudoux et al. 2013; Romig et al. 2017). AE forms discrete patches of endemicity within which transmission hot spots of much higher prevalence occur. Promotion of permanent pastures, deforestation, or privatization of the land favors *E. multilocularis* infection in small mammals and foxes (Giraudoux et al. 2003; Wang et al. 2004; Pleydell et al. 2004). Involvement of dogs in the life cycle of *E. multilocularis*, as it occurs in rural western China, and fox urbanization are responsible for higher prevalence of human AE (Torgerson et al. 2011; Vuitton 2003; WHO/OIE and Echinococcosis 2001; Robardet et al. 2011; Deplazes et al. 2004; Romig et al. 2017).

8.3.3 Genetic Diversity

E. granulosus strains were first recognized due to phenotypic variations (Romig et al. 2015). Currently, *E. granulosus* s.l. is divided into *E. granulosus* sensu stricto (the former genotypes G1–G3, "sheep strain"), *E. equinus* (G4, "horse strain"), *E. ortleppi* (G5, "cattle strain"), and *E. canadensis* (G6, "camel strain"; G7, "pig strain"; and G8 and G10, "cervid strains"—G9 is now considered a misidentification) (Romig et al. 2015; Thompson 2017; Vuitton et al. 2020). The "lion strain" has been now assigned to a different species, *E. felidis* (McManus 2013). *E. granulosus* species show different host range and geographical distribution.

The capacity of developing fertile cysts in different intermediate hosts varies between and within species. For example, horses and cattle can be infected both with *E. granulosus* sensu stricto and *E. canadensis*, but only infection with the latter results in the development of fertile cysts (Varcasia et al. 2008; Thompson et al. 1984).

The vast majority of human isolates are of the G1 and G3 strains (Deplazes et al. 2017); however, all other strains with the exception of *E. felidis*, with different relative percentages, have been reported to infect humans, although often with sterile cysts (McManus 2013; Guarnera et al. 2004).

Until the 1950s there was no clear differentiation between *E. granulosus* and *E. multilocularis* (Vuitton et al. 2011). Sister species relationships were confirmed by using genetic analysis between *E. multilocularis* and *E. shiquicus*, a new species found in animal hosts in western China but never identified in humans (Xiao et al. 2005; Nakao et al. 2007). It has long been considered that there was no diversity within the species *E. multilocularis*. Identification of the multilocus microsatellite EmsB has allowed recognition of subtle differences among isolates of *E. multilocularis* and has been used as a molecular tracker of the transmission of *E. multilocularis* among different hosts and geographical areas (Knapp et al. 2010). Phylogenetic and geographical mapping of *E. multilocularis* isolates is currently the focus of intense research work from animal and human samples (Knapp et al. 2020; Umhang et al. 2021); and a publicly available database is dedicated to worldwide cooperation in this domain (Knapp et al. 2017, 2020).

8.4 Host Response to the Parasite

Three essential mechanisms appear to be at the basis of the often long-lasting and asymptomatic cohabitation of *Echinococcus* metacestode and the intermediate host: (1) immune evasion/modulation, (2) (at least partial) protective immunity to reinfection, and (3) partial limitation of parasite growth through fibrosis which, in *E. multilocularis* infection, is also at the origin of clinical complications (Lightowlers 2010; Zhang et al. 2012).

8.4.1 Immune Response to E. granulosus *sensu lato*

Most of research work has been performed on the species *E. granulosus* sensu stricto; it likely also applies to other species of the *E. granulosus* s.l. cluster. The immune response to the parasite is conceptually divided into the pre-encystment and post-encystment phase, differentiated by the formation of the LL around the developing PSC. This occurs approximately 20 days postinfection in mice and coincides with loss of susceptibility to the host immune attack (Gottstein et al. 2017).

Both PSC and oncospheres activate a potent immune response (Breijo et al. 2008; Mourglia-Ettlin et al. 2011b; Ferreira et al. 2000a, b; Irigoin et al. 1996) that eliminates most of the infective parasites within a few days and induces high levels of protection to a subsequent challenge infection (Dempster et al. 1992; Heath and Lawrence 1996) Less than 10% of inoculated PSC and about 40% of oncospheres ingested by sheep develop into cysts, with 90% of the inoculum being killed by

3 weeks postinfection (Ferragut and Nieto 1996; Breijo et al. 1998, 2008; Zhang et al. 2003). PSC become refractory to complement-mediated killing once they start to vesiculate (Irigoin et al. 1996; Kassis and Tanner 1976).

Protection from infective stages is antibody and complement dependent (Dempster et al. 1992; Dempster and Harrison 1995; Heath and Lawrence 1996; Li et al. 2011b), is enhanced in the presence of neutrophils (Rogan et al. 1992), and requires the presence of lymphocytes (Dixon 1997). Effector mechanisms include neutrophils and macrophage activation, nitric oxide (NO), eosinophil cationic protein, complement, and antibodies (Virginio et al. 2007; Amri et al. 2007; Ferreira et al. 2000a; Heath et al. 1994; Jenkins et al. 1990; Ramos et al. 2006; Ferragut and Nieto 1996; Severi et al. 1997; Zhang et al. 2003; Riley et al. 1986; Dempster et al. 1992). Around live intact cysts, there is a remarkably scarce inflammatory reaction, as opposed to regressive cysts (Breijo et al. 2008; Coltorti and Varela-Diaz 1974; Marco et al. 2006; Sakamoto and Cabrera 2003). Although it is not clear whether the inflammatory reaction is the cause of cyst inactivation or the consequence of immune activation due to HCF spillage from spontaneously damaged cysts, some evidence exists that cysts can be damaged by an early pericystic inflammatory reaction (Fotiadis et al. 1999).

Early after infection with PSC, there is a mixed Th1 (IFNγ)/Th2 (IL-4, IL-5) response and high levels of IL-10 (Dematteis et al. 1999; Rogan 1998). The production of IL-10 and IL-4 appears actively induced by the parasite to favor its establishment by downregulating both effector arms of immunity (Dematteis et al. 1999; Haralabidis et al. 1995). High levels of circulating pro- and anti-inflammatory cytokines and higher in vitro production of a predominant Th1 response in patients with inactive infection and/or who responded to therapy are also generally reported (Bayraktar et al. 2005; Chandrasekhar and Parija 2009; Mezioug and Touil-Boukoffa 2009; Refik et al. 2005; Rigano et al. 1999b; Shan et al. 2011; Touil-Boukoffa et al. 1997, 1998). However, results and association with cyst stages are often not clear-cut (Hernandez-Pomi et al. 1997; Rigano et al. 1995b, 1999a, 2001, 2004; Piccoli et al. 2012; Tamarozzi et al. 2010; Torcal et al. 1996).

Regulatory mechanisms such as Tregs are likely to control both Th1 and Th2 (IL-5-mediated) parasite-killing effector mechanisms (Mourglia-Ettlin et al. 2011b). Increased Th1 responses (IFNγ or IL-12) impair PSC survival and cyst development, while IL-4 and IL-10 induce heavier cyst loads and impair PSC killing (Al-Qaoud and Abdel-Hafez 2008; Amri et al. 2007, 2009). Other mechanisms of immune evasion include polyclonal activation of B cells (Cox et al. 1989) and induction of IL-10, IgG4, and high levels of nonspecific antibodies and lack of antibody avidity maturation, as shown experimentally by injection of carbohydrate fractions of PSC (Miguez et al. 1996; Mourglia-Ettlin et al. 2011a; Ferragut and Nieto 1996; Severi et al. 1997; Baz et al. 1999, 2006, 2008; Cardozo et al. 2002; Dematteis et al. 2001). Moreover, PSC present Fc-binding activity (Baz et al. 1998) and express immune regulatory antigen B and EgTeg (Ortona et al. 2005; Sanchez et al. 1991; Monteiro et al. 2010). T-regulatory and myeloid-derived suppressor cells have been found to be systemically expanded in mice with *E. granulosus* infection and in CE patients (Pan et al. 2013; Tuxun et al. 2012). Taken together, these results support the

presence of an immunomodulatory environment downregulating both Th1 and Th2 responses and favoring parasite survival.

The most immune-resistant parasite form is the cyst, which may persist for decades in the immunocompetent intermediate host. The LL plays a pivotal role in the survival of cysts (Diaz et al. 2011a). This is not only due to a barrier effect, as many host molecules are found in the HCF, and parasite antigens can leave the cyst (Monteiro et al. 2010). One of the most important mechanisms is the complement-inert state of the LL, due to the sequestration of host factor H (Diaz et al. 1999). Moreover, the LL could inhibit NO production by IFNγ-activated macrophages (Steers et al. 2001). Host immunoglobulins are present in the cyst wall and in the HCF (1000–10,000 times lower levels) (Monteiro et al. 2010; Paredes et al. 2011; Coltorti and Varela-Diaz 1974). Of these, however, only a fraction are high-affinity parasite-specific, and, of note, these are of the IgG4 isotype (Coltorti and Varela-Diaz 1974; Paredes et al. 2011; Taherkhani et al. 2007). High levels of specific antibodies, predominantly IgG1 and IgG4 (Aceti et al. 1993; Wen and Craig 1994; Shambesh et al. 1997; Daeki et al. 2000), are generally present in the serum of CE patients; however, it is unclear whether they are harmful to the cyst. IgG4 and IgE have been associated with the presence of active or relapsing/unresponsive infection after treatment (Daeki et al. 2000; Hernandez-Pomi et al. 1997; Rigano et al. 1995a, b, 2001, 2002).

HCF is a complex mixture of parasite- and host-derived molecules with immunogenic properties (Monteiro et al. 2010; Siracusano et al. 1988; Hernandez-Pomi et al. 1997; Rogan et al. 1993; Hernandez and Nieto 1994; Carmena et al. 2006). The two most abundant and studied parasite-derived molecules are antigen B (AgB) and antigen 5 (Ag5), variably expressed in all parasite stages (Monteiro et al. 2010; Siracusano et al. 2008; Carmena et al. 2006). Their role in the parasite biology is unknown, but several immunomodulatory properties have been ascribed to these molecules, especially to AgB, such as inhibition of phagocyte functions, skewing immune response to the Th2 arm, and cell apoptosis (Rigano et al. 2001, 2007; Shepherd et al. 1991; Virginio et al. 2007; Kanan and Chain 2006; Spotin et al. 2012; Ioppolo et al. 1996; Daeki et al. 2000; Li et al. 2012; Mezioug and Touil-Boukoffa 2009; Siracusano et al. 1988). Other potentially immunomodulatory HCF molecules include EgTeg, EgTPx, paramyosin, and tetraspanin (Monteiro et al. 2010; Ortona et al. 2005).

8.4.2 Immune Response to E. multilocularis

What is observed in CE, an abundant host inflammatory granulomatous infiltrate between the vesicles and the liver is present in AE. Cellular immunity is pivotal for *E. multilocularis* control. In mice strains with genetic deficiencies or in case of drug-mediated impairment of cell immunity in humans or mice, susceptibility to AE increases (Baron and Tanner 1976; Liance et al. 1990, 1992), while resistance is increased by stimulation of cellular immune response (Rau and Tanner 1975;

Sarciron et al. 1992). Immunogenetic background also plays a significant role both in the susceptibility to infection and in metacestode growth in the infected experimental or human intermediate hosts (Vuitton and Gottstein 2010).

Like *E. granulosus*, *E. multilocularis* can modulate the immune response at the very early stage of antigen presentation. *E. multilocularis* modifies macrophage and DC function and interferes with antigen presentation and T-cell proliferation (Dixon 1997; Mejri and Gottstein 2009; Jenne et al. 2001). An initial Th1 response changes gradually to a mixed Th1/Th2 during the chronic phase of AE (Emery et al. 1996). Cytokines such as IL-4, IL-5, IL-13, and IFNγ are secreted in response to parasite antigens (Emery et al. 1996; Sturm et al. 1995; Godot et al. 1997), but the hallmark of *E. multilocularis* infection is the secretion of regulatory cytokines, such as IL-10 and TGF-β, which are also observed in patients with AE, especially in those with advanced and severe disease (Zhang et al. 2006). Total and specific IgE and IgG4 are elevated in patients with aggressive disease (Dreweck et al. 1997). Disappearance of IgE and decline of IgG4-specific antibodies are significantly associated with regression or surgical removal of the lesions (Gottstein et al. 1991; Wen et al. 1995).

Initiation of the Th2 (IL-4) profile alongside a Th1 (IL-12, TNFα, IFNγ) profile takes place locally, in the liver, very early during infection (Wang et al. 2014), and may be crucial for further Th2 shifting and inactivation of Th1 protective mechanisms. As in CE, enhancing Th1 immune responses, especially by acting on the innate immunity using IL-12, IFNα, and TNFα, increases resistance to *E. multilocularis* both in experimental mice and in humans (Liance et al. 1998; Jenne et al. 1998; Emery et al. 1998; Godot et al. 2003; Harraga et al. 1999).

Although the Treg function of the CD8 T-cell periparasitic infiltrate (Vuitton et al. 1989) has never been demonstrated, their cytotoxic role, as well as that of NK cells, seems to be permanently inhibited, possibly by the expression of TGF-β in lesions (Zhang et al. 2008;(Bellanger et al. 2017). immunomodulatory mechanisms by Tregs and macrophages operate in *E. multilocularis* infection of both human and mouse (Hubner et al. 2006; Vuitton and Gottstein 2010). Finally, fibrosis formation, also induced by TGF-β, appears to be the major mechanism of host protection but also the main reason for clinical complications of AE in humans (Ricard et al. 1996; Wang et al. 2013).

Recent interest has been paid to the phenomenon of T-cell exhaustion, which actually occurs in experimental models of AE after chronic infection and is related to mechanisms of checkpoint inhibition. In vitro interactions have been shown between *E. multilocularis* vesicular fluid and different immune checkpoints, such as PD-1/PD-L1, CTLA-4, LAG-3, and TIM-3 (Bellanger et al. 2017). The PD-1/PD-L1 pathway, which inhibits lymphocyte proliferation in the development of tumors, is over-expressed at the chronic stage of experimental AE (Wang et al. 2018). In both models of oral infection (primary AE) and secondary AE, the parasite load was significantly decreased in response to anti-PD-L1 antibody treatment. In secondary AE, anti-PD-L1 administration was associated with increased Th1 response and decreased Treg response, while in primary AE anti-PDL1 administration was associated with fewer lesions in the liver and decreased Treg/Th2 responses (Wang et al. 2018). Further studies in mice treated or not concurrently with albendazole have

confirmed that the parasite load was significantly reduced in response to PD-L1 blockade; this blockade contributed to T-cell activity by increasing CD4+/CD8+ effector T cells and decreasing Tregs and had the capacity to restore dendritic, Kupffer, NKT, and NK cell functions (Jebbawi et al. 2021). This highly suggests that the blockade of the PD-1/PD-L1 pathway triggers the host immune responses in favor of an immune-mediated control of *E. multilocularis* proliferation. Another checkpoint seems also very promising on the way of AE immune therapy, the T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) and its CD155 ligand. TIGIT expression is significantly increased and positively correlated with lesion activity in experimental AE and also in AE patients (Zhang et al. 2020). High TIGIT expression in both liver-infiltrating and blood T cells is associated with their functional exhaustion; its ligand CD155 is also highly expressed by hepatocytes surrounding the infiltrating lymphocytes. In co-culture experiments using human blood T cells and a hepatic cell line, CD155 induces functional impairment of TIGIT+ T cells; in vitro blockade with TIGIT antibody restores the function of T cells from AE patients. In vivo blocking TIGIT prevents T-cell exhaustion and inhibits disease progression in *E. multilocularis*-infected mice (Zhang et al. 2020). Complementary studies have shown that TIGIT was also operative in the exhaustion and functional impairment of NK cells in the AE model (Chuanshan et al. 2021). NK cells from the blood and liver tissue of AE patients close to the lesions express higher level of TIGIT and are functionally exhausted, with lower expression of granzyme B, perforin, IFN-γ, and TNF-α. Addition of anti-TIGIT mAb into AE patients' PBMC culture significantly enhanced the synthesis of IFN-γ and TNF-α by NK cells, indicating the reversion of NK cell exhaustion by TIGIT blockade. In the mouse model of *E. multilocularis* infection, the liver and splenic TIGIT+ NK cells progressively increase and become functionally deficient; NK cells from mice with persistent chronic infection express higher level of TIGIT compared to self-healing mice. TIGIT deficiency or blockade in vivo inhibits liver metacestode growth, reduces liver injury, and increases the level of IFN-γ produced by liver NK cells. Regulatory $CD56^{bright}$ and CD49a + NK cells with higher TIGIT expression are more numerous in the liver of AE patients and mice infected with *E. multilocularis*, respectively; they also co-express higher surface PD-L1 and secrete more IL-10, two strong inducers to mediate functional exhaustion of NK cells (Chuanshan et al. 2021).

As for *E. granulosus* infection, the LL has an important role in immune modulation (Vuitton and Gottstein 2010). immunomodulatory molecules are polysaccharide-containing antigens such as Em2 (G11), antigen C, EmP2, and Em492, a neutral glycosphingolipid, and novel mucin-type glycoforms. Among protein antigens, EmAP, which induces antibodies associated with disease severity and resistance to treatment in AE patients, was also shown to induce only Th2-type cytokines. Several recombinants of *E. multilocularis* proteins (such as antigen II/3 and its subfragments II/3–10 and Em18 and EM10) mainly studied for AE immunodiagnosis (Ito and Craig 2003) have all a potential biological role.

8.5 Clinical Manifestations of CE

In humans, CE cysts develop predominantly in the liver (60–70%) and lungs (20–30%), but all organs and tissues can be affected. In up to 10% of cases, CE cysts may affect the kidneys, spleen, bones, and central nervous system, but rarer localizations, such as the thyroid, pancreas, heart, muscles, breast, orbit, adrenal glands, etc., have been described; about 40% of cases have multiple locations (Pedrosa et al. 2000; Eckert 2001; Polat et al. 2003; Abdel Razek et al 2011; Nourbakhsh et al 2010a). Prevalence increases with age and was generally believed to be higher in females, especially in reports from Central Asia and western China (Craig et al. 2007b; Eckert 2001). However, a recent large survey in Eastern Africa has shown that no clear statistical differences exist between the two sexes (Solomon et al. 2017b). The observed sex ratio in a given area and/or period of time may be related to the local environment and risk factors, such as behaviors of communities, and/or to different contacts of males and females with dogs or be due to a bias related to different access rate of women to healthcare facilities.

CE symptoms are extremely variable and nonspecific (Kern et al. 2017). On average 60–75% of patients with hepatic CE are asymptomatic and can remain so for up to 10–12 years or even lifelong (Solomon et al. 2017a). When present, symptoms depend on cyst's size, number, organ infected, localization within the organ, and complications.

Symptoms may be local or systemic and are due to mass effect and compression on neighboring structures or loss of integrity of the cyst wall resulting in allergic reactions, dissemination of PSC, communication with hollow structures, and bacterial superinfection. Hepatic cysts more frequently cause abdominal discomfort or right upper quadrant pain, poor appetite, and jaundice due to compression of the common bile duct.

The most common complication is rupture, which can be subclinical or cause jaundice in case of communication with the biliary tree or anaphylactic reaction or may result in superinfection or dissemination (secondary CE). Cough, hemoptysis, and chest pain are the most common clinical symptoms of lung cysts. Cyst rupture into bronchi results in the so-called hydatid vomica, i.e., expectoration of cyst fluid and parasitic layers. Bacterial infection of the cyst is the most serious complication commonly seen after rupture. A comprehensive review of clinical manifestations and complications of hepatic CE can be found in review papers (Wen et al. 2019; Rinaldi et al. 2014; Kern et al. 2017), while the reader is referred to the following selected reviews on the clinical aspects of CE in the most common extrahepatic locations: lungs (Santivanez and Garcia 2010), bone (Neumayr et al. 2013a, b; Monge-Maillo et al. 2017), and central nervous system (Nourbakhsh et al. 2010b).

Mortality and fatality rates are difficult to estimate and vary greatly depending on cyst location, severity of disease, and health facilities. On average the reported figures show a mortality rate of 0.2/100,000 inhabitants and 2.2% fatality rate (WHO/OIE and Echinococcosis 2001; Bristow et al. 2012; Herrador et al. 2016).

Finally, available data suggest that immune suppression or coinfections with HIV or tuberculosis do not affect the clinical course of CE (Sobrino et al. 1993; Wahlers et al. 2011, 2013).

8.6 Clinical Manifestations of AE

When AE is found at an advanced stage, it is often misdiagnosed as a liver neoplasm. Jaundice is the most frequent presenting symptom, either progressive related to hilum involvement, associated with pruritus, or intermittent with pain and fever due to cholangitis/bacterial infection (Bresson-Hadni et al. 2000; Ayifuhan et al. 2012). Hypo- and asymptomatic cases are far more frequent (up to 70%). Right upper quadrant pain is the presenting symptom in ~30% of cases. Presence of massive hepatomegaly but good clinical status should raise the suspicion of AE in endemic areas. Erratic clinical signs and symptoms due to extrahepatic location of AE may also be observed at presentation (Bresson-Hadni et al. 2007; Ehrhardt et al. 2007). Lack of symptoms is more frequent in immune-suppressed patients. In these patients, the course of AE seems faster; clinical symptoms, if any, may mimic liver abscess; and both imaging and serological diagnoses may be more difficult to interpret (Gruener et al. 2008; (Kern et al. 2011, Chauchet et al. 2013).

The most frequent complications of AE are bacterial or fungal infection of the bile ducts and/or of the pseudocystic central necrotic area of lesions, with abscesses, cholangitis, and septic shock (Bresson-Hadni et al. 2007; Kern 2010; Kern et al. 2017). Locoregional extension or a hematogenous spread of parasitic tissue with distant metastases may cause a variety of symptoms ranging from dyspnea and bile-tinged sputum to seizures and stroke as well as skin nodules or bone pain or fractures. Anaphylactic reactions are extremely rare as presenting symptoms. Bleeding from esophagogastric varices due to portal hypertension, secondary to biliary cirrhosis or to chronic parasitic Budd-Chiari syndrome or portal thrombosis, is possible but has become rare (Bresson-Hadni et al. 1994, 2007).

8.7 Diagnosis

The diagnosis of CE and AE relies on imaging techniques complemented by serology, which is positive in more than 90% of immunocompetent AE patients but is much less reliable in CE. Direct microscopic analysis of parasitic material can confirm the etiology of the CE cyst, while PCR on tissue samples is mostly used in AE doubtful cases.

Monoclonal antibodies for the detection of parasite circulating antigens, cytokine release assays, and techniques for the identification of parasite nucleic acids in the blood have also been explored; however, they are at present not sensitive enough to

be used in clinical diagnosis (Liu et al. 1993; Siles-Lucas and Gottstein 2001; Devi and Parija 2003; Sunita et al. 2011; Kanwar and Vinayak 1992; Petrone et al. 2015).

8.7.1 Imaging

Imaging techniques are the mainstay of the diagnosis of CE and AE (Brunetti et al. 2010), which are often suspected after the accidental discovery of lesions during imaging exams carried out for other reasons.

Ultrasonography (US) is the cornerstone of CE screening, diagnosis, staging, and follow-up of cysts localized in organs and tissues explorable by US, especially the abdomen, while magnetic resonance imaging (MRI) and computed tomography (CT) scan, performing less well than US in the identification of CE cysts features, are used when US examination is not possible or for pre-surgical assessment (Macpherson et al. 2003b; Brunetti et al. 2010; Del Carpio et al. 2012; Stojkovic et al. 2012; Tamarozzi et al. 2018). US is also the current screening method of choice for diagnosis and regular follow-up imaging in AE (Bartholomot et al. 2002; Macpherson et al. 2003b; Bresson-Hadni et al. 2006a, b; Yang et al. 2006a; Kantarci et al. 2012a, b), while CT and MRI techniques are needed for an accurate staging of the disease (Brunetti et al. 2010; Liu et al. 2014).

The WHO-IWGE classification of CE cyst stages is based on US CE-specific imaging features (Fig. 8.1). US can visualize cysts of <5 mm with a sensitivity of 93–100% and specificity of 88–96% for hepatic cysts (Macpherson et al. 2003b; Del Carpio et al. 2000). CE cysts are generally spheric and well delimited. The pathognomonic imaging features are the following: (1) double wall defined by the parasitic layers and the adventitial layer; (2) "waterlily sign" of CE3a cysts, that is, the fluctuation of the detached parasitic layers in the cyst fluid content; (3) multivesicular cysts (CE2, CE3b) with honeycomb appearance where the "septa" are formed by the adjacent walls of daughter cysts (CE2) or where daughter cysts form in a pseudosolid cyst content with visible detached, hypoechoic parasitic layers (CE3b); and (4) the "ball of wool" sign of CE4 cysts and CE5 cysts (the latter with complete or almost complete eggshell calcification), indicative of degenerating parasitic layers folded in a pseudosolid cyst content (Macpherson et al. 2003b).

In 2/3 of cases, instead, the AE lesion is characterized by irregular limits and heterogenous content with a "geographical map" appearance of hyperechoic and hypoechoic areas. The lesion often contains scattered calcifications. Less typical US aspects include the following: (1) small hemangioma-like nodules (more frequent in asymptomatic immune-suppressed patients); (2) pseudocystic lesions with surrounding hyperechogenic ring and irregular lining, which correspond to huge AE lesions with massive necrosis; and (3) small calcified lesions which correspond to either an abortive or a very early infection (Bresson-Hadni et al. 2006a, b). US color Doppler is useful for vascular involvement evaluation. However, presence of calcifications may prevent a proper evaluation of the lesion and its real extent. Contrast-enhanced ultrasonography (CEUS) appears quite promising as it can show the periparasitic

microvascularized content of the lesions (Tao et al. 2011). An analytical classification of US images in AE has been proposed to help radiologists and clinicians detect all details of US imaging in this complex disease; however, the WHO-IWGE US classification of CE, it has no direct clinical or therapeutic counterpart (Kratzer et al. 2015). With this classification, more than 95% of AE lesions may be assigned to the following images: "hailstorm" (54.1%), "pseudocystic" (13.5%), "ossification" (13.0%), "hemangioma-like" (8.1%), and "metastasis-like" (6.5%).

CT and MRI are necessary before surgery to determine the anatomic relations of the lesion, to diagnose extra-abdominal localizations, and to demonstrate complications. MRI reproduces characteristic features of CE better than CT, especially using T2-weighted images (WI), while CT performs better in the visualization of calcifications (Stojkovic et al. 2012) (Fig. 8.5). On CT and MRI T1WI, CE inner parasitic layers are hyperdense/intense, with hypodense/intense intracystic fluid, and hypointense outer ring representing the fibrous capsule (adventitial layer), which is better seen on MRI. On T2WI, cyst content is hyperintense and the hypointense rim sign is more evident. Cyst structures do not enhance after injection of contrast agent (Pedrosa et al. 2000; Polat et al. 2003).

In AE, the typical CT aspect is a tumor-like lesion with irregular lining and heterogenous content: scattered, hyperdense calcifications and hypodense areas corresponding to necrosis and/or active parasitic tissue (Didier et al. 1985; Reuter et al. 2001). No significant contrast enhancement within the lesion and slight enhancement of the periphery are characteristic. Similar to that developed for US images, a classification of CT images of AE lesions has also been developed (Graeter et al. 2016); the classification describes both a "primary morphology" and a "pattern of calcification." The type IV of primary morphology shows an exclusive association with a central calcification; this association, seen in small lesions, seems to characterize metabolically active lesions at their very early stage of development (Brumpt et al. 2019) (Graeter et al. 2020a, b).

Intrahepatic bile duct dilation in the contralateral liver lobe indicates hilum infiltration. Hypertrophy of the contralateral lobe is also usual. MRI may facilitate the diagnosis in uncertain cases with noncalcified lesions and is the best technique to study the extension to adjacent structures (Bresson-Hadni et al. 2006a, b; Reuter et al. 2001; Kodama et al. 2003). It shows the pathognomonic aspect of multiple small vesicles, as "honeycomb" or "bunch of grapes" images, best observed on T2WI. Cholangio-MRI can detect more precisely communication of the CE cyst with the biliary tree (Hosch et al. 2008a, b) and has now replaced percutaneous cholangiography as an important part of the preoperative evaluation of AE. Perendoscopic cholangiography may be the first step of biliary drainage in patients with cholestasis or cholangitis) (Bresson-Hadni et al. 2006a, b; Ambregna et al. 2017).

Evaluation of CT images has recently been performed on 200 AE cases from 4 reference centers (2 in Europe and 2 in western China; 50 consecutive patients for each center). Although Chinese patients were younger than European patients (37 years old on average, versus 64 years old), they had significantly larger lesions, and the morphological appearance of the lesions on CT differed significantly

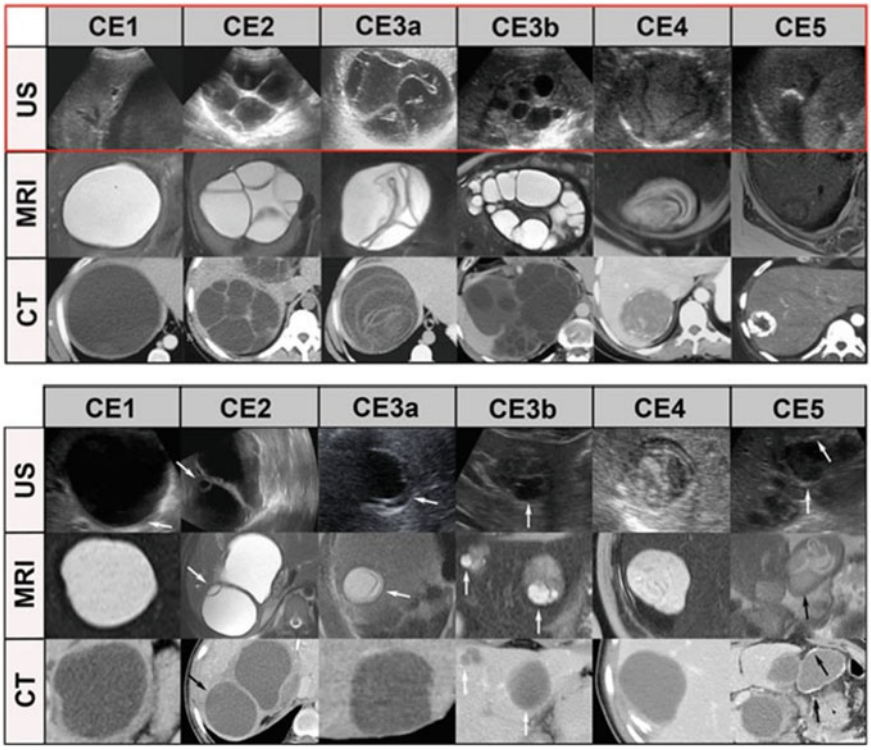

Fig. 8.5 "Best-case" (**a**) and "worst-case" (**b**) comparison of US, MRI, and CT for the diagnosis of hepatic CE. The *double line sign*, typical for CE1, is often seen in US (CE1/US) but less reliably in MRI and CT. Daughter cysts and detached parasitic layers (*water lily sign*) are often missed by CT but are clearly visible on US and MRI (see CE2 and CE3a, *arrows*). Daughter cysts inside a solid cyst matrix are often not recognized by CT (CE3b, *arrows*). The CE4-specific canalicular structure is often not visible on CT images. These cysts may be misinterpreted as CE1 cysts, i.e., staged "active" instead of "inactive." The identification of calcifications is best achieved by CT imaging, while MRI does not differentiate well between thick hyaline walls and calcifications. US detects calcifications only when a dorsal echo shadow is produced (see CE5). MRI, HASTE sequence; CT, post-contrast-enhanced images (From Stojkovic M et al. PLOS NTD 2012, with permission)

between the two groups as did the number of lesions (higher in the Chinese AE patients) (Graeter et al. 2020a, b). Distant extrahepatic AE manifestations were significantly more frequent in China than in Europe; there was a significant relationship between the presence of distant extrahepatic lesions and AE liver lesion size, and they were only seen in types I–III with a maximum of 22% for type III (Graeter et al. 2020a, b). Vascular/biliary structures were involved by the liver lesions significantly more frequently in China than in Europe, and vascular/biliary involvement depended on lesion size and of the primary morphology of the lesions, ranging from 6% of type IV liver lesions to 100% of type III lesions (Graeter et al. 2020a, b). Similar results were obtained by comparing MRI images (Graeter et al. 2021).

Chest X-ray is generally the step in diagnosis of pulmonary CE and may show the "waterlily sign" in case of detached membranes or a "meniscus" between the cyst wall and the parasitic layers in case of bronchopericystic fistula (Santivanez and Garcia 2010). Calcification of pulmonary CE cysts is rare.

CE cysts should be differentiated from simple and dysontogenetic cysts, neoplasms (including in the bone where CE does not develop and is not a well-delimited cyst but as an infiltrative lesion), metastases, postsurgical cavities, AE pseudocysts, and other parasitic lesions in the brain, hematomas, and abscesses. Differential diagnosis may be difficult, as, for example, peripheral eggshell-like calcification characteristic of CE5 cysts can be observed also in AE, and, albeit rarely, in neoplasms, and hematomas. The most difficult differential diagnostic problems for AE are intrahepatic cholangiocarcinoma and metastases, but also CE and atypical hemangiomas (Stojkovic et al. 2015).

Positron emission tomography (PET) using [18F] fluoro-deoxy-glucose (FDG) is useful to assess the viability of the lesions, at diagnosis and during follow-up of AE. FDG is not actually uptaken by the metacestode but mostly by the periparasitic inflammatory infiltrate. FDG uptake is best revealed when "delayed" images are acquired, 3 h post-FDG injection (Caoduro et al. 2013). Comparison of FDG uptake by the periphery of the lesions, as assessed by using FDG-PET and the MRI types as defined by Kodama et al. (2003), has revealed that the presence of microcysts on MRI was associated with the metabolic activity of the lesions (Azizi et al. 2015). In addition, further studies of the calcification patterns in the lesions were able to distinguish between microcalcifications ("powdery" or "feathery" in the EMUC), present in 98% of metabolically active lesions as detected by FDG-PET, and dense, macrocalcifications, associated with lesion degeneration (Brumpt et al. 2019). Such findings suggest that imaging technologies more easily available to all medical facilities than PET may provide surrogate markers of metacestode viability useful for therapeutic decision and follow-up of patients.

8.7.2 Serology

Serology for CE and AE must not be applied in the absence of a lesion with compatible features visualized on imaging; it is also important to highlight that positive serology in the absence of suggestive lesions on imaging does not justify treatment (Siles-Lucas and Gottstein 2001). Detection of circulating specific antibodies complements image-based diagnosis of CE and of AE when imaging alone is not conclusive. Many techniques have been used for serodiagnosis, based most commonly on HCF. For CE, purified or recombinant antigens are now in use and included in commercially available assays (Siles-Lucas et al. 2017; Tamarozzi et al. 2016, 2021a, c; Bartholomot et al. 2002). For AE, a commercially available ELISA test based on Em2 antigen and rII/3–10 is in routine use ($Em2^{plus}$; Bordier, Crissier, Switzerland). Several rapid tests have been used in laboratory and field settings, but they should always be used to complement imaging and are not recommended alone

for mass screening (Manciulli et al. 2021; Bartholomot et al. 2002; Tamarozzi et al. 2016). Seroassays for CE are not standardized, and reported diagnostic performances are extremely variable and depend on many factors, including the assay itself, the antigen used, and cyst characteristics including cyst stage, number, size, organ involved, and loss of integrity (spontaneous or induced by treatment). On the contrary, serology is positive in an average of 90% of immunocompetent AE patients.

In clinical practice, two paired tests are performed, with immunoblotting (IB) that can be used either as stand-alone test (if properly validated) or, more commonly, as a confirmative test in case of discordance of the first two tests (Tamarozzi et al. 2021b; Vola et al. 2019). IB has also been used to discriminate *E. granulosus* from *E. multilocularis* using band pattern interpretation; however this is possible only in a fraction of cases and is not always reliable; therefore species differentiation should not be done on the sole basis of serology (Liance et al. 2000; Tamarozzi et al. 2021b). Seropositivity in the absence of detectable lesions may be only transient and does not predict the development of an active lesion or may be a false-positive result (Hernandez et al. 2005; Grenouillet et al. 2011). It is particularly frequent in highly endemic areas where several cases of infection by *Echinococcus* spp. are likely to occur but are not associated with the successful development of a metacestode or disease because of spontaneous elimination of the metacestode at a very early stage of infection (Bartholomot et al. 2002) or abortion of lesions at a later stage (Bresson-Hadni et al. 2000).

Serological tests have important limitations, which make them ancillary to imaging techniques in CE diagnosis and of limited value in patient follow-up.

1. Test sensitivity shows wide variability between studies, depending on many factors (Carmena et al. 2006; Hernandez-Gonzalez et al. 2012; Tamarozzi et al. 2021b; Lissandrin et al. 2016). Up to 20% of patients with single hepatic and up to 50% of those with lung CE cysts may be seronegative, while patients with cysts in other localizations are often seronegative (Eckert 2001; Barbieri et al. 1998). In the case of hepatic cysts, patients with CE1 and CE4–CE5 cysts are often seronegative (30–58% and 50–87%, respectively), while rates of negativity are lower in the presence of CE2 and CE3 cysts (5–20%) (Hernandez-Gonzalez et al. 2012; Li et al. 2010, 2011b; Yang et al. 2007; Ortona et al. 2000; Lissandrin et al. 2016; Tamarozzi et al. 2021a, b, c). Presence of multiple cysts, complications, and therapy are associated with positive serological results (Hernandez-Gonzalez et al. 2012; Li et al. 2011b; Ben Nouir et al. 2008; Santivanez et al. 2012).
2. Cross-reactivity occurs with other helminthiases (especially caused by cestodes) or more rarely with nonparasitic diseases (Poretti et al. 1999; Zarzosa et al. 1999; Gonzalez-Sapienza et al. 2000; Hernandez-Gonzalez et al. 2008, 2012; de la Rue et al. 2010; Liance et al. 2000; Schweiger et al. 2012; Lin et al. 2013). Cross-reactivity with *Taenia solium* cysticercosis occurs even with very specific assays such as immunoblot (Moro et al. 1999). In any case, applying seroassays only in the case of the presence of a lesion suspect of CE increases the pretest and

therefore the posttest probability of a positive CE serology (Tamarozzi et al. 2021b).

The high rate of cross-reactivity (50–100%) between CE and AE is a problem where the two infections are co-endemic (de la Rue et al. 2010; Hernandez-Gonzalez et al. 2008; Li et al. 2010; Poretti et al. 1999; Schweiger et al. 2012). The different band pattern in HCF-based IB may discriminate between the two species in about 75% of cases (Liance et al. 2000); however, more specific tests for AE should be applied in case of high suspicion such as serology based on Em2–Em18 antigens (15% cross-reactions with CE) and microscopic/PCR analysis of parasitic material (Brunetti et al. 2010; Wang et al. 2013).

Cross-reactivity of highly sensitive tests may also be used as a diagnostic tool in particular situations, such as recurrence of AE lesions after liver transplantation (Koch et al. 2003) and in AE patients with immune suppression (Chauchet et al. 2013).

3. At present, no reliable marker of infection activity is available (Gottstein et al. 2014). In CE, specific antibodies may be detectable for over 10 years even after radical surgery (Hernandez-Gonzalez et al. 2008). Monitoring of titers over time might indicate the outcome of therapy, with decreasing antibody levels suggesting probable cure/inactivation and persistent high titers suggesting the presence of an active infection (Ben Nouir et al. 2008; Hernandez-Gonzalez et al. 2008; Li et al. 2011c; Rigano et al. 2002; Zarzosa et al. 1999). Antibody titers may increase upon relapse, but this does not always occur (Lawn et al. 2004; Zarzosa et al. 1999; Gollackner et al. 2000; Piccoli et al. 2014). In any case, imaging and not serology remains the appropriate method for the follow-up of CE cysts.
4. In AE, antibodies against Em18 have been shown to correlate best with activity of the disease (Tappe et al. 2009, 2010; Sako et al. 2011) and are recommended for the follow-up of the disease when it is treated with anti-parasitic drugs only, because morphological changes on imaging may be very slow; association of decreased Em18 antibodies and negative FDG-PET images is currently considered to be the best marker of response to treatment (Gottstein et al. 2014, 2017; Wen et al. 2019). An ELISA based on the recombinant Em18 antigen is commercially available (*E. multilocularis* recEm-18, Bordier, Crissier, Switzerland).

8.8 Treatment

Treatment approaches of CE and AE are very different and will be treated here separately. However, in both cases, the therapeutic management still relies on a moderate strength of recommendation (B) and quality of evidence III (i.e., "from opinion of respected authorities, based on clinical experience, descriptive studies, or reports of committees") (Brunetti et al. 2010).

8.8.1 Treatment of CE

For a long time, surgery has been the only available treatment for CE. In the last decades, however, with the introduction of benzimidazole therapy (mebendazole (MBZ) in the 1970s and albendazole (ABZ) in the early 1980s (Bekhti et al. 1977; Saimot et al. 1983) and the development and implementation of percutaneous treatments in the mid-1980s (Mueller et al. 1985), the use of surgery for abdominal CE was reconsidered. At present, treatment for CE of the liver is decided depending mainly on the stage and size of the cyst and on the presence of complications (Brunetti et al. 2010).

For CE, no "one-size-fits-all" or "best treatment" exists; thus, clinical decision-making should be individualized. The WHO-IWGE indicates the need for a stage-specific approach to cysts (Brunetti et al. 2010). This should take into consideration cyst features (stage, size, number, localization, complications), patient characteristics including compliance to long-term follow-up, and the availability of the different therapeutic options in the health center where the patient is followed (Brunetti et al. 2010). Four approaches are currently available for CE: surgery, medical treatment with benzimidazoles, percutaneous interventions, and the so-called watch-and-wait approach with ultrasound follow-up in the absence of treatment (Brunetti et al. 2010).

The treatment algorithm for uncomplicated hepatic CE cysts used in Pavia (Italy) WHO Collaborating Centre for Clinical Management of Cystic Echinococcosis is presented in Table 8.1.

8.8.2 Surgery

Surgery is indicated for the following: (1) complicated abdominal cysts at any stage, (2) uncomplicated CE2 and CE3b cysts (often after a failed first approach with albendazole), and (3) extra-abdominal cysts (Brunetti et al. 2010). Spleen-preserving surgery should be used in cases of splenic CE (Culafic et al. 2010). Complications include bacterial superinfection and biliary fistulae (managed by surgery when other percutaneous options are not available). Other indications for surgery include unilocular cysts at risk of rupture, when percutaneous approaches are not available, and small cysts nonresponsive to medical treatment located in anatomical locations poorly accessible to the percutaneous approach, should the cyst increase in size. In general, surgery is contraindicated in the case of very small cysts, asymptomatic and inactive cysts, and in all cases when the patient's condition would contraindicate surgery (Brunetti et al. 2010).

Surgery can be performed with a radical procedure, with removal of the entire cyst including the adventitious layer ("total cystectomy," previously known as "pericystectomy"; in that circumstance the cyst is not opened (non-opened cyst/NOP procedure) or, conservatively, leaving the adventitious layer in place ("subtotal

Table 8.1 Schematic summary of treatment indications based on cyst stage and size for intra-abdominal cysts according to the WHO-IWGE Expert Consensus

Stage	Size	Treatment
CE1 and CE3a	<5 cm	Albendazole[a]
	5–10 cm	Percutaneous aspiration + albendazole
	>10 cm	Permanent catheterization + albendazole
CE2 and CE3b	<5 cm	Albendazole (rarely successful) or surgery + albendazole
	>5 cm	Surgery and albendazole
CE4 and CE5	All	Watch and wait

[a]Albendazole is usually administered for 3 to 6 months in a continuous administration

or partial cystectomy," previously known as "endocystectomy"). The cyst may be opened (opened cyst/OC procedure), deliberately or after failure of the NOP approach; in that circumstance, the cyst content is evacuated and the cavity sterilized using a scolecidal agent; such a chemical sterilization is only possible if no communication with the biliary system is present. Laparoscopic interventions are also performed in selected cases with good results (Dziri et al. 2004). A standardized description of surgical operations in CE has been proposed by the World Association of Echinococcosis (Vuitton et al. 2020).

Reported morbidity, mortality, and recurrence rates after surgery for CE range from 3 to 84%, 0.5 to 5%, and 2 to 40%, respectively (Gollackner et al. 2000; Kapan et al. 2006; Khuroo et al. 1997; Prousalidis et al. 2012; Arif et al. 2008; Aydin et al. 2008; Bedioui et al. 2012; Chautems et al. 2003; Daradkeh et al. 2007; El Malki et al. 2008; Escola-Verge et al. 2019; Ramia et al. 2014); however the real rate of recurrence is difficult to estimate because in the vast majority of cases, the follow-up is too short and/or the patient is visited by different physicians, sometimes in different countries, so that awareness of real treatment outcome is often eluded.

The WHO-IWGE suggests that parasitic material should be removed as much as possible. Results of meta-analyses and single-center studies indicate that radical surgery is superior to "conservative" surgery (with parts of the cyst layers left in place), with lower morbidity (3–24% vs 11–25%), mortality (1–1.8% vs 2–5%), and recurrence rates (2–6.4% vs 10.4–40%) (Aydin et al. 2008; Buttenschoen and Carli Buttenschoen 2003; Daradkeh et al. 2007; Gollackner et al. 2000), although the type of surgery was not found to be predictive of postsurgery complications in another study (El Malki et al. 2008). A recent meta-analysis of partial cyst removal has however suggested that mortality and recurrence rates of conservative surgery from past studies were overestimated (Al-Saeedi et al. 2019, 2021). However, data on long-term (i.e., longer than 10 years) follow-up are difficult to obtain. Other factors associated with poor surgery outcome are large cyst size and presence of biliary fistulae (significantly higher for cysts >7.5 cm) (Kilic et al. 2008). More recent studies have found a correlation between higher cyst diameters and fistula formation (Demir et al. 2020), age >40 years, repeated surgery due to recurrence, and cyst rupture during surgery (Bedioui et al. 2012; Gollackner et al. 2000; Daradkeh et al. 2007; El Malki et al. 2008; Prousalidis et al. 2012). Relapses are particularly

frequent (up to 80%) in case of bone cysts as complete eradication is difficult due to the fact that osseous CE develops as bone infiltration with small cysts (Papanikolaou 2008; Monge-Maillo et al. 2017, 2019; Cattaneo et al. 2019). Perioperative albendazole prophylaxis is recommended to prevent secondary dissemination and reduces the rate of reactivation after surgery (4.2–6.7% vs 9.4–23.3%, with vs without albendazole, respectively); however no dedicated studies have established the optimal preoperative and/or postoperative treatment schedules (Arif et al. 2008; Gollackner et al. 2000; Kern et al. 2017). Bile leakage from biliary fistulae and cyst cavity superinfection are the most common complications of surgical interventions (4–14%) and are managed either conservatively (perendoscopic procedures) or surgically (Agarwal et al. 2005; Canyigit et al. 2011; Bedirli et al. 2002; Dziri et al. 2009; Galati et al. 2006; Manterola et al. 2003; Prousalidis et al. 2008; Caremani et al. 2007; Smego and Sebanego 2005). Important cautionary measures are the following: (1) perioperative treatment with albendazole and protection of the surgical field with pads soaked with scolecidal agents to prevent secondary CE and relapses; (2) avoidance of scolecidal agents, in case of open surgery, if cysto-bronchial or cysto-biliary fistulae are observed (the latter by visualization of the fistula, presence of bile-stained cystic fluid, detection of bilirubin in the fluid, or cholangiography); and (3) appropriate management of the residual cavity, e.g., by using omentoplasty (Al-Saeedi et al. 2019, 2021). Of note, any connection with the biliary system can be visualized only after reduction of the intracystic pressure; hence, the presence of clear fluid does not rule out cysto-biliary fistulae (Sonmez et al. 2007).

8.8.3 Medical Treatment

Benzimidazoles (BMZ)—ABZ and MBZ—are used for the medical treatment of CE, either in monotherapy or as an adjunct to invasive procedures. After intestinal absorption, ABZ is metabolized into an active product, albendazole sulfoxide, while MBZ is metabolized into inactive products. At present, ABZ is the drug of choice (Davis et al. 1989; Todorov et al. 1992a, b; Teggi et al. 1993; Franchi et al. 1999). BMZ are indicated for small (<5 cm) hepatic and lung CE1 and CE3a cysts, peritoneal cysts, multiple cysts in two or more organs, peritoneal cysts, and inoperable patients (Brunetti et al. 2010).

Whenever used alone, ABZ should be administered continuously for at least 3 months at the dosage of 10–15 mg/kg/day (maximum 800 mg/day) in two divided doses, with a fatty meal to increase absorption. The initially recommended administration with sequential interruptions should not be followed any longer (Tamarozzi et al. 2020b). Moreover, it should be administered perioperatively in case of percutaneous or surgical treatment, from a minimum of 4 hours before until a minimum of 1 month after, to prevent recurrence and secondary CE (Brunetti et al. 2010; Tamarozzi et al. 2020b). The value of the coadministration of other

drugs such as cimetidine or praziquantel is not supported, at present, by sufficient evidence (Bygott and Chiodini 2009; Lotsch et al. 2016; Velasco-Tirado et al. 2018).

BMZ are generally well tolerated, and side effects are usually mild and self-limiting, often without the need for treatment interruption (Franchi et al. 1999; Teggi et al. 1993, 1997; Tamarozzi et al. 2020b). Monthly monitoring of liver enzymes and leukocyte counts is required during therapy. Therapy can be resumed after normalization of the parameters, if therapy suspension is required. Contraindications include cysts at high risk of rupture, pregnancy (cautionary because of teratogenic effects in experimental animal models) and breastfeeding, and bone marrow depression, while they should be used cautiously in patients with other types of chronic hepatic diseases in addition to echinococcosis (Brunetti et al. 2010).

Very few controlled trials evaluated the effectiveness of BMZ (Gil-Grande et al. 1993; Keshmiri et al. 1999, 2001), and published work shows large heterogeneity of methodology. Reported outcome rates for hepatic cysts are as follows: 28–58% cure/marked improvement, 10–51% partial response, 13–37% no change, and 4–33% worsening (Todorov et al. 1992a, b; Nahmias et al. 1994; Horton 1989; Wen et al. 1994; Salinas et al. 2011; Li et al. 2011c; Teggi et al. 1993). Relapse rates range from 9 to 25% (Franchi et al. 1999; Teggi et al. 1993; Horton 1989; el-Mufti et al. 1993), and, although responsive to subsequent treatments, cysts tend to relapse multiple times (Stojkovic et al. 2009). Unilocular (CE1 and CE3a) cysts and small cysts (<6 cm) respond better and faster to ABZ treatment compared to multicystic (CE2 and CE3b) and bigger cysts, with a lower relapse rate (Franchi et al. 1999; Todorov et al. 1992a; Stojkovic et al. 2009; Liu et al. 2000; Li et al. 2011c; Larrieu et al. 2019b). Moreover, lung, spleen, and peritoneal cysts have a better response to treatment, with less relapses, while bone cysts respond very poorly (Franchi et al. 1999; Todorov et al. 1992a; Nahmias et al. 1994; Teggi et al. 1993; Liu et al. 2000). It has been observed that cyst degeneration progresses also after treatment interruption; therefore, evaluation of treatment outcome should be done not earlier than 1 year posttreatment, and long-term follow-up is required (Franchi et al. 1999; Nahmias et al. 1994; Teggi et al. 1993; Davis et al. 1989; Salinas et al. 2011). It should also be noted that spontaneous cyst degeneration occurs in up to 20% of patients (Larrieu et al. 2004; Wen and Craig 1994); therefore, the effectiveness of ABZ treatment may be overestimated (Stojkovic et al. 2009).

8.8.4 Percutaneous Treatment

Percutaneous interventions aim to evacuate the cyst content and destroy the germinal layer by means of scolecidal agents. Schematically, percutaneous techniques can be divided in two categories: needle-based techniques and catheter-based techniques.

8.8.4.1 Needle-Based Techniques

The first developed and most widely used needle-based technique is PAIR (puncture, aspiration, injection of scolecidal agent, re-aspiration) (Anonymous 2001); PAIR has been shown to work better in cyst stages with a predominantly liquid, unilocular component, i.e., CE1 and CE3a stages, especially >5 cm, and its recommended use is limited to these stages by the WHO-IWGE Expert Consensus (Brunetti et al. 2010). Other indications where PAIR can be considered include inoperable patients, failure to respond/relapse after other treatments, and patients who refuse surgery. PAIR has also been used in pregnant women with cysts at risk of rupture during labor (Ustunsoz et al. 2008) and in children (Oral et al. 2012). Several variations of PAIR have been described, including percutaneous techniques no longer using a scolecidal agent, preferring a prolonged course of benzimidazoles as adjunctive treatment to the puncture, or variations in the amount or type of scolicidal agent used, as well as elimination of the re-aspiration step. PAIR has also been applied in extrahepatic cysts but is contraindicated in lung cysts (Akhan et al 2007; Ormeci 2014; Brunetti et al. 2010; Junghanss et al. 2008; Akhan et al. 2016; Firpo et al. 2017; Ciftci et al. 2021).

Randomized, placebo-controlled trials on the use of PAIR are lacking (Nasseri Moghaddam et al. 2006). However, an increasing number of single-center retrospective studies, and a few prospective randomized trials comparing PAIR with either surgery or medical treatment (Khuroo et al. 1993, 1997), show that PAIR is safe and effective when applied to selected CE cases, with significantly lower morbidity and mortality rates, hospital stay duration, and costs compared to surgery (Khuroo et al. 1997; Smego et al. 2003; Yagci et al. 2005; Smego and Sebanego 2005; Chen et al. 2015). Reported morbidity and mortality range from 8.5 to 32% and from 0 to 1%, respectively (Giorgio et al. 2008; Khuroo et al. 1997; Yagci et al. 2005; Smego and Sebanego 2005; Filice et al. 2000) (Chen et al. 2015; Golemanov et al. 2011). Similar to surgery, biliary fistula and superinfection are the most common complications, although with lower rates (2–6%) (Smego and Sebanego 2005).

Overall response rates range from 72 to 97%, with relapse rates of 2–15% (Giorgio et al. 2008; Khuroo et al. 1997; Smego et al. 2003; Ustünsöz et al. 1999; Smego and Sebanego 2005; Chen et al. 2015). However, these figures vary greatly when cyst stages are taken into account. Indeed, unilocular CE1 and CE3a cysts respond very well to PAIR treatment (>80% response), while multivesicular CE2 and CE3b cysts have a success rate <40% (Kabaalioglu et al. 2006; Golemanov et al. 2011; Giorgio et al. 2008), with the expertise available at the center playing a great role in the success rate, as the highest figures have been reported by experienced centers. Percutaneous techniques have shown to reduce hospital stay compared to surgery (Giorgio et al. 2008; Khuroo et al. 1997; Yagci et al. 2005). PAIR has also been applied in remote resource-poor areas using portable ultrasound machines (Filice and Brunetti 1997).

8.8.4.2 Catheter-Based Techniques

In case of giant cysts (>10 cm), aspiration, followed by permanent catheterization with catheter removal when daily drainage is <10 mL, is recommended since classic PAIR is less successful in these cases (Ustünsöz et al. 1999; Men et al. 2006; Golemanov et al. 2011, Schipper 2002). This procedure is currently named standard catheterization technique (S-CAT) (Vuitton et al. 2020). A research group from Turkey also developed a procedure relying on an increased diameter catheter, placed under fluoroscopic guidance, named modified catheterization technique (MoCaT). This technique has been applied to CE2 and CE3b cysts in the liver and other abdominal organs with good results (Akhan et al. 2017; Ciftci et al. 2021), but currently data from other centers and from prospective, randomized controlled trials are lacking, which could provide information on the applicability and effectiveness of this technique also in other centers.

8.8.4.3 General Indications for Percutaneous Techniques

All percutaneous procedures on CE cysts should be performed in the presence of resuscitation equipment, and ABZ peri-interventional therapy is mandatory, as is for surgery; when ABZ treatment is contraindicated (e.g., pregnancy), a case-by-case decision should be implemented. The most used scolecidal agents are 20% saline and 95% ethanol (Smego et al. 2003). These should be applied only after excluding the presence of cysto-biliary fistulae (a risk significantly higher for cysts >7.5 cm) (Kilic et al. 2008), either with intraoperative cystoscopy or evaluating bilirubin content in the HCF. Although chemical sclerosing cholangitis, due to contact of the scolecidal agent with the biliary ducts, has never been reported after PAIR, several reports have documented it after surgery (Taranto et al. 1995; Belghiti et al. 1986; Castellano et al. 1994). Fear of anaphylactic shock because of cyst puncture has been the main reason for reluctance to perform percutaneous treatments (Yaghan et al. 2004). However, a systematic review of the literature found that more than 5500 cysts were punctured accidentally or intentionally with only 99 cases of anaphylaxis reported (1.6%), of which only 2 were fatal (0.03%) (Neumayr et al. 2011). Its pathogenesis is not completely elucidated (Li et al. 2011c), and at present the effectiveness of prophylactic measures is unknown.

8.8.5 Watch and Wait

Experts recommend that uncomplicated asymptomatic inactive CE4–CE5 cysts of the liver should be left untreated and solely monitored regularly by ultrasound, using the so-called watch-and-wait approach (Brunetti et al. 2010; Menezes da Silva 2003). The rationale follows the observation that up to 20% of cysts become

spontaneously inactive and such cysts are likely to remain stable over time (Junghanss et al. 2008; Larrieu et al. 2004; Li et al. 2011b; Wang et al. 2006; Keshmiri et al. 2001; Frider et al. 1999; Solomon et al. 2017a). CE4 and CE5 cysts, which became so spontaneously, nearly always remain inactive over time, while apparent inactivation after treatment may be only temporary in a proportion of patients (Lissandrin et al. 2018; Stojkovic et al. 2016).

Constant long-term follow-up is required especially in patients managed by watchful waiting; therefore, a careful assessment of patient adherence to this follow-up and to any prolonged medical treatment should be part of clinical decision-making.

8.8.6 Treatment of AE

Care of AE patients requires a multidisciplinary approach (Brunetti et al. 2010). A complete evaluation of disease extension (including thoracic and brain CT) is necessary before any therapeutic decision. The options may be a curative resection with a 2-year ABZ treatment, or a prolonged ABZ treatment, associated with interventional radiological or perendoscopic procedures for complications. The PNM system of classification of AE cases (Table 8.2), designed on the model of the TNM classification of cancers, helps clinicians to choose the appropriate treatment (Kern et al. 2006). A staging score is obtained as follows: stage I, P1 N0 M0; stage II, P2 N0 M0; stage IIIa, P3 N0 M0; stage IIIb, P1–3 N1 M0 or P4 N0 M0; and stage IV, P4 N1 M0 or any P, any N, and/or M1. However, the age of the patients (generally older than in CE) and contraindications due to associated conditions must also be taken into account, and this reduces the proportion of patients who are actually operated on, especially in Europe, where patients are older than in the Asian endemic areas (Graeter et al. 2020a, b).

8.8.7 Medical Treatment

MBZ and ABZ only have a parasitostatic effect on *E. multilocularis* in vitro; therefore, in most cases medical treatment will be lifelong. However, their benefit for patient survival and quality of life is now well assessed (Ammann et al. 1994; Ishizu et al. 1997). ABZ is currently preferred (Reuter et al. 2000) and administered continuously. In case of curative surgery, ABZ should be initiated before the operation and maintained for at least 2 years to avoid recurrence (Brunetti et al. 2010). In inoperable cases, long-term chemotherapy (often lifelong) significantly prolongs survival (80% at 10 years, compared to $<25\%$ in historical controls). Like in CE, blood count and liver aminotransferase levels must be checked regularly at the initiation of treatment. Fine-tuning of the dosage according to ABZ sulfoxide plasma levels is important to monitor both efficacy and the origin of possible side

Table 8.2 PNM classification of alveolar echinococcosis

P	Hepatic localization of the parasite
P X	Primary tumor cannot be assessed
P 0	No detectable tumor in the liver
P 1	Peripheral lesions without proximal vascular and/or biliary involvement
P 2	Central lesions with proximal vascular and/or biliary involvement of one lobe[a]
P 3	Central lesions with hilar vascular or biliary involvement of both lobes and/or with involvement of two hepatic veins
P 4	Any liver lesion with extension along the vessels[b] and the biliary tree
N	Extrahepatic involvement of neighboring organs [diaphragm, lung, pleura, pericardium, heart, gastric and duodenal wall, adrenal glands, peritoneum, retroperitoneum, parietal wall (muscles, skin, bone), pancreas, regional lymph nodes, liver ligaments, kidney]
N X	Not evaluable
N 1	Regional involvement of contiguous organs or tissues
M	The absence or presence of distant metastasis [lung, distant lymph nodes, spleen, CNS, orbital, bone, skin, muscle, kidney, distant peritoneum, and retroperitoneum]
M X	Not completely evaluated
M 0	No metastasis
M 1	Metastasis

Scoring system: stage I, P1 N0 M0; stage II, P2 N0 M0; stage IIIa, P3 N0 M0; stage IIIb, P1–3 N1 M0 or P4 N0 M0; stage IV, P4 N1 M0 or any P, any N, and/or M1

effects, because of the very prolonged treatment and possible interference with other drugs or life habits (such as tobacco smoking), which may change over time (Bresson-Hadni et al. 2021). In case of severe adverse effects of ABZ, switching to MBZ may be attempted; any recurrence of the adverse effect, however, imposes benzimidazole withdrawal (with unfortunately no alternative as for now).

Discontinuation of benzimidazoles in selected cases of inoperable AE now seems possible, if both FDG-PET with delayed image acquisition and serology are negative (Caoduro et al. 2013; Bresson-Hadni et al. 2011; Crouzet et al. 2010; Bardonnet et al. 2013; Reuter et al. 2004; Gottstein et al. 2017). In liver transplant patients, ABZ must be initiated before and reintroduced as soon as possible after transplantation and maintained for at least 2 years if all AE lesions were removed with the liver and lifelong in case of metacestode remnants or if new AE foci are discovered during follow-up (Bresson-Hadni et al. 2011).

8.8.8 Surgery and Interventional Nonsurgical Procedures

The only efficacious treatment for AE is partial hepatectomy (i.e., curative or so-called radical resection of lesions) (Kadry et al. 2005; Buttenschoen et al. 2009a; Crouzet et al. 2010; Ammann et al. 1998; Sato et al. 1997). This is currently possible only in one-third of AE patients. Palliative operations, such as "partial

debulking" liver resections, must be avoided due to poor results and numerous (especially biliary) complications (Bresson-Hadni et al. 2006a, b; Kadry et al. 2005; Buttenschoen et al. 2009a, b). When curative resection is not possible, percutaneous or perendoscopic procedures should be preferred to treat complications, such as drainage of abscessed lesions or bile duct decompression (Tamarozzi et al. 2014). Such drains can be maintained for years. Combined with chemotherapy, they have allowed prolonged survival in initially very severe AE cases. Biliary endoprosthesis/stent insertion is an alternative that is increasingly used and tends to replace percutaneous procedures; the use of several plastic stents inserted through bile duct stenoses allows jaundice alleviation and recalibration of the stenoses in many cases (Ambregna et al. 2017).

In very severe cases, with life-threatening complications and no other options available, liver transplantation may be proposed. The risk of recurrence or progression of extrahepatic locations in case of allogeneic transplantation is high, due to immune suppression (Koch et al. 2003). However, with early ABZ treatment after transplantation, long-term survival of more than 20 years has been observed, even in patients with residual lesions (Bresson-Hadni et al. 2011). Ex vivo liver resection followed by autotransplantation (ELRA), which allows easier resection of large-sized lesions with vascular involvement, has been proposed by Chinese surgical teams for very advanced AE lesions to avoid liver transplantation, limited by organ shortage and the increased risk of AE recurrence because of antirejection treatment (Wen et al. 2011). Evaluation of the procedure after several years of experience in more than 100 patients has demonstrated its feasibility and the acceptable results, compared to allotransplantation (Aji et al. 2018; Jiang et al. 2021). However, its use in Europe appears limited by the case profile of AE patients in Europe compared to Central Asia (Beldi et al. 2019).

8.8.9 Watch and Wait

In patients with small and calcified lesions, with no FDG uptake on PET/CT, a "watch-and-wait" attitude can reasonably be followed. It is also applied when ABZ or MBZ withdrawal has been decided based on serological and PET/CT negative results and in patients with radical resection after 2 years of ABZ or MBZ (Brunetti et al. 2010).

8.9 Prognosis and Follow-Up

The prognosis of CE is extremely variable and depends on cyst-associated factors (localization, number, stage, and complications), patient characteristics, type of therapeutic intervention required/applied, and the availability of resources in the health center where the patient is visited. Mortality and fatality rates are difficult to

estimate and vary greatly depending on these variables. On average the reported figures are mortality rate of 0.2/100,000 inhabitants and 2.2% fatality rate (Eckert 2001). Reported values for each treatment and cyst characteristics are detailed in the paragraphs on therapy.

A long-term follow-up is required in patients with CE, every 3–6 months initially and then yearly if the situation is stable. Most relapses occur within 2 years after treatment, but reactivation is reported up to >10 years from the end of therapy (Franchi et al. 1999; Prousalidis et al. 2012; Akyildiz et al. 2009). Therefore, a minimum of 2-year follow-up, if possible extended to 10 years, would be highly desirable (Junghanss et al. 2008).

In AE, a multidisciplinary reevaluation of treatment is necessary during the patient's life, since poor adherence to treatment, low plasma levels of ABZ sulfoxide, and/or adverse effects may compromise the efficacy of ABZ. Reduction of the size of lesions after percutaneous drainage of a necrotic central cavity may also enable a radical resection that had been judged unrealistic at diagnosis. Choice of complication management also requires a multidisciplinary evaluation. Whatever the type of treatment, all patients with AE should have a regular follow-up (every 3 months, then 6 months, then yearly) for at least 5 years after BMZ withdrawal. The follow-up should include US and serology, blood cell count and aminotransferase levels, and ideally FDG-PET, during the period of BMZ treatment. Monitoring of ABZ sulfoxide is also essential, both to evaluate patient's adherence to treatment and to adjust ABZ dosage.

The introduction of medical treatment, although not curative, has considerably improved the prognosis of patients with AE, at least in Europe and Japan. Years subtracted from life expectancy because of AE went from an average 18.2–21.3 years (men-women) in the mid-1970s to 3.5–2.6 years in 2005. In France, life expectancy of AE patients 1 year from diagnosis is now similar to that of their non-AE fellow citizens. Nevertheless, AE is still a lethal disease shortly after diagnosis in symptomatic patients who live in most of the endemic areas, especially in Central Asia and China, and new therapeutic options are urgently needed.

8.10 Prevention and Control

Individual prevention of CE and AE relies on hygiene: washing hands before eating, avoiding hand-mouth contact, thorough washing or cooking of vegetables, and use of safe water are all important. Chlorination and household freezing do not inactivate eggs, which are instead sensible to heat.

Control programs for CE are complex, with multiple targets, and require a long time (several years of "attack phase" followed by a "consolidation" and "maintenance" phase) and resources (Eckert 2001; Huang et al. 2011). The principal points of intervention are (1) veterinary public health actions, including appropriate management of slaughtering, (2) registration of owned dogs and control of stray dog population (however, dog culling practices should be critically evaluated) (Barnes

et al. 2012; Torgerson 2006; Johansen and Penrith 2009), (3) regular treatment of dogs with praziquantel, (4) education of animal owners and of the whole community about the purpose and importance of the program, and (5) making CE a notifiable disease.

So far, only four CE control programs have been successful, all of them carried out on islands (Iceland, New Zealand, Tasmania, and Falkland Islands). Partial success, supported by a long and sustained effort, has been achieved in some provinces of Argentina such as Rio Negro (Larrieu et al. 2019a). Elimination is difficult to obtain, and experts believe that with the current control options, achieving such a goal would take around 20 years of sustained efforts. Some independent evaluation of national control programs, especially in nomadic populations, has proved rather disappointing (van Kesteren et al. 2015). However, livestock vaccination using the highly effective EG95 vaccine, together with the elimination of older livestock, who harbor the bulk of fertile cysts, could be a useful tool to shorten the length of control programs (Craig et al. 2007b; Huang et al. 2011).

Due to its sylvatic life cycle, active control of AE relies only on definitive host treatment. Regular praziquantel treatment of dogs follows the same rule as for CE control, at family level. It was long considered that *E. multilocularis*, circulating only in wildlife, was globally beyond control (Roberts and Aubert 1995). However, control was attempted in Alaska (Rausch et al. 1990), and a few other control programs have targeted endemic rural areas, with variable results (Hegglin and Deplazes 2008). In China, control of CE may also exert some effect on AE. The problem of urban foxes is now drawing the attention of public health authorities in Europe, northern Japan, and more recently Canada; fox baiting with praziquantel attained variable results, depending mostly on the contamination pressure in the rural areas surrounding the targeted city (Hegglin and Deplazes 2008; Comte et al. 2013; Romig et al. 2017). More studies are needed to assess the best strategy to tackle this emerging public health problem.

Acknowledgments We want to thank all colleagues involved in the clinical management of patients with echinococcosis in our reference centers in Besançon and Pavia. DAV thanks all members of the FrancEchino/EchinoVista network and all those patients of the Association for Information and Research on Alveolar Echinococcosis who have so much contributed to our knowledge of AE within the past 35 years. Special thanks to Sophie Muraccioli and Lydie Belpois, from the Communication Unit of the University Hospital in Besançon, for providing Fig. 8.4, and Prof. Solange Bresson-Hadni, from the WHO Collaborating Centre on Prevention and Treatment of Human Echinococcosis, for providing Fig. 8.3 and sharing the history of her patients with us. Dedication of all participants in the WHO Informal Working Group on Echinococcosis and World Association of Echinococcosis in the research on these neglected diseases is also warmly acknowledged.

References

Abdel Razek AA, Watcharakorn A, Castillo M (2011) Parasitic diseases of the central nervous system. Neuroimaging Clin N Am 21:815–41, viii

Aydin Ü, Yazici P, Önen Z, Özsoy M, Zeytunlu M, Kiliç M, Çoker A (2008) The optimal treatment of hydatid cyst of the liver: radical surgery with a significant reduced risk of recurrence. Turkish J Gastroenterol 19:33–39

Aceti A, Pennica A, Teggi A, Fondacaro LM, Caferro M, Leri O, Tacchi G, Celestino D, Quaranta G, De Rosa F et al (1993) IgG subclasses in human hydatid disease: prominence of the IgG4 response. Int Arch Allergy Immunol 102:347–351

Agarwal S, Sikora SS, Kumar A, Saxena R, Kapoor VK (2005) Bile leaks following surgery for hepatic hydatid disease. Indian J Gastroenterol 24:55–58

Agudelo Higuita NI, Brunetti E, McCloskey C (2016) Cystic Echinococcosis. J Clin Microbiol 54(3):518–523

Aji T, Dong JH, Shao YM, Zhao JM, Li T, Tuxun TEHJ, Shalayiadang P, Ran B, Jiang TM, Zhang RQ, He YB, Huang JF, Wen H (2018) Ex vivo liver resection and autotransplantation as alternative to allotransplantation for end-stage hepatic alveolar echinococcosis. J Hepatol 69(5):1037–1046

Akhan O, Gumus B, Akinci D, Karcaaltincaba M, Ozmen M (2007) Diagnosis and percutaneous treatment of soft-tissue hydatid cysts. Cardiovasc Intervent Radiol 30:419–425

Akhan O, Akkaya S, Dagoglu MG, Akpinar B, Erbahceci A, Ciftci T, Koroglu M, Akinci D (2016) Percutaneous treatment of splenic cystic echinococcosis: results of 12 cases. Cardiovasc Intervent Radiol 39(3):441–446

Akhan O, Salik AE, Ciftci T, Akinci D, Islim F, Akpinar B (2017) Comparison of long-term results of percutaneous treatment techniques for hepatic cystic echinococcosis types 2 and 3b. AJR Am J Roentgenol 208(4):878–884

Akyildiz HY, Akcan A, Karahan I, Kucuk C, Sozuer E, Esin H (2009) Recurrent liver hydatid disease: when does it become symptomatic and how does one diagnose it? Clin Imaging 33:55–58

Al-Qaoud KM, Abdel-Hafez SK (2008) The induction of T helper type 1 response by cytokine gene transfection protects mice against secondary hydatidosis. Parasitol Res 102:1151–1155

Al-Saeedi M, Khajeh E, Hoffmann K, Ghamarnejad O, Stojkovic M, Weber TF, Golriz M, Strobel O, Junghanss T, Buchler MW, Mehrabi A (2019) Standardized endocystectomy technique for surgical treatment of uncomplicated hepatic cystic echinococcosis. PLoS Negl Trop Dis 13(6):e0007516

Al-Saeedi M, Ramouz A, Khajeh E, El Rafidi A, Ghamarnejad O, Shafiei S, Ali-Hasan-Al-Saegh S, Probst P, Stojkovic M, Weber TF, Hoffmann K, Mehrabi A (2021) Endocystectomy as a conservative surgical treatment for hepatic cystic echinococcosis: a systematic review with single-arm meta-analysis. PLoS Negl Trop Dis 15(5):e0009365

Ambregna S, Koch S, Sulz MC, Gruner B, Ozturk S, Chevaux JB, Sulima M, de Gottardi A, Napoleon B, Abergel A, Bichard P, Boytchev I, Deprez P, Dumortier J, Frossard JL, Kull E, Meny B, Moradpour D, Prat F, Vanbiervliet G, Thevenot T, Vuitton DA, Bresson-Hadni S, Vuitton L (2017) A European survey of perendoscopic treatment of biliary complications in patients with alveolar echinococcosis. Expert Rev Anti-Infect Ther 15(1):79–88

Ammann RW, Ilitsch N, Marincek B, Freiburghaus AU (1994) Effect of chemotherapy on the larval mass and the long-term course of alveolar echinococcosis. Swiss Echinococcosis Study Group. Hepatology 19:735–742

Ammann RW, Fleiner-Hoffmann A, Grimm F, Eckert J (1998) Long-term mebendazole therapy may be parasitocidal in alveolar echinococcosis. J Hepatol 29:994–998

Amri M, Aissa SA, Belguendouz H, Mezioug D, Touil-Boukoffa C (2007) In vitro antihydatic action of IFN-gamma is dependent on the nitric oxide pathway. J Interf Cytokine Res 27:781–787

Amri M, Mezioug D, Touil-Boukoffa C (2009) Involvement of IL-10 and IL-4 in evasion strategies of Echinococcus granulosus to host immune response. Eur Cytokine Netw 20:63–68

Anonymous (2001) PAIR: Puncture, Aspiration, Injection, Re-Aspiration. An option for the treatment of cystic echinococcosis.

Arif SH, Shams Ul B, Wani NA, Zargar SA, Wani MA, Tabassum R, Hussain Z, Baba AA, Lone RA (2008) Albendazole as an adjuvant to the standard surgical management of hydatid cyst liver. Int J Surg 6:448–451

Ayifuhan A, Tuerganaili A, Jun C, Ying-Mei S, Xiang-Wei L, Hao W (2012) Surgical treatment for hepatic alveolar echinococcosis: report of 50 cases. Hepato-Gastroenterology 59(115):790–793

Azizi A, Blagosklonov O, Lounis A, Berthet L, Vuitton DA, Bresson-Hadni S, Delabrousse E (2015) Alveolar echinococcosis: correlation between hepatic MRI findings and FDG-PET/CT metabolic activity. Abdom Imaging 40(1):56–63

Barbieri M, Fernandez V, Gonzalez G, Luaces VM, Nieto A (1998) Diagnostic evaluation of a synthetic peptide derived from a novel antigen B subunit as related to other available peptides and native antigens used for serology of cystic hydatidosis. Parasite Immunol 20:51–61

Bardonnet K, Vuitton DA, Grenouillet F, Mantion GA, Delabrousse E, Blagosklonov O, Miguet JP, Bresson-Hadni S (2013) 30-yr course and favorable outcome of alveolar echinococcosis despite multiple metastatic organ involvement in a non-immune suppressed patient. Ann Clin Microbiol Antimicrob 12:1

Barnes TS, Hinds LA, Jenkins DJ, Coleman GT (2007) Precocious development of hydatid cysts in a macropodid host. Int J Parasitol 37(12):1379–1389

Barnes TS, Deplazes P, Gottstein B, Jenkins DJ, Mathis A, Siles-Lucas M, Torgerson PR, Ziadinov I, Heath DD (2012) Challenges for diagnosis and control of cystic hydatid disease. Acta Trop 123:1–7

Baron RW, Tanner CE (1976) The effect of immunosuppression on secondary Echinococcus multilocularis infections in mice. Int J Parasitol 6:37–42

Bartholomot G, Vuitton DA, Harraga S, Shida Z, Giraudoux P, Barnish G, Wang YH, MacPherson CN, Craig PS (2002) Combined ultrasound and serologic screening for hepatic alveolar echinococcosis in central China. Am J Trop Med Hyg 66(1):23–29

Bayraktar MR, Mehmet N, Durmaz R (2005) Th1 and Th2 inducing cytokines in Cystic echinococcosis. Turkiye Parazitol Derg 29:167–170

Baz A, Carol H, Marco M, Casabo L, Jones F, Dunne D, Nieto A (1998) Fc-binding molecules specific for human IgG1 and IgG3 are present in Echinococcus granulosus protoscoleces. Parasite Immunol 20:399–404

Baz A, Richieri A, Puglia A, Nieto A, Dematteis S (1999) Antibody response in CD4-depleted mice after immunization or during early infection with Echinococcus granulosus. Parasite Immunol 21:141–150

Baz A, Ettlin GM, Dematteis S (2006) Complexity and function of cytokine responses in experimental infection by Echinococcus granulosus. Immunobiology 211:3–9

Baz A, Carol H, Fernandez V, Mourglia-Ettlin G, Nieto A, Orn A, Dematteis S (2008) Echinococcus granulosus: induction of T-independent antibody response against protoscolex glycoconjugates in early experimental infection. Exp Parasitol 119:460–466

Bedioui H, Bouslama K, Maghrebi H, Farah J, Ayari H, Hsairi H, Kacem M, Jouini M, Bensafta Z (2012) Predictive factors of morbidity after surgical treatment of hepatic hydatid cyst. Pan Afr Med J 13:29

Bedirli A, Sakrak O, Sozuer EM, Kerek M, Ince O (2002) Surgical management of spontaneous intrabiliary rupture of hydatid liver cysts. Surg Today 32:594–597

Bekhti A, Schaaps JP, Capron M, Dessaint JP, Santoro F, Capron A (1977) Treatment of hepatic hydatid disease with mebendazole: preliminary results in four cases. Br Med J 2:1047–1051

Beldi G, Vuitton D, Lachenmayer A, Heyd B, Dufour JF, Richou C, Candinas D, Bresson-Hadni S (2019) Is ex vivo liver resection and autotransplantation a valid alternative treatment for end-stage hepatic alveolar echinococcosis in Europe? J Hepatol 70(5):1030–1031

Belghiti J, Benhamou JP, Houry S, Grenier P, Huguier M, Fekete F (1986) Caustic sclerosing cholangitis. A complication of the surgical treatment of hydatid disease of the liver. Arch Surg 121:1162–1165

Bellanger AP, Mougey V, Pallandre JR, Gbaguidi-Haore H, Godet Y, Millon L (2017) Echinococcus multilocularis vesicular fluid inhibits activation and proliferation of natural killer cells. Folia Parasitol (Praha) 64

Ben Nouir N, Nunez S, Gianinazzi C, Gorcii M, Muller N, Nouri A, Babba H, Gottstein B (2008) Assessment of Echinococcus granulosus somatic protoscolex antigens for serological follow-up of young patients surgically treated for cystic echinococcosis. J Clin Microbiol 46:1631–1640

Bottcher D, Bangoura B, Schmaschke R, Muller K, Fischer S, Vobis V, Meiler H, Wolf G, Koller A, Kramer S, Overhoff M, Gawlowska S, Schoon HA (2013) Diagnostics and epidemiology of alveolar echinococcosis in slaughtered pigs from large-scale husbandries in Germany. Parasitol Res 112(2):629–636

Breijo M, Spinelli P, Sim RB, Ferreira AM (1998) Echinococcus granulosus: an intraperitoneal diffusion chamber model of secondary infection in mice. Exp Parasitol 90:270–276

Breijo M, Anesetti G, Martinez L, Sim RB, Ferreira AM (2008) Echinococcus granulosus: the establishment of the metacestode is associated with control of complement-mediated early inflammation. Exp Parasitol 118(2):188–196

Bresson-Hadni S, M. J. P. M. G. A. V. D. A. (2007). Echinococcosis of the liver

Bresson-Hadni S, Laplante JJ, Lenys D, Rohmer P, Gottstein B, Jacquier P, Mercet P, Meyer JP, Miguet JP, Vuitton DA (1994) Seroepidemiologic screening of Echinococcus multilocularis infection in a European area endemic for alveolar echinococcosis. Am J Trop Med Hyg 51(6): 837–846

Bresson-Hadni S, Vuitton DA, Bartholomot B, Heyd B, Godart D, Meyer JP, Hrusovsky S, Becker MC, Mantion G, Lenys D, Miguet JP (2000) A twenty-year history of alveolar echinococcosis: analysis of a series of 117 patients from eastern France. Eur J Gastroenterol Hepatol 12(3): 327–336

Bresson-Hadni S, Delabrousse E, Blagosklonov O, Bartholomot B, Koch S, Miguet JP, Mantion GA, Vuitton DA (2006a) Imaging aspects and non-surgical interventional treatment in human alveolar echinococcosis. Parasitol Int 55(Suppl):S267–S272

Bresson-Hadni S, Delabrousse E, Blagosklonov O, Bartholomot B, Koch S, Miguet JP, Andre Mantion G, Angele Vuitton D (2006b) Imaging aspects and non-surgical interventional treatment in human alveolar echinococcosis. Parasitol Int 55(Suppl):S267–S272

Bresson-Hadni S, Miguet JP, Mantion G, Vuitton D (2007) Echinococcosis of the liver. In: Rodes J, Benhamou J, Blei A, Reichen J, Rizzetto M (eds) Textbook of hepatology: from basic science to clinical practice. Blackwell, Oxford

Bresson-Hadni S, Blagosklonov O, Knapp J et al (2011) Should possible recurrence of disease contraindicate liver transplantation in patients with end-stage alveolar echinococcosis? A 20-year follow-up study. Liver Transpl 17(7):855–865. https://doi.org/10.1002/lt.22299

Bresson-Hadni S, Montange D, Richou C, Brumpt E, Fillion A, Bartholomot B, Blagosklonov O, Delabrousse E, Grenouillet F, Vuitton DA, Millon L (2021) Tobacco, cannabis, and liquorice: Hidden players altering albendazole metabolism in patients with hepatic alveolar echinococcosis. J Hepatol 74(2):471–473

Bristow BN, Lee S, Shafir S, Sorvillo F (2012) Human echinococcosis mortality in the United States, 1990–2007. PLoS Negl Trop Dis 6(2):e1524

Brumpt E, Blagosklonov O, Calame P, Bresson-Hadni S, Vuitton DA, Delabrousse E (2019) AE hepatic lesions: correlation between calcifications at CT and FDG-PET/CT metabolic activity. Infection 47(6):955–960

Brunetti E, Kern P, Vuitton DAI (2010) Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Trop 114(1):1–16

Brunetti E, Garcia HH, Junghanss T (2011) Cystic echinococcosis: chronic, complex, and still neglected. PLoS Negl Trop Dis 5(7):e1146

Bruzinskaite R, Marcinkute A, Strupas K, Sokolovas V, Deplazes P, Mathis A, Eddi C, Sarkunas M (2007) Alveolar echinococcosis, Lithuania. Emerg Infect Dis 13(10):1618–1619

Budke CM (2006) Global socioeconomic impact of cystic echinococcosis. Emerg Infect Dis 12(2): 296–303

Budke CM, Deplazes P, Torgerson PR (2006) Global socioeconomic impact of cystic echinococcosis. Emerg Infect Dis 12(2):296–303

Buttenschoen K, Carli Buttenschoen D (2003) Echinococcus granulosus infection: the challenge of surgical treatment. Langenbeck's Arch Surg 388:218–230

Buttenschoen K, Carli Buttenschoen D, Gruener B, Kern P, Beger HG, Henne-Bruns D, Reuter S (2009a) Long-term experience on surgical treatment of alveolar echinococcosis. Langenbeck's Arch Surg 394:689–698

Buttenschoen K, Gruener B, Carli Buttenschoen D, Reuter S, Henne-Bruns D, Kern P (2009b) Palliative operation for the treatment of alveolar echinococcosis. Langenbeck's Arch Surg 394: 199–204

Bygott JM, Chiodini PL (2009) Praziquantel: neglected drug? Ineffective treatment? Or therapeutic choice in cystic hydatid disease? Acta Trop 111:95–101. https://doi.org/10.1016/j.actatropica.2009.04.006

Campos-Bueno A, Lopez-Abente G, Andres-Cercadillo AM (2000) Risk factors for Echinococcus granulosus infection: a case-control study. Am J Trop Med Hyg 62(3):329–334

Canyigit M, Gumus M, Cay N, Erol B, Karaoglanoglu M, Akhan O (2011) Refractory cystobiliary fistula secondary to percutaneous treatment of hydatid cyst: treatment with N-butyl 2-cyanoacrylate embolization. Cardiovasc Intervent Radiol 34(Suppl 2):S266–S270

Caoduro C, Porot C, Vuitton DA, Bresson-Hadni S, Grenouillet F, Richou C, Boulahdour H, Blagosklonov O (2013) The role of delayed 18F-FDG PET imaging in the follow-up of patients with alveolar echinococcosis. J Nucl Med 54:358–363

Cardozo G, Tucci P, Hernandez A (2002) Characterization of the immune response induced by a carbohydrate enriched fraction from Echinococcus granulosus protoscoleces in patients with cystic hydatid disease. Parasitol Res 88:984–990

Caremani M, Lapini L, Tacconi D, Giorni P, Corradini S, Giaccherini R (2007) Sonographic management of complicated cystic echinococcosis. J Ultrasound 10:179–185

Carmena D, Benito A, Eraso E (2006) Antigens for the immunodiagnosis of Echinococcus granulosus infection: an update. Acta Trop 98:74–86

Castellano G, Moreno-Sanchez D, Gutierrez J, Moreno-Gonzalez E, Colina F, Solis-Herruzo JA (1994) Caustic sclerosing cholangitis. Report of four cases and a cumulative review of the literature. Hepato-Gastroenterology 41:458–470

Cattaneo L, Manciulli T, Cretu CM, Giordani MT, Angheben A, Bartoloni A, Zammarchi L, Bartalesi F, Richter J, Chiodini P, Godbole G, Junghanss T, Stojkovic M, Sammarchi L, Dore R, Vercelli A, Benazzo F, Cuzzocrea F, Tamarozzi F, Brunetti E (2019) Cystic Echinococcosis of the Bone: A European Multicenter Study. Am J Trop Med Hyg 100(3):617–621

Chandrasekhar S, Parija SC (2009) Serum antibody & Th2 cytokine profiles in patients with cystic echinococcosis. Indian J Med Res 130:731–735

Chauchet A, Grenouillet F, Knapp J, Richou C, Delabrousse E, Dentan C, Capelle S, Di Martino V, Deconinck E, Blagosklonov O, Vuitton DA, Bresson-Hadni S (2013) Emergence of a new opportunistic infection in Europe: hepatic alveolar echinococcosis. A fifty-case report. J Hepatol 58:S381–S381

Chautems R, Buhler L, Gold B, Chilcott M, Morel P, Mentha G (2003) Long term results after complete or incomplete surgical resection of liver hydatid disease. Swiss Med Wkly 133:258–262

Chen X, Cen C, Xie H, Zhou L, Wen H, Zheng S (2015) The comparison of 2 new promising weapons for the treatment of hydatid cyst disease: PAIR and laparoscopic therapy. Surg Laparosc Endosc Percutan Tech 25(4):358–362

Chuanshan Z, Wang H, Jing L, Hou X, Li L, Wang W, Shi Y, Li D, Zhao Z, Aji T, Lin R, Shao Y, Vuitton DA, Tian Z, Sun H, Wen H (2021) Involvement of TIGIT in NK cell exhaustion and immune escape in patients and mouse model with liver E. multilocularis infection. Hepatology 74(6):3376–3393

Ciftci TT, Unal E, Azizova A, Ayyildiz VA, Akinci D, Akhan O (2021) Renal cystic Echinococcosis: long-term outcomes of percutaneous treatment. J Vasc Interv Radiol 32(6):890–895.e2. https://doi.org/10.1016/j.jvir.2021.03.519

Coltorti E, Varela-Diaz V (1974) Echinococcus granulosus: penetration of macromolecules and their localization on the parasite membranes of cysts. Exp Parasitol 35:225–231

Comte S, Raton V, Raoul F, Hegglin D, Giraudoux P, Deplazes P, Favier S, Gottschek D, Umhang G, Boue F, Combes B (2013) Fox baiting against Echinococcus multilocularis: contrasted achievements among two medium size cities. Prev Vet Med 111(1–2):147–155

Cox DA, Marshall-Clarke S, Dixon JB (1989) Activation of normal murine B cells by Echinococcus granulosus. Immunology 67:16–20

Craig PS (2006) Epidemiology of human alveolar echinococcosis in China. Parasitol Int 55(Suppl): S221–S225

Craig PS, Budke CM, Schantz P, Li T, Qiu J, Yang JY, Zeyhle E, Rogan MT, Ito A (2007a) Human Echinococcosis: a neglected disease? Trop Med Health 35(4):283–292

Craig PS, Mcmanus DP, Lightowlers MW, Chabalgoity JA, Garcia HH, Gavidia CM, Gilman RH, Gonzalez AE, Lorca M, Naquira C, Nieto A, Schantz PM (2007b) Prevention and control of cystic echinococcosis. Lancet Infect Dis 7:385–394

Crouzet J, Grenouillet F, Delabrousse E, Blagosklonov O, Thevenot T, Di Martino V, Piarroux R, Mantion GA, Bresson-Hadni S (2010) Personalized management of patients with inoperable alveolar echinococcosis undergoing treatment with albendazole: usefulness of positron-emission-tomography combined with serological and computed tomography follow-up. Clin Microbiol Infect 16:788–791

Culafic DM, Kerkez MD, Mijac DD, Lekic NS, Rankovic VI, Lekic DD, Dordevic Z (2010) Spleen cystic echinococcosis: clinical manifestations and treatment. Scand J Gastroenterol 45:186–190

D'Alessandro A, Rausch RL (2008) New aspects of neotropical polycystic (Echinococcus vogeli) and unicystic (Echinococcus oligarthrus) echinococcosis. Clin Microbiol Rev 21(2):380–401

da Silva AM (2011) Hydatid cyst/cystic echinococcosis: anatomical and surgical nomenclature and method to quantify the cyst content solidification. Chin Med J (Engl) 124:2806–2812

Daeki AO, Craig PS, Shambesh MK (2000) IgG-subclass antibody responses and the natural history of hepatic cystic echinococcosis in asymptomatic patients. Ann Trop Med Parasitol 94:319–328

Daradkeh S, El-Muhtaseb H, Farah G, Sroujieh AS, Abu-Khalaf M (2007) Predictors of morbidity and mortality in the surgical management of hydatid cyst of the liver. Langenbeck's Arch Surg 392:35–39

Davis A, Dixon H, Pawlowski ZS (1989) Multicentre clinical trials of benzimidazole-carbamates in human cystic echinococcosis (phase 2). Bull World Health Organ 67:503–508

De la Rue ML, Yamano K, Almeida CE, Iesbich MP, Fernandes CD, Goto A, Kouguchi H, Takahashi K (2010) Serological reactivity of patients with Echinococcus infections (E. granulosus, E. vogeli, and E. multilocularis) against three antigen B subunits. Parasitol Res 106:741–745

Del Carpio M, Moguilansky S, Costa M, Panomarenko H, Bianchi G, Bendersky S, Lazcano M, Frider B, Larrieu E (2000) Diagnosis of human hydatidosis. Predictive value of a rural ultrasonographic survey in an apparently healthy population. Medicina 60:466–468

Del Carpio M, Hugo Mercapide C, Salvitti JC, Uchiumi L, Sustercic J, Panomarenko H, Moguilensky J, Herrero E, Talmon G, Volpe M, Araya D, Mujica G, Calabro A, Mancini S, Chiosso C, Labanchi JL, Saad R, Goblirsch S, Brunetti E, Larrieu E (2012) Early diagnosis, treatment and follow-up of cystic echinococcosis in remote rural areas in Patagonia: Impact of ultrasound training of non-specialists. PLoS Negl Trop Dis 6(1)

Dematteis S, Baz A, Rottenberg M, Fernandez C, Orn A, Nieto A (1999) Antibody and Th1/Th2-type responses in BALB/c mice inoculated with live or dead Echinococcus granulosus protoscoleces. Parasite Immunol 21:19–26

Dematteis S, Pirotto F, Marques J, Nieto A, Orn A, Baz A (2001) Modulation of the cellular immune response by a carbohydrate rich fraction from Echinococcus granulosus protoscoleces in infected or immunized Balb/c mice. Parasite Immunol 23:1–9

Demir S, Ilikan GB, Erturk A, Oztorun CI, Guney D, Azili MN, Senel E, Tiryaki HT (2020) A serious complication of liver hydatid cysts in children: cystobiliary fistulas. Pediatr Surg Int 36(5):611–620

Dempster RP, Harrison GB (1995) Maternal transfer of protection from Echinococcus granulosus infection in sheep. Res Vet Sci 58:197–202

Dempster RP, Harrison GB, Berridge MV, Heath DD (1992) Echinococcus granulosus: use of an intermediate host mouse model to evaluate sources of protective antigens and a role for antibody in the immune response. Int J Parasitol 22:435–441

Deplazes P, Hegglin D, Gloor S, Romig T (2004) Wilderness in the city: the urbanization of Echinococcus multilocularis. Trends Parasitol 20(2):77–84

Deplazes P, Rinaldi L, Alvarez Rojas CA, Torgerson PR, Harandi MF, Romig T, Antolova D, Schurer JM, Lahmar S, Cringoli G, Magambo J, Thompson RCA, Jenkins EJ (2017) Global Distribution of Alveolar and Cystic Echinococcosis. Adv Parasitol 95:315–493

Devi CS, Parija SC (2003) A new serum hydatid antigen detection test for diagnosis of cystic echinococcosis. Am J Trop Med Hyg 69:525–528

Diaz A, Irigoin F, Ferreira F, Sim RB (1999) Control of host complement activation by the Echinococcus granulosus hydatid cyst. Immunopharmacology 42:91–98

Diaz A, Casaravilla C, Irigoin F, Lin G, Previato JO, Ferreira F (2011a) Understanding the laminated layer of larval Echinococcus I: structure. Trends Parasitol 27(5):204–213

Diaz A, Casaravilla C, Allen JE, Sim RB, Ferreira AM (2011b) Understanding the laminated layer of larval Echinococcus II: immunology. Trends Parasitol 27(6):264–273

Didier D, Weiler S, Rohmer P, Lassegue A, Deschamps JP, Vuitton D, Miguet JP, Weill F (1985) Hepatic alveolar echinococcosis: correlative US and CT study. Radiology 154:179–186

Dixon JB (1997) Echinococcosis. Comp Immunol Microbiol Infect Dis 20:87–94

Dreweck CM, Luder CG, Soboslay PT, Kern P (1997) Subclass-specific serological reactivity and IgG4-specific antigen recognition in human echinococcosis. Tropical Med Int Health 2:779–787

Dziri C, Haouet K, Fingerhut A (2004) Treatment of hydatid cyst of the liver: where is the evidence? World J Surg 28:731–736

Dziri C, Haouet K, Fingerhut A, Zaouche A (2009) Management of cystic echinococcosis complications and dissemination: where is the evidence? World J Surg 33:1266–1273

Eckert J (2001) WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern

Eckert J, Deplazes P (2004) Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin Microbiol Rev 17(1):107–135

El Malki HO, El Mejdoubi Y, Souadka A, Mohsine R, Ifrine L, Abouqal R et al (2008) Predictive factors of deep abdom- inal complications after operation for hydatid cyst of the liver: 15 years of experience with 672 patients. J Am Coll Surg Papanikolaou, A. Osseous hydatid disease. Iran J Parasitol 3:60–64. https://doi.org/10.1016/j.trstmh.2007.09.012

Ehrhardt AR, Reuter S, Buck AK, Haenle MM, Mason RA, Gabelmann A, Kern P, Kratzer W (2007) Assessment of disease activity in alveolar echinococcosis: a comparison of contrast enhanced ultrasound, three-phase helical CT and [(18)F] fluorodeoxyglucose positron emission tomography. Abdom Imaging (1432–0509 (Electronic))

El-Mufti M, Kamag A, Ibrahim H, Taktuk S, Swaisi I, Zaidan A, Sameen A, Shimbish F, Bouzghaiba W, Haasi S et al (1993) Albendazole therapy of hydatid disease: 2-year follow-up of 40 cases. Ann Trop Med Parasitol 87:241–246

Emery I, Liance M, Deriaud E, Vuitton DA, Houin R, Leclerc C (1996) Characterization of T-cell immune responses of Echinococcus multilocularis-infected C57BL/6J mice. Parasite Immunol 18:463–472

Emery I, Leclerc C, Sengphommachanh K, Vuitton DA, Liance M (1998) In vivo treatment with recombinant IL-12 protects C57BL/6J mice against secondary alveolar echinococcosis. Parasite Immunol 20:81–91

Escola-Verge L, Salvador F, Sanchez-Montalva A, Escudero-Fernandez JM, Sulleiro E, Rando A, Bilbao I, Lazaro JL, Serres X, Salvador J, Molina I (2019) Retrospective study of cystic echinococcosis in a recent cohort of a referral center for liver surgery. J Gastrointest Surg 23(6):1148–1156

Ferragut G, Nieto A (1996) Antibody response of Echinococcus granulosus infected mice: recognition of glucidic and peptidic epitopes and lack of avidity maturation. Parasite Immunol 18: 393–402

Ferreira AM, Breijo M, Sim RB, Nieto A (2000a) Contribution of C5-mediated mechanisms to host defence against Echinococcus granulosus hydatid infection. Parasite Immunol 22:445–453

Ferreira AM, Irigoin F, Breijo M, Sim RB, Diaz A (2000b) How Echinococcus granulosus deals with complement. Parasitol Today 16:168–172

Filice C, Brunetti E (1997) Use of PAIR in human cystic echinococcosis. Acta Trop 64:95–107

Filice C, Brunetti E, Bruno R, Crippa FG (2000) Percutaneous drainage of echinococcal cysts (PAIR–puncture, aspiration, injection, reaspiration): results of a worldwide survey for assessment of its safety and efficacy. WHO-Informal Working Group on Echinococcosis-Pair Network. Gut 47:156–157

Firpo G, Vola A, Lissandrin R, Tamarozzi F, Brunetti E (2017) Preliminary evaluation of percutaneous treatment of echinococcal cysts without injection of scolicidal agent. Am J Trop Med Hyg 97:1818–1826. https://doi.org/10.4269/ajtmh.17-0468

Fotiadis C, Sergiou C, Kirou J, Troupis TG, Tselentis J, Doussaitou P, Gorgoulis VG, Sechas MN (1999) Experimental echinococcus infection in the mouse model: pericystic cellular immunity reaction and effects on the lymphoid organs of immunocompetent and thymectomized mice. In Vivo 13:541–546

Franchi C, Di Vico B, Teggi A (1999) Long-term evaluation of patients with hydatidosis treated with benzimidazole carbamates. Clin Infect Dis 29:304–309

Frider B, Larrieu E, Odriozola M (1999) Long-term outcome of asymptomatic liver hydatidosis. J Hepatol 30:228–231

Galati G, Sterpetti AV, Caputo M, Adduci M, Lucandri G, Brozzetti S, Bolognese A, Cavallaro A (2006) Endoscopic retrograde cholangiography for intrabiliary rupture of hydatid cyst. Am J Surg 191:206–210

Galindo M, Gonzalez MJ, Galanti N (2002) Echinococcus granulosus protoscolex formation in natural infections. Biol Res 35(3–4):365–371

Gemmell MA, Lawson JR, Roberts MG (1986) Population dynamics in echinococcosis and cysticercosis: biological parameters of Echinococcus granulosus in dogs and sheep. Parasitology 92(Pt 3):599–620

Gil-Grande LA, Rodriguez-Caabeiro F, Prieto JG, Sanchez-Ruano JJ, Brasa C, Aguilar L, Garcia-Hoz F, Casado N, Barcena R, Alvarez AI et al (1993) Randomised controlled trial of efficacy of albendazole in intra-abdominal hydatid disease. Lancet 342:1269–1272

Giorgio A, De Stefano G, Esposito V, Liorre G, Di Sarno A, Giorgio V, Sangiovanni V, Iannece MD, Mariniello N (2008) Long-term results of percutaneous treatment of hydatid liver cysts: a single center 17 years experience. Infection 36:256–261

Giraudoux, P., P. S. Craig, P. Delattre, G. Bao, B. Bartholomot, S. Harraga, J. P. Quere, F. Raoul, Y. Wang, D. Shi and D. A. Vuitton (2003). Interactions between landscape changes and host communities can regulate Echinococcus multilocularis transmission. Parasitology 127(Suppl): S121–131

Giraudoux P, Raoul F, Afonso E, Ziadinov I, Yang Y, Li L, Li T, Quere JP, Feng X, Wang Q, Wen H, Ito A, Craig PS (2013) Transmission ecosystems of Echinococcus multilocularis in China and Central Asia. Parasitology 140(13):1655–1666

Godot V, Harraga S, Deschaseaux M, Bresson-Hadni S, Gottstein B, Emilie D, Vuitton DA (1997) Increased basal production of interleukin-10 by peripheral blood mononuclear cells in human alveolar echinococcosis. Eur Cytokine Netw 8:401–408

Godot V, Harraga S, Podoprigora G, Liance M, Bardonnet K, Vuitton DA (2003) IFN alpha-2a protects mice against a helminth infection of the liver and modulates immune responses. Gastroenterology 124:1441–1450

Golemanov B, Grigorov N, Mitova R, Genov J, Vuchev D, Tamarozzi F, Brunetti E (2011) Efficacy and safety of PAIR for cystic echinococcosis: experience on a large series of patients from Bulgaria. Am J Trop Med Hyg 84(1):48–51

Gollackner B, Langle F, Auer H, Maier A, Mittlbock M, Agstner I, Karner J, Langer F, Aspock H, Loidolt H, Rockenschaub S, Steininger R (2000) Radical surgical therapy of abdominal cystic hydatid disease: factors of recurrence. World J Surg 24:717–721

Gonzalez-Sapienza G, Lorenzo C, Nieto A (2000) Improved immunodiagnosis of cystic hydatid disease by using a synthetic peptide with higher diagnostic value than that of its parent protein, Echinococcus granulosus antigen B. J Clin Microbiol 38:3979–3983

Gottstein B, Wilson JF, Tanner I et al (1991) Specific cellular and humoral immune responses in patients with different long-term courses of Alveolar Echinococcosis (Infection with Echinococcus Multilocularis). Am J Trop Med Hyg 45(6):734–742. https://doi.org/10.4269/ajtmh.1991.45.734

Gottstein B, Wang J, Blagosklonov O, Grenouillet F, Millon L, Vuitton DA, Muller N (2014) Echinococcus metacestode: in search of viability markers. Parasite 21:63

Gottstein B, Soboslay P, Ortona E, Wang J, Siracusano A, Vuitton D (2017) Immunology of alveolar and cystic Echinococcosis (AE and CE). Adv Parasitol 96:1–54

Graeter T, Kratzer W, Oeztuerk S, Haenle MM, Mason RA, Hillenbrand A, Kull T, Barth TF, Kern P, Gruener B (2016) Proposal of a computed tomography classification for hepatic alveolar echinococcosis. World J Gastroenterol 22(13):3621–3631

Graeter T, Bao H, Delabrousse E, Brumpt E, Shi R, Li W, Jiang Y, Schmidberger J, Kratzer W, Liu W (2020a) Hepatic alveolar echinococcosis: Comparative computed tomography study between two Chinese and two European centres. Food Waterborne Parasitol 19:e00082

Graeter T, Bao HH, Shi R, Liu WY, Li WX, Jiang Y, Schmidberger J, Brumpt E, Delabrousse E, Kratzer W (2020b) Evaluation of intrahepatic manifestation and distant extrahepatic disease in alveolar echinococcosis. World J Gastroenterol 26(29):4302–4315

Graeter T, Shi R, Bao H, Liu W, Li W, Jiang Y, Schmidberger J, Brumpt E, Delabrousse E, Kratzer W (2021) Intrahepatic manifestation and distant extrahepatic disease in alveolar echinococcosis: a multicenter cohort study. Acta Radiol 62(8):997–1005

Grenouillet F, Mouzon L, Guillou N, Laplante J, Bardonnet K, Meyer J, Knapp J, Millon L, Giraudoux P, Bresson-Hadni S, Vuitton DA (2011) Long-term follow-up after mass screening for alveolar echinococcosis in eastern France: outcome in seropositive subject. XXIVth World Congress of Hydatidology, Sept 14–18, 2011, Urumqi, China

Grenouillet F, Frider B, Alvarez Rodriguez J, Amante M, Pestalardo ML, Cazorla A, Bresson-Hadni S, Millon L (2013) Molecular diagnosis of polycystic echinococcosis due to Echinococcus vogeli in a Paraguayan immigrant in Argentina. J Clin Microbiol 51(9):3151–3153

Gruener B, Cretu CM, Brunetti E, Menezes CN, Haerter G, Grobusch MP, Kern P (2008) Accelerated larval growth of Echinococcus spp in the immunodeficient host? Am J Trop Med Hyg 79:125

Guarnera EA, Parra A, Kamenetzky L, Garcia G, Gutierrez A (2004) Cystic echinococcosis in Argentina: evolution of metacestode and clinical expression in various Echinococcus granulosus strains. Acta Trop 92(2):153–159

Haralabidis S, Karagouni E, Frydas S, Dotsika E (1995) Immunoglobulin and cytokine profile in murine secondary hydatidosis. Parasite Immunol 17:625–630

Harraga S, Godot V, Bresson-Hadni S, Pater C, Beurton I, Bartholomot B, Vuitton DA (1999) Clinical efficacy of and switch from T helper 2 to T helper 1 cytokine profile after interferon alpha2a monotherapy for human echinococcosis. Clin Infect Dis 29:205–206

Heath DD, Lawrence SB (1996) Antigenic polypeptides of Echinococcus granulosus oncospheres and definition of protective molecules. Parasite Immunol 18:347–357
Heath DD, Holcman B, Shaw RJ (1994) Echinococcus granulosus: the mechanism of oncosphere lysis by sheep complement and antibody. Int J Parasitol 24:929–935
Hegglin D, Deplazes P (2008) Control strategy for Echinococcus multilocularis. Emerg Infect Dis 14(10):1626–1628
Hernandez A, Nieto A (1994) Induction of protective immunity against murine secondary hydatidosis. Parasite Immunol 16:537–544
Hernandez A, Cardozo G, Dematteis S, Baz A, Trias N, Nunez H, Barrague A, Lopez L, Fuentes J, Lopez O, Ferreira C (2005) Cystic echinococcosis: analysis of the serological profile related to the risk factors in individuals without ultrasound liver changes living in an endemic area of Tacuarembo, Uruguay. Parasitology 130:455–460
Hernandez-Gonzalez A, Muro A, Barrera I, Ramos G, Orduna A, Siles-Lucas M (2008) Usefulness of four different Echinococcus granulosus recombinant antigens for serodiagnosis of unilocular hydatid disease (UHD) and postsurgical follow-up of patients treated for UHD. Clin Vaccine Immunol 15:147–153
Hernandez-Gonzalez A, Santivanez S, Garcia HH, Rodriguez S, Munoz S, Ramos G, Orduna A, Siles-Lucas M (2012) Improved serodiagnosis of cystic echinococcosis using the new recombinant 2B2t antigen. PLoS Negl Trop Dis 6:e1714
Hernandez-Pomi A, Borras-Salvador R, Mir-Gisbert A (1997) Analysis of cytokine and specific antibody profiles in hydatid patients with primary infection and relapse of disease. Parasite Immunol 19:553–561
Herrador Z, Siles-Lucas M, Aparicio P, Lopez-Velez R, Gherasim A, Garate T, Benito A (2016) Cystic echinococcosis epidemiology in Spain based on hospitalization records, 1997–2012. PLoS Negl Trop Dis 10(8):e0004942
Horton RJ (1989) Chemotherapy of Echinococcus infection in man with albendazole. Trans R Soc Trop Med Hyg 83:97–102
Hosch W, Stojkovic M, Janisch T, Kauffmann GW, Junghanss T (2007) The role of calcification for staging cystic echinococcosis (CE). Eur Radiol 17:2538–2545
Hosch W, Stojkovic M, Janisch T, Heye T, Werner J, Friess H, Kauffmann GW, Junghanss T (2008a) MR imaging for diagnosing cysto-biliary fistulas in cystic echinococcosis. Eur J Radiol 66(2):262–267
Hosch W, Junghanss T, Stojkovic M, Brunetti E, Heye T, Kauffmann GW, Hull WE (2008b) Metabolic viability assessment of cystic echinococcosis using high-field (1)H MRS of cyst contents. NMR Biomed 21(7):734–754
Hosch W, Junghanss T, Stojkovic M, Brunetti E, Heye T, Kauffmann GW, Hull WE (2008c) Metabolic viability assessment of cystic echinococcosis using high-field 1H MRS of cyst contents. NMR Biomed 21(7):734–754
Houston S, Belga S, Buttenschoen K, Cooper R, Girgis S, Gottstein B, Low G, Massolo A, MacDonald C, Muller N, Preiksaitis J, Sarlieve P, Vaughan S, Kowalewska-Grochowska K (2021) Epidemiological and clinical characteristics of alveolar echinococcosis: an emerging infectious disease in Alberta, Canada. Am J Trop Med Hyg
Huang L, Huang Y, Wang Q, Xiao N, Yi D, Yu W, Qiu D (2011) An agent-based model for control strategies of Echinococcus granulosus. Vet Parasitol 179:84–91
Hubner MP, Manfras BJ, Margos MC, Eiffler D, Hoffmann WH, Schulz-Key H, Kern P, Soboslay PT (2006) Echinococcus multilocularis metacestodes modulate cellular cytokine and chemokine release by peripheral blood mononuclear cells in alveolar echinococcosis patients. Clin Exp Immunol 145:243–251
Ioppolo S, Notargiacomo S, Profumo E, Franchi C, Ortona E, Rigano R, Siracusano A (1996) Immunological responses to antigen B from Echinococcus granulosus cyst fluid in hydatid patients. Parasite Immunol 18:571–578
Irigoin F, Wurzner R, Sim RB, Ferreira AM (1996) Comparison of complement activation in vitro by different Echinococcus granulosus extracts. Parasite Immunol 18:371–375

Ishizu H, Uchino J, Sato N, Aoki S, Suzuki K, Kuribayashi H (1997) Effect of albendazole on recurrent and residual alveolar echinococcosis of the liver after surgery. Hepatology 25:528–531

Ito A, Craig PS (2003) Immunodiagnostic and molecular approaches for the detection of taeniid cestode infections. Trends Parasitol 19:377–381

Jebbawi F, Bellanger AP, Lunstrom-Stadelmann B, Rufener R, Dosch M, Goepfert C, Gottstein B, Millon L, Grandgirard D, Leib SL, Beldi G, Wang J (2021) Innate and adaptive immune responses following PD-L1 blockade in treating chronic murine alveolar echinococcosis. Parasite Immunol 43(8):e12834

Jenkins P, Dixon JB, Rakha NK, Carter SD (1990) Regulation of macrophage-mediated larvicidal activity in Echinococcus granulosus and Mesocestoides corti (Cestoda) infection in mice. Parasitology 100(Pt 2):309–315

Jenne L, Kilwinski J, Radloff P, Flick W, Kern P (1998) Clinical efficacy of and immunologic alterations caused by interferon gamma therapy for alveolar echinococcosis. Clin Infect Dis 26: 492–494

Jenne L, Arrighi JF, Sauter B, Kern P (2001) Dendritic cells pulsed with unfractionated helminthic proteins to generate antiparasitic cytotoxic T lymphocyte. Parasite Immunol 23:195–201

Jiang TM, Ran B, Guo Q, Zhang RQ, Duan S, Zhong K, Wen H, Shao YM, Aji T (2021) Use of the ligamentum teres hepatis for outflow reconstruction during ex vivo liver resection and autotransplantation in patients with hepatic alveolar echinococcosis: a case series of 24 patients. Surgery 170(3):822–830

Johansen MV, Penrith ML (2009) Has culling been properly assessed as a valid and justified control intervention measure for zoonotic diseases? PLoS Negl Trop Dis 3:e541

Junghanss T, Da Silva AM, Horton J, Chiodini PL, Brunetti E (2008) Clinical management of cystic echinococcosis: state of the art, problems, and perspectives. Am J Trop Med Hyg 79:301–311

Kabaalioglu A, Ceken K, Alimoglu E, Apaydin A (2006) Percutaneous imaging-guided treatment of hydatid liver cysts: do long-term results make it a first choice? Eur J Radiol 59:65–73

Kadry Z, Renner EC, Bachmann LM, Attigah N, Renner EL, Ammann RW, Clavien PA (2005) Evaluation of treatment and long-term follow-up in patients with hepatic alveolar echinococcosis. Br J Surg 92:1110–1116

Kanan JH, Chain BM (2006) Modulation of dendritic cell differentiation and cytokine secretion by the hydatid cyst fluid of Echinococcus granulosus. Immunology 118:271–278

Kantarci M, Bayraktutan U, Karabulut N, Aydinli B, Ogul H, Yuce I, Calik M, Eren S, Atamanalp SS, Oto A (2012a) Alveolar echinococcosis: spectrum of findings at cross-sectional imaging. Radiographics 32:2053–2070

Kantarci, M., U. Bayraktutan, N. Karabulut, B. Aydinli, H. Ogul, I. Yuce, M. Calik, S. Eren, S. S. Atamanalp and A. Oto (2012b). Alveolar echinococcosis: spectrum of findings at cross-sectional imaging using high field 1H MRS of cyst contents. NMR Biomed 21:734–54

Kanwar JR, Vinayak VK (1992) The significance of free and immune-complexed hydatid-specific antigen(s) as an immunodiagnostic tool for human hydatidosis. J Med Microbiol 37:396–403

Kapan M, Kapan S, Goksoy E, Perek S, Kol E (2006) Postoperative recurrence in hepatic hydatid disease. J Gastrointest Surg 10:734–739

Kassis AI, Tanner CE (1976) The role of complement in hydatid disease: in vitro studies. Int J Parasitol 6:25–35

Kern P (2010) Clinical features and treatment of alveolar echinococcosis. Curr Opin Infect Dis 23(5):505–512

Kern P, Bardonnet K, Renner E, Auer H, Pawlowski Z, Ammann RW, Vuitton DA (2003) European echinococcosis registry: human alveolar echinococcosis, Europe, 1982–2000. Emerg Infect Dis 9(3):343–349

Kern P, Wen H, Sato N, Vuitton DA, Gruener B, Shao Y, Delabrousse E, Kratzer W, Bresson-Hadni S (2006) WHO classification of alveolar echinococcosis: principles and application. Parasitol Int 55(Suppl):S283–S287

Kern P, Grüner B, Wahlers K (2011). Diagnosis and course of echinococcocal diseases in the transplant setting. Transpl Infect Dis (1399–3062 (Electronic))

Kern P, Menezes da Silva A, Akhan O, Mullhaupt B, Vizcaychipi KA, Budke C, Vuitton DA (2017) The echinococcoses: diagnosis, clinical management and burden of disease. Adv Parasitol 96:259–369

Keshmiri M, Baharvahdat H, Fattahi SH, Davachi B, Dabiri RH, Baradaran H, Ghiasi T, Rajabimashhadi MT, Rajabzadeh F (1999) A placebo controlled study of albendazole in the treatment of pulmonary echinococcosis. Eur Respir J 14(3):503–507

Keshmiri M, Baharvahdat H, Fattahi SH, Davachi B, Dabiri RH, Baradaran H, Rajabzadeh F (2001) Albendazole versus placebo in treatment of echinococcosis. Trans R Soc Trop Med Hyg 95(2): 190–194

Khuroo MS, Dar MY, Yattoo GN, Zargar SA, Javaid G, Khan BA, Boda MI (1993) Percutaneous drainage versus albendazole therapy in hepatic hydatidosis: a prospective, randomized study. Gastroenterology 104:1452–1459

Khuroo MS, Wani NA, Javid G, Khan BA, Yattoo GN, Shah AH, Jeelani SG (1997) Percutaneous drainage compared with surgery for hepatic hydatid cysts. N Engl J Med 337:881–887

Kilic M, Yoldas O, Koc M, Keskek M, Karakose N, Ertan T, Gocmen E, Tez M (2008) Can biliary-cyst communication be predicted before surgery for hepatic hydatid disease: does size matter? Am J Surg 196:732–735

Knapp J, Bart JM, Maillard S, Gottstein B, Piarroux R (2010) The genomic Echinococcus microsatellite EmsB sequences: from a molecular marker to the epidemiological tool. Parasitology 137(3):439–449

Knapp J, Damy S, Brillaud J, Tissot JD, Navion J, Melior R, Afonso E, Hormaz V, Gottstein B, Umhang G, Casulli A, Dadeau F, Millon L, Raoul F (2017) EWET: Data collection and interface for the genetic analysis of Echinococcus multilocularis based on EmsB microsatellite. PLoS One 12(10):e0183849

Knapp J, Gottstein B, Bretagne S, Bart JM, Umhang G, Richou C, Bresson-Hadni S, Millon L (2020) Genotyping Echinococcus multilocularis in human alveolar echinococcosis patients: an EmsB microsatellite analysis. Pathogens 9(4):282

Koch S, Bresson-Hadni S, Miguet JP, Crumbach JP, Gillet M, Mantion GA, Heyd B, Vuitton DA, Minello A, Kurtz S (2003) Experience of liver transplantation for incurable alveolar echinococcosis: a 45-case European collaborative report. Transplantation 75(6):856–863

Kodama Y, Fujita N, Shimizu T, Endo H, Nambu T, Sato N, Todo S, Miyasaka K (2003) Alveolar echinococcosis: MR findings in the liver. Radiology 228(1):172–177

Kratzer W, Gruener B, Kaltenbach TE, Ansari-Bitzenberger S, Kern P, Fuchs M, Mason RA, Barth TF, Haenle MM, Hillenbrand A, Oeztuerk S, Graeter T (2015) Proposal of an ultrasonographic classification for hepatic alveolar echinococcosis: Echinococcosis multilocularis Ulm classification-ultrasound. World J Gastroenterol 21(43):12392–12402

Larrieu EJ, Costa MT, del Carpio M, Moguillansky S, Bianchi G, Yadon ZE (2002) A case-control study of the risk factors for cystic echinococcosis among the children of Rio Negro province, Argentina. Ann Trop Med Parasitol 96(1):43–52

Larrieu E, Del Carpio M, Salvitti JC, Mercapide C, Sustersic J, Panomarenko H, Costa M, Bigatti R, Labanchi J, Herrero E, Cantoni G, Perez A, Odriozola M (2004) Ultrasonographic diagnosis and medical treatment of human cystic echinococcosis in asymptomatic school age carriers: 5 years of follow-up. Acta Trop 91(1):5–13

Larrieu E, Mujica G, Araya D, Labanchi JL, Arezo M, Herrero E, Santillan G, Vizcaychipi K, Uchiumi L, Salvitti JC, Grizmado C, Calabro A, Talmon G, Sepulveda L, Galvan JM, Cabrera M, Seleiman M, Crowley P, Cespedes G, Cachau MG, Gino L, Molina L, Daffner J, Gauci CG, Donadeu M, Lightowlers MW (2019a) Pilot field trial of the EG95 vaccine against ovine cystic echinococcosis in Rio Negro, Argentina: 8 years of work. Acta Trop 191:1–7

Larrieu E, Uchiumi L, Salvitti JC, Sobrino M, Panomarenko O, Tissot H, Mercapide CH, Sustercic J, Arezo M, Mujica G, Herrero E, Labanchi JL, Grizmado C, Araya D, Talmon G, Galvan JM, Sepulveda L, Seleiman M, Cornejo T, Echenique H, Del Carpio M (2019b) Epidemiology, diagnosis, treatment and follow-up of cystic echinococcosis in asymptomatic carriers (vol 113, pg 74, 2019). Trans R Soc Trop Med Hyg 113(5):291–291

Lawn SD, Bligh J, Craig PS, Chiodini PL (2004) Human cystic echinococcosis: evaluation of post-treatment serologic follow-up by IgG subclass antibody detection. Am J Trop Med Hyg 70:329–335

Lawson JR, Gemmell MA (1990) Transmission of taeniid tapeworm eggs via blowflies to intermediate hosts. Parasitology 100(Pt 1):143–146

Li T, Ito A, Chen X, Sako Y, Qiu J, Xiao N, Qiu D, Nakao M, Yanagida T, Craig PS (2010) Specific IgG responses to recombinant antigen B and em18 in cystic and alveolar echinococcosis in china. Clin Vaccine Immunol 17:470–475

Li T, Ito A, Pengcuo R, Sako Y, Chen X, Qiu D, Xiao N, Craig PS (2011a) Post-treatment follow-up study of abdominal cystic echinococcosis in Tibetan communities of northwest Sichuan Province, China. PLoS Negl Trop Dis 5(10):e1364

Li RY, Peng Q, Jia B, Shi GQ, Zhao ZS, Shen H, Li HT (2011b) Antibody and cytokine responses to hydatid in experimentally infected Kazakh sheep with hydatidosis resistance haplotype. Parasitol Res 108:1131–1137

Li Y, Zheng H, Cao X, Liu Z, Chen L (2011c) Demographic and clinical characteristics of patients with anaphylactic shock after surgery for cystic echinococcosis. Am J Trop Med Hyg 85:452–5

Li Y, Xu H, Chen J, Gan W, Wu W, Hu X (2012) Gene cloning, expression, and localization of antigen 5 in the life cycle of Echinococcus granulosus. Parasitol Res 110:2315–2323

Liance M, Vuitton DA, Guerret-Stocker S, Carbillet JP, Grimaud JA, Houin R (1984) Experimental alveolar echinococcosis. Suitability of a murine model of intrahepatic infection by Echinococcus multilocularis for immunological studies. Experientia 40(12):1436–1439

Liance M, Bresson-Hadni S, Meyer JP, Houin R, Vuitton DA (1990) Cellular immunity in experimental Echinococcus multilocularis infection. I. Sequential and comparative study of specific in vivo delayed-type hypersensitivity against E. multilocularis antigens in resistant and sensitive mice. Clin Exp Immunol 82:373–377

Liance M, Bresson-Hadni S, Vuitton DA, Lenys D, Carbillet JP, Houin R (1992) Effects of cyclosporin A on the course of murine alveolar echinococcosis and on specific cellular and humoral immune responses against Echinococcus multilocularis. Int J Parasitol 22:23–28

Liance M, Ricard-Blum S, Emery I, Houin R, Vuitton DA (1998) Echinococcus multilocularis infection in mice: in vivo treatment with a low dose of IFN-gamma decreases metacestode growth and liver fibrogenesis. Parasite 5:231–237

Liance M, Janin V, Bresson-Hadni S, Vuitton DA, Houin R, Piarroux R (2000) Immunodiagnosis of Echinococcus infections: confirmatory testing and species differentiation by a new commercial Western Blot. J Clin Microbiol 38:3718–3721

Lightowlers MW (2010) Fact or hypothesis: concomitant immunity in taeniid cestode infections. Parasite Immunol 32(8):582–589

Lin G, Todeschini AR, Koizumi A, Neves JL, Gonzalez H, Dematteis S, Hada N, Previato JO, Ferreira F, Mendonca-Previato L, Diaz A (2013) Further structural characterization of the Echinococcus granulosus laminated layer carbohydrates: the blood-antigen P1-motif gives rise to branches at different points of the O-glycan chains. Glycobiology 23:438–452

Lissandrin R, Tamarozzi F, Piccoli L, Tinelli C, De Silvestri A, Mariconti M, Meroni V, Genco F, Brunetti E (2016) Factors influencing the serological response in hepatic Echinococcus granulosus infection. Am J Trop Med Hyg 94(1):166–171

Lissandrin R, Tamarozzi F, Mariconti M, Manciulli T, Brunetti E, Vola A (2018) Watch and wait approach for inactive Echinococcal cyst of the liver: an update. Am J Trop Med Hyg 99(2): 375–379

Liu W, Delabrousse E, Blagosklonov O, Wang J, Zeng H, Jiang Y, Qin Y, Vuitton DA, Wen H (2014) Innovation in hepatic alveolar echinococcosis imaging: best use of old tools, and necessary evaluation of new ones. Parasite 21:74

Lotsch F, Naderer J, Skuhala T, Groger M, Auer H, Kaczirek K, Waneck F, Ramharter M (2016) Intra-cystic concentrations of albendazole-sulphoxide in human cystic echinococcosis: a systematic review and analysis of individual patient data. Parasitol Res 115(8):2995–3001

Macpherson CNL, Bartholomot B, Frider B (2003a) Application of ultrasound in diagnosis, treatment, epidemiology, public health and control of Echinococcus granulosus and E. multilocularis. Parasitology 127(Suppl):21–35

Macpherson CNL, Vuitton DA, Gharbi HA, Caremani M, Frider B, Brunettii E, Perdomo R, Schantz PM, Felice C, Teggi A et al (2003b) International classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiological settings. Acta Trop 85:253–261. https://doi.org/10.1016/S0001-706X(02)00223-1

Manciulli T, Enriquez-Laurente R, Tamarozzi F, Lissandrin R, Elizalde M, Sedano C, Bardales K, Vola A, De Silvestri A, Tinelli C, Brunetti E, Santivanez S, Mariconti M (2021) Field performance of a rapid diagnostic test for the serodiagnosis of abdominal cystic echinococcosis in the Peruvian highlands. Am J Trop Med Hyg 105(1):181–187

Manterola C, Barroso M, Vial M, Bustos L, Munoz S, Losada H, Bello N, Hernandez F, Carrasco R (2003) Liver abscess of hydatid origin: clinical features and results of aggressive treatment. ANZ J Surg 73:220–224

Marco M, Baz A, Fernandez C, Gonzalez G, Hellman U, Salinas G, Nieto A (2006) A relevant enzyme in granulomatous reaction, active matrix metalloproteinase-9, found in bovine Echinococcus granulosus hydatid cyst wall and fluid. Parasitol Res 100:131–139

Matoff K, Kolev G (1964) The role of the hairs, muzzle and paws of Echinococcic dogs in the epidemiology of Echinococcosis. Z Tropenmed Parasitol 15:452–460

McManus DP (2013) Current status of the genetics and molecular taxonomy of Echinococcus species. Parasitology 140(13):1617–1623

Mejri N, Gottstein B (2009) Echinococcus multilocularis metacestode metabolites contain a cysteine protease that digests eotaxin, a CC pro-inflammatory chemokine. Parasitol Res 105: 1253–1260

Men S, Yucesoy C, Edguer TR, Hekimoglu B (2006) Percutaneous treatment of giant abdominal hydatid cysts: long-term results. Surg Endosc 20:1600–1606

Menezes da Silva A (2003) Hydatid cyst of the liver-criteria for the selection of appropriate treatment. Acta Trop 85:237–242

Mezioug D, Touil-Boukoffa C (2009) Cytokine profile in human hydatidosis: possible role in the immunosurveillance of patients infected with Echinococcus granulosus. Parasite 16:57–64

Miguez M, Baz A, Nieto A (1996) Carbohydrates on the surface of Echinococcus granulosus protoscoleces are immunodominant in mice. Parasite Immunol 18:559–569

Monge-Maillo B, Chamorro Tojeiro S, López-Vélez R (2017) Management of osseous cystic echinococcosis. Expert Rev Anti-Infect Ther 15(12):1075–1082

Monge-Maillo B, Olmedo Samperio M, Pérez-Molina JA, Norman F, Mejía CR, Tojeiro SC, López-Vélez R (2019) Osseous cystic echinococcosis: a case series study at a referral unit in Spain. PLoS Negl Trop Dis 13(2):e0007006

Monteiro KM, De Carvalho MO, Zaha A, Ferreira HB (2010) Proteomic analysis of the Echinococcus granulosus metacestode during infection of its intermediate host. Proteomics 10:1985–1999

Moro PL, Gilman RH, Verastegui M, Bern C, Silva B, Bonilla JJ (1999) Human hydatidosis in the central Andes of Peru: evolution of the disease over 3 years. Clin Infect Dis 29:807–812

Mourglia-Ettlin G, Amezcua-Vesely MC, Fraga R, Baz A, Merino MC, Gruppi A, Dematteis S (2011a) Echinococcus granulosus glycoconjugates induce peritoneal B cell differentiation into antibody-secreting cells and cytokine production. Parasite Immunol 33:621–631

Mourglia-Ettlin G, Marques JM, Chabalgoity JA, Dematteis S (2011b) Early peritoneal immune response during Echinococcus granulosus establishment displays a biphasic behavior. PLoS Negl Trop Dis 5:e1293

Mueller PR, Dawson SL, Ferrucci JT Jr, Nardi GL (1985) Hepatic echinococcal cyst: successful percutaneous drainage. Radiology 155:627–628

Nahmias J, Goldsmith R, Soibelman M, El-On J (1994) Three- to 7-year follow-up after albendazole treatment of 68 patients with cystic echinococcosis (hydatid disease). Ann Trop Med Parasitol 88:295–304

Nakao M, McManus DP, Schantz PM, Craig PS, Ito A (2007) A molecular phylogeny of the genus Echinococcus inferred from complete mitochondrial genomes. Parasitology 134(Pt 5):713–722

Nasseri Moghaddam S, Abrishami A, Malekzadeh R (2006) Percutaneous needle aspiration, injection, and reaspiration with or without Benzimidazole coverage for uncomplicated hepatic hydatid cysts. Cochrane Database Syst Rev CD003623

Neumayr A, Troia G, De Bernardis C, Tamarozzi F, Goblirsch S, Piccoli L, Hatz C, Filice C, Brunetti E (2011) Justified concern or exaggerated fear: the risk of anaphylaxis in percutaneous treatment of cystic echinococcosis-a systematic literature review. PLoS Negl Trop Dis 5:e1154

Neumayr A, Tamarozzi F, Goblirsch S, Blum J, Brunetti E (2013a) Spinal cystic echinococcosis – a systematic analysis and review of the literature: part 2. Treatment, follow-up and outcome. PLoS Negl Trop Dis 7:e2458

Neumayr A, Tamarozzi F, Goblirsch S, Blum J, Brunetti E (2013b) Spinal cystic echinococcosis – a systematic analysis and review of the literature: part 1. Epidemiology and anatomy. PLoS Negl Trop Dis 7:e2450

Nourbakhsh A, Vannemreddy P, Minagar A, Toledo EG, Palacios E, Nanda A (2010a) Hydatid disease of the central nervous system: a review of literature with an emphasis on Latin

Nourbakhsh A, Vannemreddy P, Minagar A, Toledo EG, Palacios E, Nanda A (2010b) Hydatid disease of the central nervous system: a review of literature with an emphasis on Latin American countries. Neurol Res 32:245–251

Paredes R, Godoy P, Rodriguez B et al (2011) Bovine (Bos taurus) humoral immune response against Echinococcus granulosus and hydatid cyst infertility. J Cell Biochem 112:189–199

Oral A, Yigiter M, Yildiz A, Yalcin O, Dikmen T, Eren S, Kantarci M, Salman AB (2012) Diagnosis and management of hydatid liver disease in children: a report of 156 patients with hydatid disease. J Pediatr Surg 47:528–534

Örmeci N (2014) PAIR vs Örmeci technique for the treatment of hydatid cyst 2014:358–364

Ortona E, Rigano R, Margutti P, Notargiacomo S, Ioppolo S, Vaccari S, Barca S, Buttari B, Profumo E, Teggi A, Siracusano A (2000) Native and recombinant antigens in the immunodiagnosis of human cystic echinococcosis. Parasite Immunol 22:553–559

Ortona E, Margutti P, Delunardo F, Nobili V, Profumo E, Rigano R, Buttari B, Carulli G, Azzara A, Teggi A, Bruschi F, Siracusano A (2005) Screening of an Echinococcus granulosus cDNA library with IgG4 from patients with cystic echinococcosis identifies a new tegumental protein involved in the immune escape. Clin Exp Immunol 142:528–538

Pan W, Zhou HJ, Shen YJ, Wang Y, Xu YX, Hu Y, Jiang YY, Yuan ZY, Ugwu CE, Cao JP (2013) Surveillance on the status of immune cells after Echinococcus granulosus protoscoleces infection in Balb/c mice. PLoS One 8:e59746

Papanikolaou A (2008) Osseous hydatid disease. Iran J Parasitol 3(4):60–64. https://doi.org/10.1016/j.trstmh.2007.09.012

Pedrosa I, Saiz A, Arrazola J, Ferreiros J, Pedrosa CS (2000) Hydatid disease: radiologic and pathologic features and complications. Radiographics 20(3):795–817

Petrone L, Vanini V, Petruccioli E, Ettorre GM, Busi Rizzi E, Schinina V, Girardi E, Ludovisi A, Gomez-Morales MA, Pozio E, Teggi A, Goletti D (2015) IL-4 specific-response in whole blood associates with human Cystic Echinococcosis and cyst activity. J Infect 70(3):299–306

Piarroux M, Piarroux R, Giorgi R, Knapp J, Bardonnet K, Sudre B, Watelet J, Dumortier J, Gerard A, Beytout J, Abergel A, Mantion G, Vuitton DA, Bresson-Hadni S (2011) Clinical features and evolution of alveolar echinococcosis in France from 1982 to 2007: results of a survey in 387 patients. J Hepatol 55:1025–1033

Piarroux M, Piarroux R, Knapp J, Bardonnet K, Dumortier J, Watelet J, Gerard A, Beytout J, Abergel A, Bresson-Hadni S, Gaudart J (2013) Populations at risk for alveolar echinococcosis, France. Emerg Infect Dis 19(5):721–728

Piccoli L, Meroni V, Genco F, Tamarozzi F, Tinelli C, Filice C, Brunetti E (2012) Serum cytokine profile by ELISA in patients with echinococcal cysts of the liver: a stage-specific approach to assess their biological activity. Clin Dev Immunol 2012:483935

Piccoli L, Tamarozzi F, Cattaneo F, Mariconti M, Filice C, Bruno A, Brunetti E (2014) Long-term sonographic and serological follow-up of inactive echinococcal cysts of the liver: hints for a "watch-and-wait" approach. PLoS Negl Trop Dis 8(8):e3057

Pleydell DR, Raoul F, Tourneux F, Danson FM, Graham AJ, Craig PS, Giraudoux P (2004) Modelling the spatial distribution of Echinococcus multilocularis infection in foxes. Acta Trop 91(3):253–265

Polat P, Kantarci M, Alper F, Suma S, Koruyucu MB, Okur A (2003) Hydatid disease from head to toe. Radiographics 23:475–94, quiz 536–7

Poretti D, Felleisen E, Grimm F, Pfister M, Teuscher F, Zuercher C, Reichen J, Gottstein B (1999) Differential immunodiagnosis between cystic hydatid disease and other cross-reactive pathologies. Am J Trop Med Hyg 60:193–198

Possenti A, Manzano-Roman R, Sanchez-Ovejero C, Boufana B, La Torre G, Siles-Lucas M, Casulli A (2016) Potential risk factors associated with human cystic echinococcosis: systematic review and meta-analysis. PLoS Negl Trop Dis 10(11):e0005114

Prousalidis J, Kosmidis C, Anthimidis G, Fachantidis E, Harlaftis N, Aletras H (2008) Forty-four years' experience (1963–2006) in the management of primarily infected hydatid cyst of the liver. HPB (Oxford) 10:18–24

Prousalidis J, Kosmidis C, Anthimidis G, Kapoutzis K, Karamanlis E, Fachantidis E (2012) Postoperative recurrence of cystic hydatidosis. Can J Surg 55:15–20

Ramia JM, Serrablo A, De la Plaza R, Esarte J, Gijon L, Sarria L, Figueras J, Garcia-Parreno J (2014) Is radical surgery feasible in liver hydatid cysts in contact with the inferior vena cava? World J Surg 38(11):2940–2945

Ramos AL, Discipio RG, Ferreira AM (2006) Eosinophil cationic protein damages protoscoleces in vitro and is present in the hydatid cyst. Parasite Immunol 28:347–355

Rau ME, Tanner CE (1975) BCG suppresses growth and metastasis of hydatid infections. Nature 256:318–319

Rausch RL, Wilson JF, Schantz PM (1990) A programme to reduce the risk of infection by Echinococcus multilocularis: the use of praziquantel to control the cestode in a village in the hyperendemic region of Alaska. Ann Trop Med Parasitol 84:239–250

Refik M, Mehmet N, Durmaz R (2005) Postoperative changes in serum cytokines profile and nitric oxide levels in patients with cystic echinococcosis. Parasite 12:265–269

Rehmann P, Grone A, Gottstein B, Sager H, Muller N, Vollm J, Bacciarini LN (2005) Alveolar echinococcosis in the zoological garden Basle. Schweiz Arch Tierheilkd 147(11):498–502

Reuter S, Jensen B, Buttenschoen K, Kratzer W, Kern P (2000) Benzimidazoles in the treatment of alveolar echinococcosis: a comparative study and review of the literature. J Antimicrob Chemother 46:451–456

Reuter S, Nussle K, Kolokythas O, Haug U, Rieber A, Kern P, Kratzer W (2001) Alveolar liver echinococcosis: a comparative study of three imaging techniques. Infection 29:119–125

Reuter S, Buck A, Manfras B, Kratzer W, Seitz HM, Darge K, Reske SN, Kern P (2004) Structured treatment interruption in patients with alveolar echinococcosis. Hepatology 39:509–517

Reyes MM, Taramona CP, Saire-Mendoza M, Gavidia CM, Barron E, Boufana B, Craig PS, Tello L, Garcia HH, Santivanez SJ (2012) Human and canine echinococcosis infection in informal, unlicensed abattoirs in Lima, Peru. PLoS Negl Trop Dis 6(4):e1462

Ricard-Blum S, Bresson-Hadni S, Guerret S, Grenard P, Volle PJ, Risteli L, Grimaud JA, Vuitton DA (1996) Mechanism of collagen network stabilization in human irreversible granulomatous liver fibrosis. Gastroenterology 111:172–182

Rigano R, Profumo E, Di Felice G, Ortona E, Teggi A, Siracusano A (1995a) In vitro production of cytokines by peripheral blood mononuclear cells from hydatid patients. Clin Exp Immunol 99: 433–439

Rigano R, Profumo E, Ioppolo S, Notargiacomo S, Ortona E, Teggi A, Siracusano A (1995b) Immunological markers indicating the effectiveness of pharmacological treatment in human hydatid disease. Clin Exp Immunol 102:281–285

Rigano R, Profumo E, Buttari B, Teggi A, Siracusano A (1999a) Cytokine gene expression in peripheral blood mononuclear cells (PBMC) from patients with pharmacologically treated cystic echinococcosis. Clin Exp Immunol 118:95–101

Rigano R, Profumo E, Ioppolo S, Notargiacomo S, Teggi A, Siracusano A (1999b) Serum cytokine detection in the clinical follow up of patients with cystic echinococcosis. Clin Exp Immunol 115:503–507

Rigano R, Profumo E, Bruschi F, Carulli G, Azzara A, Ioppolo S, Buttari B, Ortona E, Margutti P, Teggi A, Siracusano A (2001) Modulation of human immune response by Echinococcus granulosus antigen B and its possible role in evading host defenses. Infect Immun 69:288–96 R

Rigano R, Ioppolo S, Ortona E, Margutti P, Profumo E, Ali MD, Di Vico B, Teggi A, Siracusano A (2002) Long-term serological evaluation of patients with cystic echinococcosis treated with benzimidazole carbamates. Clin Exp Immunol 129:485–492

Rigano R, Buttari B, De Falco E, Profumo E, Ortona E, Margutti P, Scotta C, Teggi A, Siracusano A (2004) Echinococcus granulosus-specific T-cell lines derived from patients at various clinical stages of cystic echinococcosis. Parasite Immunol 26:45–52

Rigano R, Buttari B, Profumo E, Ortona E, Delunardo F, Margutti P, Mattei V, Teggi A, Sorice M, Siracusano A (2007) Echinococcus granulosus antigen B impairs human dendritic cell differentiation and polarizes immature dendritic cell maturation towards a Th2 cell response. Infect Immun 75:1667–1678

Riley EM, Dixon JB, Jenkins P, Ross G (1986) Echinococcus granulosus infection in mice: host responses during primary and secondary infection. Parasitology 92(Pt 2):391–403

Rinaldi F, Brunetti E, Neumayr A, Maestri M, Goblirsch S, Tamarozzi F (2014) Cystic echinococcosis of the liver: A primer for hepatologists. World J Hepatol 6(5):293–305

Robardet E, Giraudoux P, Caillot C, Augot D, Boue F, Barrat J (2011) Fox defecation behaviour in relation to spatial distribution of voles in an urbanised area: An increasing risk of transmission of Echinococcus multilocularis? Int J Parasitol 41(2):145–154

Roberts MG, Aubert MF (1995) A model for the control of Echinococcus multilocularis in France. Vet Parasitol 56:67–74

Rogan MT (1998) T-cell activity associated with secondary infections and implanted cysts of Echinococcus granulosus in BALB/c mice. Parasite Immunol 20:527–533

Rogan MT, Craig PS, Zehyle E, Masinde G, Wen H, Zhou P (1992) In vitro killing of taeniid oncospheres, mediated by human sera from hydatid endemic areas. Acta Trop 51:291–296

Rogan MT, Marshall I, Reid GD, Macpherson CN, Craig PS (1993) The potential of vervet monkeys (Cercopithecus aethiops) and baboons (Papio anubis) as models for the study of the immunology of Echinococcus granulosus infections. Parasitology 106(Pt 5):511–517

Rogan MT, Hai WY, Richardson R, Zeyhle E, Craig PS (2006) Hydatid cysts: does every picture tell a story? Trends Parasitol

Romig T (2009) Echinococcus multilocularis in Europe – state of the art. Vet Res Commun 33 (Suppl 1):31–34

Romig T, Zeyhle E, Macpherson CN, Rees PH, Were JB (1986) Cyst growth and spontaneous cure in hydatid disease. Lancet 1(8485):861

Romig T, Ebi D, Wassermann M (2015) Taxonomy and molecular epidemiology of Echinococcus granulosus sensu lato. Vet Parasitol 213(3–4):76–84

Romig T, Deplazes P, Jenkins D, Giraudoux P, Massolo A, Craig PS, Wassermann M, Takahashi K, de la Rue M (2017) Ecology and life cycle patterns of echinococcus species. Adv Parasitol 95:213–314

Saimot AG, Meulemans A, Cremieux AC, Giovanangeli MD, Hay JM, Delaitre B, Coulaud JP (1983) Albendazole as a potential treatment for human hydatidosis. Lancet 2:652–656

Sakamoto T, Cabrera PA (2003) Immunohistochemical observations on cellular response in unilocular hydatid lesions and lymph nodes of cattle. Acta Trop 85(2):271–279

Sako Y, Tappe D, Fukuda K, Kobayashi Y, Itoh S, Frosch M, Gruner B, Kern P, Ito A (2011) Immunochromatographic test with recombinant Em18 antigen for the follow-up study of alveolar echinococcosis. Clin Vaccine Immunol 18:1302–1305

Salinas JL, Vildozola Gonzales H, Astuvilca J, Arce-Villavicencio Y, Carbajal-Gonzalez D, Talledo L, Willig JH (2011) Long-term albendazole effectiveness for hepatic cystic echinococcosis. Am J Trop Med Hyg 85:1075–1079

Sanchez F, March F, Mercader M, Coll P, Munoz C, Prats G (1991) Immunochemical localization of major hydatid fluid antigens in protoscoleces and cysts of Echinococcus granulosus from human origin. Parasite Immunol 13:583–592

Santivanez S, Garcia HH (2010) Pulmonary cystic echinococcosis. Curr Opin Pulm Med 16(3): 257–261

Santivanez SJ, Arias P, Portocarrero M, Rodriguez S, Gonzalez AE, Gilman RH, Gavidia CM, Garcia HH (2012) Serological diagnosis of lung cystic hydatid disease using the synthetic p176 peptide. Clin Vaccine Immunol 19:944–947

Sarciron ME, Delabre I, Walbaum S, Raynaud G, Petavy AF (1992) Effects of multiple doses of isoprinosine on Echinococcus multilocularis metacestodes. Antimicrob Agents Chemother 36: 191–194

Sato N, Uchino J, Takahashi M, Aoki S, Takahashi H, Yamashita K, Matsushita M, Suzuki K, Namieno T (1997) Surgery and outcome of alveolar echinococcosis of the liver: historical comparison of mass screening systems in Japan. Int Surg 82:201–204

Scharf G, Deplazes P, Kaser-Hotz B, Borer L, Hasler A, Haller M, Fluckiger M (2004) Radiographic, ultrasonographic, and computed tomographic appearance of alveolar echinococcosis in dogs. Vet Radiol Ultrasound 45(5):411–418

Schipper HG, Lameris JS, Van Delden OM, Rauws EA, Kager PA (2002) Percutaneous evacuation (PEVAC) of multivesicular echinococcal cysts with or without cystobiliary fistulas which contain non-drainable material: first results of a modified PAIR method. Gut 50:718–723

Schweiger A, Grimm F, Tanner I, Mullhaupt B, Bertogg K, Muller N, Deplazes P (2012) Serological diagnosis of echinococcosis: the diagnostic potential of native antigens. Infection 40:139–152

Severi MA, Ferragut G, Nieto A (1997) Antibody response of Echinococcus granulosus infected mice: protoscolex specific response during infection is associated with decreasing specific IgG1/IgG3 ratio as well as decreasing avidity. Parasite Immunol 19:545–552

Shambesh MK, Craig PS, Wen H, Rogan MT, Paolillo E (1997) IgG1 and IgG4 serum antibody responses in asymptomatic and clinically expressed cystic echinococcosis patients. Acta Trop 64:53–63

Shan JY, Ji WZ, Li HT, Tuxun T, Lin RY, Wen H (2011) TLR2 and TLR4 expression in peripheral blood mononuclear cells of patients with chronic cystic echinococcosis and its relationship with IL-10. Parasite Immunol 33:692–696

Shepherd JC, Aitken A, Mcmanus DP (1991) A protein secreted in vivo by Echinococcus granulosus inhibits elastase activity and neutrophil chemotaxis. Mol Biochem Parasitol 44: 81–90

Siles-Lucas MM, Gottstein BB (2001) Molecular tools for the diagnosis of cystic and alveolar echinococcosis. Tropical Med Int Health 6:463–475

Siles-Lucas M, Casulli A, Conraths FJ, Muller N (2017) Laboratory diagnosis of Echinococcus spp. in human patients and infected animals. Adv Parasitol 96:159–257

Siracusano A, Teggi A, Quintieri F, Notargiacomo S, De Rosa F, Vicari G (1988) Cellular immune responses of hydatid patients to Echinococcus granulosus antigens. Clin Exp Immunol 72:400–405

Siracusano A, Margutti P, Delunardo F, Profumo E, Rigano R, Buttari B, Teggi A, Ortona E (2008) Molecular cross-talk in host-parasite relationships: the intriguing immunomodulatory role of Echinococcus antigen B in cystic echinococcosis. Int J Parasitol 38:1371–1376

Smego RA Jr, Sebanego P (2005) Treatment options for hepatic cystic echinococcosis. Int J Infect Dis 9:69–76

Smego RA Jr, Bhatti S, Khaliq AA, Beg MA (2003) Percutaneous aspiration-injection-reaspiration drainage plus albendazole or mebendazole for hepatic cystic echinococcosis: a meta-analysis. Clin Infect Dis 37:1073–1083

Sobrino JM, Pulpón LA, Crespo MG, Silva L, Segovia J, Serrano-Fiz S, Burgos R, Montero CG, Perafán A, Téllez G (1993) Heart transplantation in a patient with liver hydatidosis (1053–2498 (Print))

Solomon N, Kachani M, Zeyhle E, Macpherson CNL (2017a) The natural history of cystic echinococcosis in untreated and albendazole-treated patients. Acta Trop 171:52–57

Solomon N, Zeyhle E, Carter J, Wachira J, Mengiste A, Romig T, Fields PJ, Macpherson CNL (2017b) Cystic Echinococcosis in Turkana, Kenya: the role of cross-sectional screening surveys in assessing the prevalence of human infection. Am J Trop Med Hyg 97(2):587–595

Solomon N, Zeyhle E, Subramanian K, Fields PJ, Romig T, Kern P, Carter JY, Wachira J, Mengiste A, Macpherson CNL (2018) Cystic echinococcosis in Turkana, Kenya: 30 years of imaging in an endemic region. Acta Trop 178:182–189

Sonmez K, Karabulut R, Turkyilmaz Z, Basaklar AC, Kale N (2007) Clear cystic fluid in hepatic hydatidosis does not rule out communication between cysts and the biliary system. Adv Ther 24: 291–295

Spotin A, Majdi MM, Sankian M, Varasteh A (2012) The study of apoptotic bifunctional effects in relationship between host and parasite in cystic echinococcosis: a new approach to suppression and survival of hydatid cyst. Parasitol Res 110:1979–1984

Steers NJ, Rogan MT, Heath S (2001) In-vitro susceptibility of hydatid cysts of Echinococcus granulosus to nitric oxide and the effect of the laminated layer on nitric oxide production. Parasite Immunol 23:411–417

Stojkovic M, Zwahlen M, Teggi A, Vutova K, Cretu CM, Virdone R, Nicolaidou P, Cobanoglu N, Junghanss T (2009) Treatment response of cystic echinococcosis to benzimidazoles: a systematic review. PLoS Negl Trop Dis 3(9):e524

Stojkovic M, Rosenberger K, Kauczor HU, Junghanss T, Hosch W (2012) Diagnosing and staging of cystic Echinococcosis: how do CT and MRI perform in comparison to ultrasound? PLoS Negl Trop Dis 6(10):1–8

Stojkovic M, Mickan C, Weber TF, Junghanss T (2015) Pitfalls in diagnosis and treatment of alveolar echinococcosis: a sentinel case series. BMJ Open Gastroenterol 2(1):e000036

Stojkovic M, Rosenberger KD, Steudle F, Junghanss T (2016) Watch and wait management of inactive cystic echinococcosis – does the path to inactivity matter – analysis of a prospective patient cohort. PLoS Negl Trop Dis 10(12):1–10. https://doi.org/10.1371/journal.pntd.0005243

Sturm D, Menzel J, Gottstein B, Kern P (1995) Interleukin-5 is the predominant cytokine produced by peripheral blood mononuclear cells in alveolar echinococcosis. Infect Immun 63:1688–97

Sunita T, Khurana S, Malla N, Dubey ML (2011) Immunodiagnosis of cystic echinococcosis by antigen detection in serum, urine, and saliva samples. Trop Parasitol 1:33–38

Taherkhani H, Zeyhle E, Rogan MT (2007) Antibody responses in human cystic hydatid disease to the laminated layer of Echinococcus granulosus. Parasitol Res 101:647–652

Tamarozzi F, Meroni V, Genco F, Piccoli L, Tinelli C, Filice C, Brunetti E (2010) Ex vivo assessment of serum cytokines in patients with cystic echinococcosis of the liver. Parasite Immunol 32:696–700

Tamarozzi F, Vuitton L, Brunetti E, Vuitton DA, Koch S (2014) Non-surgical and non-chemical attempts to treat echinococcosis: do they work? Parasite 21:75

Tamarozzi F, Covini I, Mariconti M, Narra R, Tinelli C, De Silvestri A, Manzoni F, Casulli A, Ito A, Neumayr A, Brunetti E (2016) Comparison of the diagnostic accuracy of three rapid tests for the serodiagnosis of hepatic cystic echinococcosis in humans. PLoS Negl Trop Dis 10(2): e0004444

Tamarozzi F, Akhan O, Cretu CM, Vutova K, Akinci D, Chipeva R, Ciftci T, Constantin CM, Fabiani M, Golemanov B, Janta D, Mihailescu P, Muhtarov M, Orsten S, Petrutescu M, Pezzotti P, Popa AC, Popa LG, Popa MI, Velev V, Siles-Lucas M, Brunetti E, Casulli A (2018) Prevalence of abdominal cystic echinococcosis in rural Bulgaria, Romania, and Turkey: a cross-sectional, ultrasound-based, population study from the HERACLES project. Lancet Infect Dis 18(7):769–778

Tamarozzi F, Akhan O, Cretu CM, Vutova K, Fabiani M, Orsten S, Pezzotti P, Popa GL, Velev V, Siles-Lucas M, Brunetti E, Casulli A (2019) Epidemiological factors associated with human cystic echinococcosis: a semi-structured questionnaire from a large population-based ultrasound cross-sectional study in eastern Europe and Turkey. Parasit Vectors 12(1):371

Tamarozzi F, Deplazes P, Casulli A (2020a) Reinventing the wheel of Echinococcus granulosus sensu lato transmission to humans. Trends Parasitol 36(5):427–434

Tamarozzi F, Horton J, Muhtarov M, Ramharter M, Siles-Lucas M, Gruener B, Vuitton DA, Bresson-Hadni S, Manciulli T, Brunetti E (2020b) A case for adoption of continuous albendazole treatment regimen for human echinococcal infections. PLoS Negl Trop Dis 14(9):e0008566

Tamarozzi F, Silva R, Fittipaldo VA, Buonfrate D, Gottstein B, Siles-Lucas M. Serology for the diagnosis of human hepatic cystic echinococcosis and its relation with cyst staging: A systematic review of the literature with meta-analysis. Basáñez M-G, ed. PLoS Negl Trop Dis. 2021a;15(4):e0009370. https://doi.org/10.1371/journal.pntd.0009370

Tamarozzi F, Longoni SS, Vola A, Degani M, Tais S, Rizzi E, Prato M, Scarso S, Silva R, Brunetti E, Bisoffi Z, Perandin F (2021b) Evaluation of nine commercial serological tests for the diagnosis of human hepatic cyst echinococcosis and the differential diagnosis with other focal liver lesions: a diagnostic accuracy study. Diagnostics (Basel) 11(2)

Tamarozzi F, Silva R, Fittipaldo VA, Buonfrate D, Gottstein B, Siles-Lucas M (2021c) Serology for the diagnosis of human hepatic cystic echinococcosis and its relation with cyst staging: a systematic review of the literature with meta-analysis. PLoS Negl Trop Dis 15(4):e0009370

Tao S, Qin Z, Hao W, Yongquan L, Lanhui Y, Lei Y (2011) Usefulness of gray-scale contrast-enhanced ultrasonography (SonoVue(R)) in diagnosing hepatic alveolar echinococcosis. Ultrasound Med Biol 37:1024–1028

Tappe D, Frosch M, Sako Y, Itoh S, Gruner B, Reuter S, Nakao M, Ito A, Kern P (2009) Close relationship between clinical regression and specific serology in the follow-up of patients with alveolar echinococcosis in different clinical stages. Am J Trop Med Hyg 80:792–797

Tappe D, Sako Y, Itoh S, Frosch M, Gruner B, Kern P, Ito A (2010) Immunoglobulin G subclass responses to recombinant Em18 in the follow-up of patients with alveolar echinococcosis in different clinical stages. Clin Vaccine Immunol 17:944–948

Taranto D, Beneduce F, Vitale LM, Loguercio C, Del Vecchio BC (1995) Chemical sclerosing cholangitis after injection of scolicidal solution. Ital J Gastroenterol 27:78–79

Teggi A, Lastilla MG, De Rosa F (1993) Therapy of human hydatid disease with mebendazole and albendazole. Antimicrob Agents Chemother 37:1679–1684

Teggi A, Giattino M, Franchi C, Lastilla M (1997) A hypothesis on the significance of an increase in serum transaminases in patients with hydatidosis treated with benzimidazole carbamates. Recenti Prog Med 88:452–458

Thompson RC (2017) Biology and systematics of Echinococcus. Adv Parasitol 95:65–109

Thompson RC, Kumaratilake LM, Eckert J (1984) Observations on Echinococcus granulosus of cattle origin in Switzerland. Int J Parasitol 14(3):283–291

Todorov T, Mechkov G, Vutova K, Georgiev P, Lazarova I, Tonchev Z, Nedelkov G (1992a) Factors influencing the response to chemotherapy in human cystic echinococcosis. Bull World Health Organ 70:347–358

Todorov T, Vutova K, Mechkov G, Georgiev P, Petkov D, Tonchev Z, Nedelkov G (1992b) Chemotherapy of human cystic echinococcosis: comparative efficacy of mebendazole and albendazole. Ann Trop Med Parasitol 86:59–66

Torcal J, Navarro-Zorraquino M, Lozano R, Larrad L, Salinas JC, Ferrer J, Roman J, Pastor C (1996) Immune response and in vivo production of cytokines in patients with liver hydatidosis. Clin Exp Immunol 106:317–322

Torgerson PR (2006) Canid immunity to Echinococcus spp.: impact on transmission. Parasite Immunol 28:295–303

Torgerson PR, Schweiger A, Deplazes P, Pohar M, Reichen J, Ammann RW, Tarr PE, Halkik N, Mullhaupt B (2008) Alveolar echinococcosis: from a deadly disease to a well-controlled

infection. Relative survival and economic analysis in Switzerland over the last 35 years. J Hepatol 49(1):72–77
Torgerson PR, Keller K, Magnotta M, Ragland N (2010) The global burden of alveolar echinococcosis. PLoS Negl Trop Dis 4(6):e722
Torgerson P, Macpherson C, Vuitton D (2011) In: Palmer SSLT, Brown P, DWG. (eds) Cystic Echinococcosis. Oxford textbook on zoonoses. Oxford University Press, Oxford
Touil-Boukoffa C, Sanceau J, Tayebi B, Wietzerbin J (1997) Relationship among circulating interferon, tumor necrosis factor-alpha, and interleukin-6 and serologic reaction against parasitic antigen in human hydatidosis. J Interf Cytokine Res 17:211–217
Touil-Boukoffa C, Bauvois B, Sanceau J, Hamrioui B, Wietzerbin J (1998) Production of nitric oxide (NO) in human hydatidosis: relationship between nitrite production and interferon-gamma levels. Biochimie 80:739–744
Tuxun T, Wang JH, Lin RY, Shan JY, Tai QW, Li T, Zhang JH, Zhao JM, Wen H (2012) Th17/Treg imbalance in patients with liver cystic echinococcosis. Parasite Immunol 34:520–527
Uchiumi L, Mujica G, Araya D, Salvitti JC, Sobrino M, Moguillansky S, Solari A, Blanco P, Barrera F, Lamunier J, Arezo M, Seleiman M, Yadon ZE, Tamarozzi F, Casulli A, Larrieu E (2021) Prevalence of human cystic echinococcosis in the towns of Norquinco and Ramos Mexia in Rio Negro Province, Argentina, and direct risk factors for infection. Parasit Vectors 14(1):262
Ueno M, Kuroda N, Yahagi K, Ohtaki T, Kawanaka M (2012) Analysis of antibody responses by commercial western blot assay in horses with alveolar echinococcosis. J Vet Med Sci 74(6):813–815
Umhang G, Bastid V, Avcioglu H, Bagrade G, Bujanic M, Bjelic Cabrilo O, Casulli A, Dorny P, van der Giessen J, Guven E, Harna J, Karamon J, Kharchenko V, Knapp J, Kolarova L, Konyaev S, Laurimaa L, Losch S, Miljevic M, Miterpakova M, Moks E, Romig T, Saarma U, Snabel V, Sreter T, Valdmann H, Boue F (2021) Unravelling the genetic diversity and relatedness of Echinococcus multilocularis isolates in Eurasia using the EmsB microsatellite nuclear marker. Infect Genet Evol 92:104863
Ustünsöz B, Akhan O, Kamiloğlu MA, Somuncu I, Uğurel MS, Cetiner S (1999) Percutaneous treatment of hydatid cysts of the liver: long-term results. Am J Roentgenol 172(1):91–96. https://doi.org/10.2214/ajr.172.1.9888746
Ustunsoz B, Ugurel MS, Uzar AI, Duru NK (2008) Percutaneous treatment of hepatic hydatid cyst in pregnancy: long-term results. Arch Gynecol Obstet 277:547–550
van Kesteren F, Qi XW, Tao J, Feng XH, Mastin A, Craig PS, Vuitton DA, Duan XY, Chu XD, Zhu JL, Wen H (2015) Independent evaluation of a canine Echinococcosis Control Programme in Hobukesar County, Xinjiang, China. Acta Trop 145:1–7
Varcasia A, Garippa G, Pipia AP, Scala A, Brianti E, Giannetto S, Battelli G, Poglayen G, Micagni G (2008) Cystic echinococcosis in equids in Italy. Parasitol Res 102(4):815–818
Velasco-Tirado V, Alonso-Sardon M, Lopez-Bernus A, Romero-Alegria A, Burguillo FJ, Muro A, Carpio-Perez A, Bellido JLM, Pardo-Lledias J, Cordero M, Belhassen-Garcia M (2018) Medical treatment of cystic echinococcosis: systematic review and meta-analysis. BMC Infect Dis 18
Virginio VG, Taroco L, Ramos AL, Ferreira AM, Zaha A, Ferreira HB, Hernandez A (2007) Effects of protoscoleces and AgB from Echinococcus granulosus on human neutrophils: possible implications on the parasite's immune evasion mechanisms. Parasitol Res 100:935–42
Vuitton DA, Bresson-Hadni S, Laroche L, Kaiserlian D, Guerret-Stocker S, Bresson JL, Gillet M (1989) Cellular immune response in Echinococcus multilocularis infection in humans. II. Natural killer cell activity and cell subpopulations in the blood and in the periparasitic granuloma of patients with alveolar echinococcosis. Clin Exp Immunol 78:67–74
Vola A, Manciulli T, De Silvestri A, Lissandrin R, Mariconti M, Siles-Lucas M, Brunetti E, Tamarozzi F (2019) Diagnostic performances of commercial ELISA, indirect hemagglutination, and western blot in differentiation of hepatic echinococcal and non-echinococcal lesions: a retrospective analysis of data from a single referral centre. Am J Trop Med Hyg 101(6):1345–1349
Vuitton DA (2003) The ambiguous role of immunity in echinococcosis: protection of the host or of the parasite? Acta Trop 85(2):119–132

Vuitton DA, Gottstein B (2010) Echinococcus multilocularis and its intermediate host: a model of parasite-host interplay. J Biomed Biotechnol 2010:923193

Vuitton DA, Zhou H, Bresson-Hadni S, Wang Q, Piarroux M, Raoul F, Giraudoux P (2003) Epidemiology of alveolar echinococcosis with particular reference to China and Europe. Parasitology 127(Suppl): S87–S107

Vuitton DA, Wang Q, Zhou HX, Raoul F, Knapp J, Bresson-Hadni S, Wen H, Giraudoux P (2011) A historical view of alveolar echinococcosis, 160 years after the discovery of the first case in humans: part 1. What have we learnt on the distribution of the disease and on its parasitic agent? Chin Med J 124(18):2943–2953

Vuitton DA, McManus DP, Rogan MT, Romig T, Gottstein B, Naidich A, Tuxun T, Wen H, Menezes da Silva A (2020) International consensus on terminology to be used in the field of echinococcoses. Parasite 27:41

Wahlers K, Menezes CN, Wong M, Mogoye B, Frean J, Romig T, Kern P, Grobusch MP (2011) Human cystic echinococcosis in South Africa. Acta Trop 120(3):179–184

Wahlers K, Menezes CN, Romig T, Kern P, Grobusch MP (2013) Cystic echinococcosis in South Africa: the worst yet to come? Acta Trop 128(1):1–6

Wang Q, Vuitton DA, Qiu J, Giraudoux P, Xiao Y, Schantz PM, Raoul F, Li T, Yang W, Craig PS (2004) Fenced pasture: a possible risk factor for human alveolar echinococcosis in Tibetan pastoralist communities of Sichuan, China. Acta Trop 90(3):285–293

Wang Y, He T, Wen X, Li T, Waili A, Zhang W, Xu X, Vuitton DA, Rogan MT, Wen H, Craig PS (2006) Post-survey follow-up for human cystic echinococcosis in northwest China. Acta Trop 98(1):43–51

Wang J, Zhang C, Wei X, Blagosklonov O, Lv G, Lu X, Mantion G, Vuitton DA, Wen H, Lin R (2013) TGF-beta and TGF-beta/Smad signaling in the interactions between Echinococcus multilocularis and its hosts. PLoS One 8:e55379

Wang JH, Lin RY, Zhang WB, Li L, Gottstein B, Blagosklonov O, Lü GD, Zhang CS (2014) Transcriptional profiles of cytokine/chemokine factors of immune cell-homing to the parasitic lesions: a comprehensive one-year course study in the liver of E. multilocularis-infected mice. PLoS One

Wang J, Jebbawi F, Bellanger AP, Beldi G, Millon L, Gottstein B (2018) Immunotherapy of alveolar echinococcosis via PD-1/PD-L1 immune checkpoint blockade in mice. Parasite Immunol 40(12):e12596

Wen H, Craig PS (1994) Immunoglobulin G subclass responses in human cystic and alveolar echinococcosis. Am J Trop Med Hyg 51:741–748

Wen H, Tian WL, Zou PF, Xiang MX (1992) A rare case of mixed cystic and alveolar hydatidosis. Trans R Soc Trop Med Hyg 86(3):290–291

Wen H, Zou PF, Yang WG, Lu J, Wang YH, Zhang JH, New RR, Craig PS (1994) Albendazole chemotherapy for human cystic and alveolar echinococcosis in north-western China. Trans R Soc Trop Med Hyg 88(3):340–343

Wen H, Bresson-Hadni S, Vuitton DA, Lenys D, Yang BM, Ding ZX, Craig PS (1995) Analysis of immunoglobulin G subclass in the serum antibody responses of alveolar echinococcosis patients after surgical treatment and chemotherapy as an aid to assessing the outcome. Trans R Soc Trop Med Hyg 89:692–697

Wen H, Dong JH, Zhang JH, Zhao JM, Shao YM, Duan WD, Liang YR, Ji XW, Tai QW, Aji T, Li T (2011) Ex vivo liver resection followed by autotransplantation for end-stage hepatic alveolar echinococcosis. Chin Med J 124:2813–2817

Wen H, Vuitton L, Tuxun T, Li J, Vuitton DA, Zhang W, McManus DP (2019) Echinococcosis: advances in the 21st century. Clin Microbiol Rev 32(2):1–39

WHO/OIE and M. o. Echinococcosis (2001) Echinococcosis in humans and animals: a public health problem of global concern. Paris, World Organisation for Animal Health (Office International des Epizooties) and World Health Organisation)

Xiao N, Qiu J, Nakao M, Li T, Yang W, Chen X, Schantz PM, Craig PS, Ito A (2005) Echinococcus shiquicus n. sp., a taeniid cestode from Tibetan fox and plateau pika in China. Int J Parasitol 35(6):693–701

Yagci G, Ustunsoz B, Kaymakcioglu N, Bozlar U, Gorgulu S, Simsek A, Akdeniz A, Cetiner S, Tufan T (2005) Results of surgical, laparoscopic, and percutaneous treatment for hydatid disease of the liver: 10 years experience with 355 patients. World J Surg 29:1670–1679

Yaghan R, Heis H, Bani-Hani K, Matalka I, Shatanawi N, Gharaibeh K, Bani-Hani A (2004) Is fear of anaphylactic shock discouraging surgeons from more widely adopting percutaneous and laparoscopic techniques in the treatment of liver hydatid cyst? Am J Surg 187:533–537

Yang YR, Ellis M, Sun T, Li Z, Liu X, Vuitton DA, Bartholomot B, Giraudoux P, Craig PS, Boufana B, Wang Y, Feng X, Wen H, Ito A, McManus DP (2006a) Unique family clustering of human echinococcosis cases in a Chinese community. Am J Trop Med Hyg 74(3):487–494

Yang YR, Williams GM, Craig PS, Sun T, Yang SK, Cheng L, Vuitton DA, Giraudoux P, Li X, Hu S, Liu X, Pan X, McManus DP (2006b) Hospital and community surveys reveal the severe public health problem and socio-economic impact of human echinococcosis in Ningxia Hui Autonomous Region, China. Tropical Med Int Health 11(6):880–888

Yang YR, Craig PS, Ito A, Vuitton DA, Giraudoux P, Sun T, Williams GM, Huang Z, Li Z, Wang Y et al (2007) A correlative study of ultrasound with serology in an area in China co-endemic for human alveolar and cystic echinococcosis. Trop Med Int Heal 12:637–646. https://doi.org/10.1111/j.1365-3156.2007.01834.x

Yang YR, Craig PS, Vuitton DA, Williams GM, Sun T, Liu TX, Boufana B, Giraudoux P, Teng J, Li Y, Huang L, Zhang W, Jones MK, McManus DP (2008) Serological prevalence of echinococcosis and risk factors for infection among children in rural communities of southern Ningxia, China. Tropical Med Int Health 13(8):1086–1094

Zarzosa MP, Orduna Domingo A, Gutierrez P, Alonso P, Cuervo M, Prado A, Bratos MA, Garcia-Yuste M, Ramos G, Rodriguez Torres A (1999) Evaluation of six serological tests in diagnosis and postoperative control of pulmonary hydatid disease patients. Diagn Microbiol Infect Dis 35:255–262

Zhang W, You H, Li J, Zhang Z, Turson G, Aili H, Wang J, Mcmanus DP (2003) Immuno-globulin profiles in a murine intermediate host model of resistance for Echinococcus granulosus infection. Parasite Immunol 25:161–168

Zhang Y, Bart JM, Giraudoux P, Craig P, Vuitton D, Wen H (2006) Morphological and molecular characteristics of Echinococcus multilocularis and Echinococcus granulosus mixed infection in a dog from Xinjiang, China. Vet Parasitol 139(1–3):244–248

Zhang S, Hue S, Sene D, Penfornis A, Bresson-Hadni S, Kantelip B, Caillat-Zucman S, Vuitton DA (2008) Expression of major histocompatibility complex class I chain-related molecule A, NKG2D, and transforming growth factor-beta in the liver of humans with alveolar echinococcosis: new actors in the tolerance to parasites? J Infect Dis 197:1341–1349

Zhang W, Wen H, Li J, Lin R, McManus DP (2012) Immunology and immunodiagnosis of cystic echinococcosis: an update. Clin Dev Immunol 2012:101895

Zhang C, Lin R, Li Z, Yang S, Bi X, Wang H, Aini A, Zhang N, Abulizi A, Sun C, Li L, Zhao Z, Qin R, Li X, Aji T, Shao Y, Vuitton DA, Tian Z, Wen H (2020) Immune exhaustion of T cells in Alveolar Echinococcosis patients and its reversal by blocking checkpoint receptor TIGIT in a murine model. Hepatology 71(4):1297–1315

Chapter 9
Taeniasis and Cysticercosis

Elizabeth Ferrer and María Jesús Perteguer

Abstract Taeniasis and cysticercosis are zoonotic diseases caused by *Taenia saginata* and *Taenia solium.* Tapeworms can infect the human intestine and have a wide geographic distribution. *Taenia asiatica*, another tapeworm species, was described in Southeast Asia. Larval stages of these cestodes (metacestodes or cysticerci) cause cysticercosis; *T. saginata* causes bovine cysticercosis, *T. asiatica* larvae develop in pig viscera, and *T. solium* may lead to cysticercosis in pig and human. Invasion of the central nervous system (CNS) by parasite larvae may cause neurocysticercosis (NCC), one of the most prevalent parasitic infections of the human CNS. Taeniasis and cysticercosis continue to cause health problems and livestock industry losses in endemic areas as well as in non-endemic regions associated with travel and migrations. There are few symptoms associated with taeniasis. On the other hand, neurocysticercosis is pleomorphic and may be life-threatening, depending on the location, number, stage of cysticerci, and host immune response. Diagnosis of taeniasis is generally done by microscopic examinations of stool; detection of cysticercosis is generally performed by neuroimaging and immunoassays. Both conventional coprological techniques and immunological assays show limitations, and new more specific and sensitive diagnostic tools have been developed such as specific monoclonal antibodies, recombinant antigens, synthetic peptides, and PCR. Considering the clinical impact, veterinary problems, and economic losses derived from taeniasis/cysticercosis, control programs have been implemented. Moreover, several vaccine candidates have been characterized to complement control measures.

E. Ferrer (✉)
Instituto de Investigaciones Biomédicas "Dr. Francisco J. Triana Alonso" (BIOMED) and Departamento de Parasitología, Facultad de Ciencias de la Salud, Universidad de Carabobo Sede Aragua, Maracay, Venezuela
e-mail: eferrer1@uc.edu.ve

M. J. Perteguer
Instituto de Salud Carlos III, Centro Nacional de Microbiología, Servicio de Parasitología, Majadahonda, Madrid, Spain

F. Bruschi (ed.), *Helminth Infections and their Impact on Global Public Health*,
https://doi.org/10.1007/978-3-031-00303-5_9

9.1 Introduction

Taeniasis is a human intestinal parasitic infection, caused by adult stages of the *Taenia saginata* and *Taenia solium*. The human being is the only known definitive host for both taeniids. Cysticercosis is an infection by parasitic larval stages (cysticerci or metacestodes) in tissues of intermediate host. Cysticerci of *T. saginata* (*Cysticercus bovis*) cause bovine cysticercosis and those of *T. solium* (*Cysticercus cellulosae*) porcine and human cysticercosis. Man can accidentally act as intermediate host of *T. solium* (WHO 2020).

Taenia asiatica is the last tapeworm species found to infect humans. Initially, there were debates over its taxonomic status, which were finally solved. The larvae of *T. asiatica* (*Cysticercus viscerotropica*) infect pig liver and other viscera and have characteristic geographical distribution, epidemiology, genomics, and immunodiagnosis. Recently, the name *Taenia asiaticus* has been proposed to replace *T. asiatica* (Eom and Rim 1993; Eom et al. 2020).

9.2 The Agent

The life cycle of cestodes, or tapeworms, includes three distinct stages: adult, egg, and larva (metacestode or cysticercus). Adult tapeworm is flattened, ribbon-shaped, and hermaphrodite; *T. solium* are very thin and measure 2–8 meters (m) long, while *T. saginata* and *T. asiatica* are longer, thicker, and wider, with an average size of 5 m although they may reach up to 16 meters. The adult has three parts: scolex, neck, and body or strobila. The scolex is a muscular "head" at the anterior end, with globular shape and 1-mm average diameter; the scolex of *T. solium* has 4 suckers, a rostellum, and a double crown of 22 to 32 hooks, from where it gets the name *armed scolex*; in contrast in *T. saginata*, the scolex presents four suckers but does not have hooks nor rostellum (*unarmed scolex*). The scolex in *T. asiatica* is smaller than that of *T. saginata* and has four suckers with a cupsidal rostellum with two rudimentary hooks in a wartlike formation. The scolex serves to maintain the position of the parasite in the host's gut (Náquira 1999; Flisser 2013; Mendlovic et al. 2021).

The neck is short, 5–10 mm, and slender; it contains the germinative cells apparently responsible for producing proglottids or segments (strobilation). The strobili (tapeworm body) are large, measuring several meters and consisting of hundreds of proglottids classified as immature, mature, or gravid, based on their reproductive system development. Mature proglottids, located between immature and gravid segments, are slightly wider than long, while immature proglottids are narrower (6 mm). Mature proglottids have genital organs consisting of about 150 to 1200 testes depending on the species, bilobed or trilobed ovary, and a genital pore. Gravid proglottids are longer than wide, arranged in the last fifth of the worm, and have eggs in the lateral uterine branches. *T. solium* and *T. saginata* differ in the number of primary lateral uterine branches: *T. solium* have 7–15 lateral branches and

T. saginata 15–30 lateral branches. Proglottids of *T. asiatica*, with more than 15 primary uterine branches, are similar to those of *T. saginata* although with a protuberance at the posterior end (Pawlowski 2002; Eom et al. 2020).

Eggs pass out with feces of taeniasis carriers, either from the gravid proglottids or freely. It should be noted that *T. saginata* proglottids might be found in undergarments of tapeworm carriers after their release from the anus. Eggs are spherical, 30–50 μm, with a very thick riddled outer keratin embryophore that protects the hexacanth embryo (oncosphere) from environmental conditions and fully embryonated when eliminated. Morphologically, human taeniid eggs are identical making species identification impossible if based on this feature alone (Pawlowski 2002).

Cysticercus or metacestode is the larval stage of this type of taeniids. *T. solium* (*C. cellulosae*) cysticerci are rounded or oval vesicles, 6–15 mm in diameter, whitish, fluid-filled, with an invaginated scolex (hooks and four suckers) which can be seen as small eccentric solid granule. Occasionally, large, irregular, fluid-filled, and round or lobulated vesicles, similar to a bunch of grapes, may develop, known as racemose cysticercus. Cysticerci of *T. saginata* (*C. bovis*) are akin to *T. solium* vesicular cysticercus, although the scolex does not show the double row of hooks (Náquira 1999). *T. saginata asiatica* metacestodes (*C. viscerotropica*) are smaller and covered by wartlike formations (Eom and Rim 1993; Eom et al. 2020).

The eggs eliminated by *Taenia* carriers can survive for days to months in the environment, in soil or water. Cattle (*T. saginata*) and pigs (*T. solium* and *T. asiatica*) become infected by ingesting eggs or gravid proglottids. In the animal's intestine, the oncospheres hatch, activate, invade the intestinal wall, and migrate to the tissues and some organs, where they develop into cysticerci, e.g., pork muscle and brain (*T. solium*); pork's hepatic and extrahepatic visceral organs, mainly liver (*T. saginata asiatica*); and muscle and viscera of cattle (*T. saginata*). A cysticercus can survive for several years in the animal. Humans become infected by the ingestion of raw or undercooked infected meat, pork (*T. solium/T. asiatica*), and cattle (*T. saginata*). In the human intestine, the cysticercus becomes an adult tapeworm over a period of 2 months; the adult may survive for years and transmit the infection through the eggs. In contrast to *T. saginata*, *T. solium* eggs may infect humans and develop into cysticerci, which can infect the brain and lead to a serious condition known as neurocysticercosis (Pawlowski 2002; Sato et al. 2018).

9.3 Epidemiology of Infection

Taeniasis and cysticercosis are endemic in some Latin American, Asian, and African countries, especially in rural areas, where socioeconomic status is low and health requirements and meat inspection infrastructure are insufficient. Human neurocysticercosis (NCC) is one of the most common parasitic diseases of the central nervous system (CNS) and has been associated with late-onset epilepsy cases in endemic regions. Moreover, it is the most frequent preventable cause of epilepsy worldwide. The number of individuals with NCC is estimated to be between 2.56

and 8.30 million, with great economic relevance, associated with treatment costs, lost workdays, and decrease in profitability for the livestock industry. In endemic areas, *T. solium* is the leading cause of deaths from foodborne diseases with an estimated two to five million loss measured in disability-adjusted life years (Del Brutto and Garcia 2013; WHO 2020). Regarding cattle and porcine cysticercosis, they overlap in many countries causing costly condemnations and important economic losses as previously explained (Fleury et al. 2013b).

The scene is different in high-income countries such the United States and many European countries. In the United States, human cysticercosis has always been predominantly an imported disease, highly prevalent in young Hispanic adult immigrants, occasionally, local transmission through tapeworm carriers from endemic areas (Serpa and White 2012; Spallone et al. 2020). In Western Europe, cysticercosis was almost under control in the last century, but a significant increase has been observed in association with immigration. Imported human cases have been reported from 1990 to the present in all countries except Iceland; most cases have been found in Portugal and Spain, but suspected autochthonous cases are infrequent. Sporadic porcine cysticercosis cases have been reported (Laranjo-González et al. 2017; Ursini et al. 2020; Herrador et al. 2020). In Eastern European countries, from were less and fragment information, the prevalence of cysticercosis is higher in comparison to Western countries. Cases have been reported in 15 of the 22 countries, with the largest number diagnosed being Romania and Serbia, as well as the highest number of suspected autochthonous cases. Furthermore, frequently, there is no species identification in taeniasis cases. Overall, bovine and porcine cysticercosis has decreased over the years and has been reported in 15 and 8 countries, respectively (Trevisan et al. 2018b).

In Asia, where pig production is central to many rural communities; the prevalence of the disease is moderately endemic but variable between neighboring countries and within each country according to different risk factors. Thus, it is almost absent in countries like Japan and Singapore, with high living standards, where only imported cases of NCC are reported. Prevalence is low in Islamic countries, although present in hotspot areas mainly related to other religious minorities. In countries known to be endemic, improvement of the control measures and diagnostic tools and rapid economic growth of some of these Asian countries have led to a decline in cysticercosis prevalence with spatial variation, i.e., mostly occurring in rural and remote areas and highly defined endemic areas within each country (Aung and Spelman 2016; Robertson et al. 2017; Qian et al. 2020). *T. saginata*, *T. asiatica*, and *T. solium* overlap in some Asiatic countries. Moreover, the Asian *T. solium* genotype differs from the African-American genotype (Ito et al. 2003; Sato et al. 2011).

African countries report the highest global prevalence of active infection (circulating *T. solium* antigens) (Coral-Almeida et al. 2015). Africa's changing societal conditions (urbanization expansion due to a population growth and increase in demand for pig meat) and favorable environment for parasite transmission (backyard pig farming systems, unqualified meat inspectors, illegal slaughtering, open field defecation practices, and poor personal hygiene) have caused cysticercosis to appear

as a persistent, uncontrolled health problem. In West Africa, human and porcine cysticercosis is largely present in most countries. However, there are information gaps on prevalence, with no official data in some countries. Persistent high prevalence is reported in Nigeria, Senegal, and Burkina Faso, despite the latter being a predominantly Muslim country. Epilepsy is relatively common and more frequent in *T. solium* serology-positive patients but rarely reported due to fear of stigmatization (Melki et al. 2018; Weka et al. 2019). Some Eastern and South African countries, e.g., Tanzania, Mozambique, and Zambia, where *T. solium* is recognized as a public health threat, are making great efforts to gather evidence of distribution and burden of the disease, a compulsory prerequisite for assessing the cost-effectiveness of control programs (Trevisan et al. 2017, 2018a). In Madagascar, sympatric distribution of the Asiatic and the African-American genotype has been found (Vega et al. 2003).

In Latin American countries, where several epidemiological studies still document the disease as a public health problem (Martins-Melo et al. 2017; Rodríguez-Morales et al. 2018; Cortez et al. 2020), consistent association between epilepsy and NCC is widely accepted and has been systematically described. Thus, in Latin America and the Caribbean, with an estimated 14.9 million people with NCC, between 450,000 and 1.35 million people suffer from NCC-associated epilepsy. A recent study from Peru's northern coast describes high percentages – between 30% and 50% – of epilepsy cases associated with NCC infection not related to gender (Bruno et al. 2013; PAHO/WHO 2019; Pesantes et al. 2020). Ecuador is one of the few South America countries that has carried out studies to estimate the burden of NCC combined with spatial analysis, identifying "hotspots" of neurological disorders associated with NCC mainly in the southern provinces of the country (Coral-Almeida et al. 2020). In Mexico, assessment of costs associated with *T. solium* infection of both humans and pigs revealed considerable expenses. The cost per epilepsy patient was similar to the estimates for South Africa, with similar medical services as Mexico, and higher than the ones for Cameroon and Tanzania, with less access to expensive imaging diagnostic tests, such as computed tomography and magnetic resonance imaging, and surgical interventions (Bhattarai et al. 2019). In Central America and the Caribbean basin, where cysticercosis might be undiagnosed and underreported, human *T. solium* cases and porcine cysticercosis have been reported in 16 and 6 countries out of 41, respectively (Braae et al. 2017b).

Finally, the taeniasis/cysticercosis complex is considered a neglected tropical disease (NTD). The WHO recognizes that people with epilepsy (the most common clinical signs in NCC patients) suffer stigmatization and discrimination and are promoting actions to manage epilepsy and control cysticercosis (WHO 2020).

9.4 The Host Response to the Parasite and Pathogenesis

Few studies have assessed the immune response in taeniasis, most focusing on antibody detection. Taeniasis carriers can also suffer cysticercosis; thus, it is difficult to determine whether the antibodies were produced in response to adult parasites or cysticerci (Correa and Medina 1999). Experimental models of *T. solium* taeniasis in hamsters, gerbils, and chinchillas have showed that chinchillas are the most successful experimental definitive model since tapeworms with gravid proglottids were obtained (Flisser et al. 2010). On the other hand, many studies have been carried out to determine immune response mechanisms against *T. solium* cysticerci. Response has been evaluated in murine models (mouse *T. crassiceps*), NCC rat model, pigs, and humans as reviewed below.

9.4.1 Innate Immunity

The relevance of the innate immune system in response to NCC has been increasingly acknowledged. Toll-like receptors (TLRs) appear to be important for recognizing these parasites and induce inflammatory responses. Studies in mixed symptomatic NCC patients suggest a role for TLR4 in regulating the levels of inflammatory and anti-inflammatory cytokines by showing a relationship between TLR-4 gene polymorphisms (Asp299Gly and Thr399Ile) and occurrence of NNC and development of symptoms. These polymorphisms have also been associated with calcified NCC and seizures and occurrence of disseminated cysticercosis (Verma et al. 2010; Lachuriya et al. 2016). Participation of complement component 5 (C5) and the TRAF1 gene in the risk of developing severe NCC is firmly sustained in TRAF1/C5 locus polymorphism studies with NCC patients (Villegas et al. 2019). Dendritic cells (DCs) treated with parasite cysticerci excretory/secretory (E/S) antigens are unresponsiveness to lipopolysaccharides (LPS), thus impairing subsequent TLR4-mediated response induced in DCs (Terrazas et al. 2011).

Parasite produces substances that modulate the complement activation or block inflammation response (White et al. 1992). Sulfated polysaccharides, teniastatin, and paramyosin interfere with complement system activation, besides blocking other immunological responses (Laclette et al. 1992).

9.4.2 Adaptive Immunity

Peripheral (serum) and local cerebrospinal fluid (CSF) adaptive immunity with different immune profiles is induced in asymptomatic and symptomatic NCC patients with marked differences depending on the location of the parasite and its stage (Sciutto et al. 2013c; Prodjinotho et al. 2020).

9.4.3 Humoral Responses

Humoral response in NCC patients has been mainly studied as an immunodiagnostic tool. Most infected individuals produce antibodies of distinct specificities generated at different periods of infection in response to changes in antigen release during parasite development (Flisser et al. 1980; Dorny et al. 2003). Anti-cysticerci IgG antibodies are the most prevalent and have been detected in serum, CSF, and saliva, throughout the infection; IgM, IgA, and IgE antibodies are generally less common. In extraparenchymal cases, associated with high parasite antigen burden and consequently very strong antibody reactions, IgG, IgM, and IgE are detected even in CFS. In general, antibodies against this parasite seem to be poorly effective in clearing parasite, and only activated oncospheres showed susceptibility to specific antibody attack mediated by complement system (Molinari et al. 1993b).

9.4.4 Cellular Responses

Cysticerci may cause asymptomatic infection in the host and persist for many years without triggering an inflammatory response. Histological studies in pigs and humans have shown viable cysticerci, with or without mild inflammatory reaction (Carpio 2002). This has been associated with the prevalence of a Th2 response with high levels of IL-4, IL-5, IL-13, and anti-*Taenia*-specific IgG4 (Terrazas et al. 2012). Thus, in chronic asymptomatic patients, Th2-associated immunological memory, induced by recurrent contacts with the parasite, may promote an effective immune response that regulates local tissue damage and interferes with a successful parasite establishment (Sciutto et al. 2013c). On the contrary, symptomatic NCC is significantly associated with the development of granulomas and degenerating cysts that lose their ability to regulate host's response and are important components of the neuropathology leading to neurological symptoms. In the colloidal stage, cysts release immunogenic molecules; the initiation of granulomas has been related with robust Th1 response with local expression of TNF-alpha, IL-12, IL-18, and IFN-γ and peripheral expression of TNFα, IL-1ß, and IL-6. The granular-nodular stage is linked with mixed Th1 and Th2 response, as well as with TGF-ß and cytokine profile changes (TNFα, IL-1ß, IL-4, IL-6, and IL-10), as long as collagenous structures are generated to develop the calcified stage (Terrazas et al. 2012; Prodjinotho et al. 2020).

9.4.5 Other Immunomodulation and Evasion Mechanisms

T. solium metacestodes modulate the host's immune response to ensure their survival (Toenjes and Kuhn 2003). Cysticerci secrete molecules to induce apoptosis in

immune cells, e.g., cysteine proteases and annexin B1, produce cysteine proteases (cathepsin L-like) that break down IgG, or use antioxidative enzymes to protect them from oxidative damages (Terrazas et al. 2012). Moreover, the cysticerci surface can adsorb host molecules (antibodies, complement units) and mimic the host repertoire (White et al. 1992; Spolski et al. 2002). Immunomodulation through the production of alternately activated macrophages, which leads to the production of downregulatory cytokines and activation of the alternative arginase 1 pathway, has also been suggested as an immune protection mechanism of the parasite (Rodríguez-Sosa et al. 2006). In murine models, micro-RNAs from *T. solium* has been shown to modulate macrophages cytokine expression (Landa et al. 2019). In porcine model, apoptotic cells were observed in the inflammatory infiltrate, but not with dead parasites (Sikasunge et al. 2008).

Studies in humans report immune response variations based on patient's age and gender, response being stronger in females and young people (Kelvin et al. 2009). It is hypothesized that population genetics (HLA polymorphisms) and parasite genotypes may be involved in disease progression (Pal et al. 2000).

9.5 Inmunopathological Processes

Neuroinflammation is critical in NCC pathogenesis. Several studies indicate that NCC symptom severity is associated with the intensity of the immune response; thus, symptomatic parenchymal disease occurs with larval degeneration or death following cysticidal therapy. Initially, there is an asymptomatic period during which the immune response seems unable to resolve the infection, as the parasite modulates host responses releasing immunomodulatory components directly into the brain tissue or indirectly through the induction of regulatory networks. Host immune responses to viable and degenerating cysts are heterogeneous, possibly due to different immune responses, suppression responses to viable cysts, and activation responses to the degenerating ones. Host factors, combined with those related to cyst (number, localization, and development stage), determine the magnitude of the inflammatory response and thus the severity of the disease (Carpio et al. 2013; Fleury et al. 2016; Prodjinotho et al. 2020).

Inflammatory reaction around cysts is associated with increased permeability of the blood-brain barrier (BBB), allowing the entry of different peripheral cells into the CNS. Vascular alteration and BBB disruption contribute to NCC pathology. A study with an in vitro model of human umbilical vein endothelial cells has shown that cysticerci E/S products alone can stimulate angiogenesis (Fleury et al. 2016; Carmen-Orozco et al. 2019).

Two primary processes seem to be important in the development of NCC-related epilepsy: inflammation and calcification. The inflammatory response triggers epileptogenesis through glycolysis and/or persistent areas of BBB dysfunction. Calcifications may be associated or not with hippocampal sclerosis. Calcified lesions

with perilesional edema are associated with recurrent seizures despite treatment with anticonvulsant drugs (Herrick et al. 2020).

9.6 Clinical Manifestations

9.6.1 Taeniasis

Taeniasis is usually asymptomatic; minimal lesions may develop in the intestinal mucosa. Although some clinicians described abdominal pain, weight loss, nausea, constipation, or diarrhea during taeniasis, others do not recognize specific symptoms associated with this infection. It is important to consider that *T. solium* taeniasis patients might also suffer cysticercosis, and differential diagnosis is essential before prescribing pharmacological treatment (Bustos et al. 2012).

9.6.2 Cysticercosis

Most infections are asymptomatic. Symptomatic cases include several clinical presentation forms depending on environmental factors, the individual (genetic background, age, gender), and the type of parasite (Pal et al. 2000; Chavarria et al. 2006). Prognosis of the disease will depend on the number, size, type, condition, and site of metacestodes, as well immunological responses to cysticercosis (Sotelo 2011). *T. solium* cysticerci can invade CNS, eyes, skeletal muscle, and subcutaneous tissues. Muscle and subcutaneous locations are the most benign forms of cysticercosis, while ocular cysticercosis and especially NCC are the most serious conditions.

The main symptom of ocular cysticercosis is blurred vision; when left untreated, it may lead to impaired vision or blindness (Madigubba et al. 2007). NCC is a clinically heterogeneous disease with a wide spectrum of potential manifestations, from asymptomatic to life-threatening.

Different criteria are applied to classify NCC:

(i) *Location of cysticerci*. *Parenchymal*: Parasites are in the brain parenchyma or in the subarachnoid space of the convexity or in the sulcus of the convexity. It is the most common form, and its main symptoms are epilepsy and headache. *Extraparenchymal*: The parasite locates in the basal cisterns of the subarachnoid space or in the ventricular system. It is the most severe form that may cause permanent sequelae; the main symptom is intracranial hypertension.

(ii) *Viability of cysticerci*. *Active*: viable cysticerci. *Transitional*: degenerating cysticerci. *Inactive:* calcified cysticerci (Carpio et al. 2016). In NCC, onset of symptoms usually occurs years after the infection, although they may develop at very early stages. In parenchymal NCC, where symptoms arise when cysticerci begin to degenerate, the incubation period is approximately 2–5 years;

while in extraparenchymal NCC, where parasites have plenty of space to develop before mass effect or inflammation give rise to symptoms, the incubation period may vary from 10 to 20 years or even more (Nash et al. 2018; Hamamoto Filho et al. 2020).

(iii) *Progression of parenchymal lesions by neuroimaging techniques*. *Viable or vesicular form*: small single or multiple lesions, hypodense rounded images, with an inner hyperdense nodule (scolex), lacking surrounding edema. *Colloidal*: lesions are surrounded by edema and represent the acute encephalitis phase in which the host's immune system is reacting against the parasite. *Nodular-granular*: hyperdense lesions surrounded by edema, known as "cysticercus granuloma." *Calcified lesions*: small hyperdense nodules without perilesional edema, being the most common finding in NCC (García and Del Brutto 2003; Nash 2012).

In general, *parenchymal cysticercosis* is the most frequent presentation form in patients who suffer seizures, characterized by a host-induced inflammatory pericystic lesion and different prognosis depending on the number (single or multiple lesions), stage, and localization of cyst and type of seizures (new-onset seizures or established epilepsy). Early-onset seizures are transient seizures triggered by active NCC infection (viable or degenerating cyst) usually within multiple relapses (related to episodic lesion-associated inflammation) which stop if early controlled. In other cases, established epilepsy, due to permanent damage, leads to recurrent seizure episodes that do not respond to antiparasitic and anticonvulsant medications. Calcified cysts are mostly clinically silent although in a small proportion of patients established epilepsy is elicited, most of the time associated with perilesional edema host-induced (Gonzales et al. 2016; Nash et al. 2017; Herrick et al. 2020). Symptoms of *extraparenchymal disease* vary according to the parasite locations, although intracranial hypertension is the most frequent; when cysticerci lodge within the ventricular system, they cause a complete, partial, or transient obstruction of CFS flow, leading to acute intracranial hypertension secondary to hydrocephalus that can be life-threatening. *Subarachnoid* NCC is also characterized by hydrocephalus, a consequence of inflammatory occlusion, and ischemic cerebrovascular complications. *Ventricular* cysticercosis sometimes triggers seizures or hydrocephalus, meningeal inflammation, nausea, vomiting, headache, ataxia, and confusion. *Spinal* cisticercosis occurs with inflammatory and demyelinating changes and is characterized by radicular pain or paresthesiae or progressive cord compression (Pal et al. 2000; Del Brutto and Garcia 2013; Nash et al. 2018).

Computed tomography (CT) and magnetic resonance imaging (MRI) studies allow illustrating the heterogeneity of NCC. Figure 9.1 exemplifies the diversity of this neurological pathology:

(i) *Number of cysticerci*: There are cases with just one cyst (Images 1–1 and 1–6) and others with dozens (Images 1–2, 1–5, 1–7).

(ii) *Location of cysticerci* parenchymal (Images 1–1 and 1–2), ventricular (Image 1–3), subarachnoid space (Image 1–4).

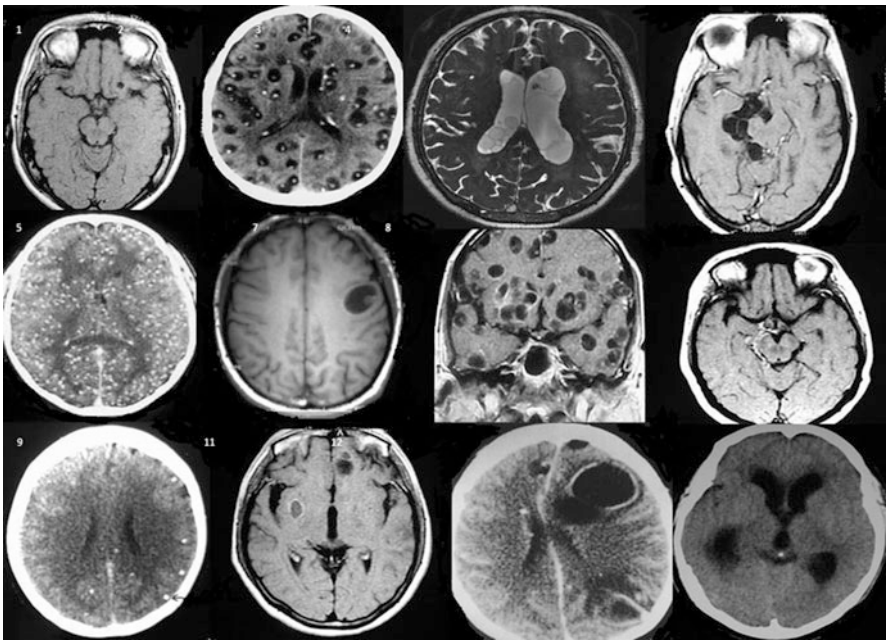

Fig. 9.1 Heterogeneity of neurocysticercosis by magnetic resonance imaging and computer tomography (images provided by Dr. Agnès Fleury, *Instituto de Investigaciones Biomédicas, UNAM, Instituto Nacional de Neurología y Neurociencias, SS,* Mexico City, Mexico)

(iii) *Cysticerci viability*, vesicular (Images 1–2 and 1–6), colloidal (Images 1–8 and 1–9), or calcified (Images 1–5 and 1–9).
(iv) *Inflammatory response*, weak (Image 1–10) and strong (Image 1–11).

It has not been demonstrated that *T. saginata* and *T. asiatica* produce human cysticercosis, although some authors suggest that the latter may occur (Galán-Puchades and Fuentes 2013).

9.7 Diagnosis

9.7.1 Diagnosis of Taeniasis

Diagnosis of human tapeworm carriers is essential. Patients with *T. solium* taeniasis may also suffer cysticercosis, which should be always considered in the diagnosis and treatment not only of the patient but also of the people in close contact. Patients with *T. saginata* taeniasis are the source of cattle cysticercosis, while individuals carrying *T. solium* are the origin of human and porcine cysticercosis (Mendlovic et al. 2021).

9.7.1.1 Parasitological Diagnosis

Parasitological diagnosis of taeniasis is based on the identification of gravid proglottids. Proglottids with less than 15 uterine branches are characteristic of *T. solium*, while those with more than 15 branches correspond to *T. saginata*. Proglottids are frequently deformed, hindering species-specific morphological identification (Mayta et al. 2000; Guezala et al. 2009). Microscopic observation of eggs in stool examination (coprological diagnosis by either the Kato-Katz thick smear or Willis techniques) only indicates taeniasis. The diagnosis of the species is not possible when using either of these techniques, because taeniid eggs are morphologically indistinguishable. Parasitological methods have low sensitivity (Webb and Cabada 2017).

9.7.1.2 Immunodiagnosis

Coproantigen detection was developed using polyclonal antibodies (against adult crude extracts) in antigen capture assays. The assay exhibited good sensitivity (100%–85%) and specificity to detect taeniasis carriers although it was not possible to distinguish between *T. solium* and *T. saginata* infections (Allan et al. 1990). Coproantigen detection has been used in epidemiological studies, determination of taeniasis prevalence, and assessment of mass drug administration efficacy in endemic regions (Bustos et al. 2012; Okello et al. 2014; Braae et al. 2017a; Gabriël et al. 2018). Moreover, rabbit polyclonal antibodies were generated using E/S or surface antigens of adult tapeworms (Machnicka et al. 1996), but they did not allow species-specific taeniid diagnosis. An ELISA to detect *T. solium*-specific coproantigens have been developed using a hybrid system, first antibody against *T. solium* adult crude extract and second antibody anti-E/S adult antigens (Guezala et al. 2009). The specificity of rabbit polyclonal antibodies has recently been questioned, as a considerable number of false positives were obtained in samples from several communities (Parkhouse et al. 2020). Currently, there are methods that employ monoclonal antibodies to identify *T. solium* eggs (Montenegro et al. 1996) or coproantigens (Praet et al. 2013; Parkhouse et al. 2020), with excellent sensitivity and specificity.

On the other hand, several techniques have been developed for antibody detection in sera of individuals with taeniasis. The EITB assay with *T. solium* adult E/S yielded 95% sensitivity and 100% specificity (Wilkins et al. 1999). Two E/S antigens were cloned, TSES38 and TSES33, expressed in a baculovirus system showing excellent sensitivities and specificities in EITB (Levine et al. 2004, 2007). TSES33, or TSES38, was used in a magnetic immunochromatographic test to identify *T. solium* taeniasis carriers with good results (Handali et al. 2010). Furthermore, an immunoblot assay was developed for the diagnosis of *T. asiatica* taeniasis (Jeon and Eom 2009). Tso31 is another antigen that allows differentiating *Taenia solium* and *Taenia saginata* in human feces. However, its application with environmental

samples is limited, as the Tso31 gene has also been identified in the genome of *T. multiceps* (Vargas-Calla et al. 2019).

9.7.1.3 Molecular Diagnosis

Several molecular targets have been cloned, characterized, and used for the molecular diagnosis of human taeniids. Ribosomal, mitochondrial, repetitive DNA sequences have been used in the development of different PCR (*polymerase chain reaction*) protocols (Harrison et al. 1990; Gottstein et al. 1991; Zarlenga et al. 1991; Bowles and McManus 1994). PCR-RFLP (*PCR-restriction fragment length polymorphism*) (Mayta et al. 2000; Cho et al. 2014), Multiplex-PCR (González et al. 2000, 2010; Jeon et al. 2009, 2011, 2013; Jeon and Eom 2013; Pornruseetriratn et al. 2017; Li et al. 2019), PCR sequencing (Jeon et al. 2013; Cho et al. 2014; Okello et al. 2014; Song et al. 2019), and real-time PCR (Praet et al. 2013) have allowed species-specific identification of *T solium*, *T. saginata*, and *T. asiatica*. Additionally, some molecular markers have permitted to differentiate between *T. saginata* and *T. asiatica*, as well as two genotypes within *T. solium*, the African-American genotype and Asian genotype (Gasser et al. 1999; Hancock et al. 2001; Yamasaki et al. 2004; Ito et al. 2003; Jeon et al. 2009, 2011; Sato et al. 2011; Jeon and Eom 2013). Moreover, the rapid and simple loop-mediated isothermal amplification (LAMP) protocol has been established for the differential diagnosis of human taeniids (Nkouawa et al. 2010). Recently, a triplex real-time PCR able to discriminate between the three *Taenia* species in human stool and, with superior performance than the KK thick smear and cAgELISA in terms of sensitivity and specificity, has been described (Ng-Nguyen et al. 2017). Most of these molecular protocols have been used with infected stools, showing excellent sensitivity and specificity.

9.7.2 Diagnosis of Cysticercosis

9.7.2.1 Parasitological Diagnosis

Parasitological diagnosis (direct visualization of parasite/lesion) of NCC is usually carried out during autopsies (postmortem) or biopsies (Schantz et al. 1992). Ophthalmologic examination is of great use in ocular cysticercosis. When cysticerci are located in muscle or subcutaneous tissue, palpation, biopsies, and fine-needle aspiration cytology are employed, with rapid onsite evaluation. These methods enable early disease detection, particularly when lesions are found in anatomically approachable superficial sites, although differential diagnosis from other pathogens should be made (Handa et al. 2008; Santosh et al. 2019).

9.7.2.2 Neuroimaging

CT and MRI are key for the diagnosis of NCC, since they allow knowing the number, size, evolutionary stage, and location of the lesions, as the inflammation response. Differential diagnosis from other neurological disorders is essential considering that neuroimaging cost and technical complexity hamper their use in some endemic areas (Del Brutto et al. 2017). Ultrasonography and MRI allow identifying intramuscular cysticerci even solitary specimens (Tripathy et al. 2012). Imaging techniques have improved the detection of scolices and visualization of cysts in extraparenchymal spaces (Carpio et al. 2013; Del Brutto et al. 2017).

9.7.2.3 Immunodiagnosis

Immunodiagnostic techniques include detection of antibodies, and antigens, in human serum and CSF samples, supporting NCC diagnosis (Del Brutto et al. 2017). These techniques are also used in the diagnosis of cattle and porcine cysticercosis.

Harrison et al. (1989) developed an antigen capture immunodiagnostic assay based on HP10 monoclonal antibody (HP10-Ag-ELISA), specific for repetitive glyco-residues in secretions of *T. saginata* and other taeniid metacestodes. The assay has been used with serum and CSF samples, showing the best sensitivity when patients have several alive cysticerci and severe NCC (Ferrer et al. 2003a; Fleury et al. 2007, 2013a, b; Wardrop et al. 2015; Hernández et al. 2019; Cortez et al. 2020). Additionally, this Ag-ELISA assay permits monitoring NCC treatment. Based on the same monoclonal antibody, a lateral flow assay (HP10-Ag-LFA) for the diagnosis of extraparenchymal neurocysticercosis (EP-NCC) has been developed and successfully tested with CSF and serum samples, providing an encouraging field test for rapid identification of endemic human and bovine cysticercosis (Fleury et al. 2016; Parkhouse et al. 2018, 2019). It was recently found that the HP10-Ag-ELISA also detects a similar protein in the vesicular fluid of *T. hydatigena*; therefore, whereas specific for human cysticercosis, this assay should not be used for the diagnosis of porcine cysticercosis in areas where *T. solium* and *T. hydatigena* coexist (Cortez et al. 2018).

Other authors have prepared monoclonal antibodies (Wang et al. 1992; Brandt et al. 1992) and rabbit polyclonal antibodies (Pardini et al. 2001; Parija and Rajesh Reddy 2006) for circulating antigen immunodiagnosis. Van Kerckhoven et al. (1998) used the B158 monoclonal antibody, an anti-*T. saginata* reagent, in a Ag-ELISA for cysticercosis detection. The system has been used for cattle and porcine diagnosis, seroepidemiological surveys in endemic regions, and NCC detection and differentiation between active and inactive NCC (Dorny et al. 2003; Nguekam et al. 2003; Mwape et al. 2013). The authors have confirmed the utility of urine samples for cysticercosis diagnosis (Castillo et al. 2009; Mwape et al. 2011). The monoclonal antibody-based B158/B60 Ag-ELISA has been used for cattle,

porcine, and human cysticercosis diagnosis in several epidemiological studies (Porphyre et al. 2016; Jansen et al. 2017, 2018; Garvey et al. 2018; Kabululu et al. 2020a). In most *T. hydatigena*-positive pigs, circulating antigens were detected by the B158/B60 Ag-ELISA, confirming that this test cannot be used to diagnose porcine *T. solium* cysticercosis in areas where these parasites are coendemic (Nguyen et al. 2020). Another study in which several new monoclonal antibodies raised against *T. solium* (TsmAbs) as capture antibodies and a rabbit polyclonal anti-*T. solium* whole cyst as a detector antibody were used showed that eight TsmAbs detected antigens in NCC-positive human sera and three of these also in urine samples, pointing out a potential utility of some of these TsmAbs for the diagnosis and posttreatment monitoring of patients with viable NCC infections (Paredes et al. 2016).

There are many tested methods and techniques for NCC immunodiagnosis by antibody detection in serum, CSF, saliva, and urine samples. They are probably the first choice in a routine microbiology laboratory, although it is worth considering that antibody detection indicates parasite exposure but not always active infection and works better for active NCC diagnosis than for inactive NCC (García et al. 2001; White and Garcia 2018).

For many years, *T. solium* crude antigens, complete extract or vesicular fluid, have been used (Larralde et al. 1990). These techniques show poor specificity, mainly due to cross-reactions with related helminth infections (Gottstein et al. 1987). Moreover, heterologous antigenic extracts have been employed. *T. saginata* (Harrison and Parkhouse 1989; Oliveira et al. 2010; Nunes et al. 2017), *T. crassiceps* (Larralde et al. 1990; Espíndola et al. 2002; Suzuki et al. 2007; da Silva et al. 2017), and *T. hydatigena* (Hayunga et al. 1991) have been used to detect human cysticercosis.

Purified antigens were introduced as diagnostic tools. Antigen B (AgB or paramyosin) and glycoproteins have been studied for NCC immunodiagnosis (Flisser et al. 1980; Laclette et al. 1992; Tsang et al. 1989). Based on a lentil-lectin chromatography, metacestode glycoproteins were purified and used to diagnose NCC in either EITB (LLGP-EITB) or ELISA protocols, with serum or CSF samples. Several studies, as well as the Pan American Health Organization, have recognized LLGP-EITB as the gold standard for NCC (Greene et al. 2000; White and Garcia 2018; Carod and Dorny 2020; Nguyen et al. 2020). LLGP-EITB has 100% specificity and an overall sensitivity of 98%. However, it maintains the major problems of other serological techniques for NCC, i.e., approximately 30% of patients with a single brain parasite, or calcified lesions, may test negative (Wilson et al. 1991) and *T. solium* genotypes displays distinct glycoprotein patterns (Sato et al. 2006; Romo et al. 2020). The main drawback of this technique is the complexity to obtain and purify the LLGP extract and its high cost, hampering its use in endemic areas (Suzuki and Rossi 2011; Hernández-González et al. 2017). A meta-analysis of NCC diagnostic tests has shown that detection of antibodies in serum and CSF by ELISA or EITB has a similar diagnostic value irrespective of the antigen used, but the study does not identify all potential sources of heterogeneity (Cardona-Arias et al. 2017). Conversely, other studies have revealed improved

efficiency of EITB when purified native antigen mix extracted from the fluid of cysticercus is used (Ribeiro Vda et al. 2014; Ayala-Sulca and Miranda-Ulloa 2015). Moreover, purified *T. solium* E/S antigens have been employed in ELISA and FAST-ELISA with very promising results in NCC antibody detection (Ng and Ko 1994; Sahu et al. 2009; Atluri et al. 2009). Low molecular weight metacestode secretion proteins, and especially glycoproteins, have showed best performances in NCC diagnosis assays. Thus, 14- and 18-kDa antigens (Espíndola et al. 2002; Molinari et al. 2002; Sahu et al. 2009) and 8–30 kDa protein fraction (Gottstein et al. 1987; Yang et al. 1998; Park et al. 2000; Atluri et al. 2009; Jeon and Eom 2009; Sako et al. 2015; Nkouawa et al. 2017; Li et al. 2019) have been described as the best candidates for developing an antibody detection system aimed at detecting NCC. Although these systems have worked properly, some difficulties (biochemical purification, requirement of big parasite amounts, reproducibility) restrict their uses.

Biotechnological approaches have been used to solve the scarcity of *T. solium* parasitic material for the preparation and purification of diagnostic antigen candidates. Cloning and expression of *T. solium* metacestode genes relevant for diagnosis have allowed circumventing this limitation.

Many genes have been studied over the past decades. Paramyosin, sHSP, TSA18/HP6, F18, TS14, TS18, T24, 50-kDa glycoprotein, TsAg5, and other molecules were cloned and expressed in prokaryotic and eukaryotic systems and evaluated with collections of serum and CSF samples. The recombinant products were checked by ELISA, western blot (WB), EITB, or multiplex bead-based assay, with good sensitivity and specificity for NCC diagnosis (Greene et al. 2000; Vazquez-Talavera et al. 2001; Ferrer et al. 2003b, 2005a, 2007a; Montero et al. 2003; Hancock et al. 2003, 2004, 2006; Hernández-González et al. 2017). Although most worked better with active NCC samples, Tsag-HP6 expressed in a baculovirus system showed the best sensitivity (60%) for inactive NCC immunodetection. Furthermore, some recombinant antigens have been used for porcine and bovine cysticercosis identification. The design of an antemortem immunodiagnostic method for bovine cysticercosis by ELISA based on Tsag3 has been suggested. This recombinant antigen was obtained from a phage display peptide library, exhibiting similar results to *T. saginata* metacestode crude antigen (TsCa) when used as a capture antigen in an ELISA (Fogaça et al. 2014). Besides, a novel cathepsin L-like cysteine protease from *T. solium* metacestode was expressed successfully in a baculovirus system and was evaluated as a candidate antigen to diagnose porcine cysticercosis by ELISA immunoassay (León-Janampa et al. 2019).

Regarding recombinant products, one of the most promising NCC diagnostic antigens is the 8-kDa family of proteins. Their members are metacestode excretory/secretory glycoproteins (65–90 amino acid residues and 7–12 kDa), which elicit strong specific antibody reactions in the infected individuals. They appear to be expressed as variant arrays, with both sequence heterogeneity and homology in clusters of small domains that determine epitope differences among them (Hancock et al. 2003; Ferrer et al. 2007b, 2009, 2012). The main members of this antigen family are the 10-kDa molecule (cysticercosis diagnosis antigen (CyDA)) (Chung et al. 1999); NC-3(8 kDa)/NC-9(13 kDa) antigens (Hubert et al. 1999);

glycoproteins TS14 (14 kDa) and TS18 (18 kDa) (Greene et al. 2000); Ag1, Ag1V1, Ag2, Ag2V1, and chimeric Ag1V1–Ag2 molecules (Sako et al. 2000; Sato et al. 2011); and Ts8B1, Ts8B2, and Ts8B3 (Ferrer et al. 2007b, 2009, 2012).

Recently, new recombinant antigens have been described, showing good sensitivity and specificity for NCC diagnosis. A recombinant truncated form of the native gp24 (Hancock et al. 2006) has been suggested (rT24H-EITB) as an alternative to native antigen LLGP-EITB for the diagnosis of NCC. rT24H has been successfully tested in classical (ELISA, EITB) and innovative formats (multiplex bead-based (MBA) assay) (Hernández-González et al. 2017; Dermauw et al. 2018). A novel portable lateral flow assay has been developed with this antigen for point-of-care testing of specific antibodies in human serum with the aid of a mobile phone (Lee et al. 2019). Three other new recombinant antigens of *T. solium* metacestode have been described for the immunodiagnosis of cysticercosis, TsF78 (filamin), TsP43 (peroxidase), and TsC28 (collagen XV), with diagnostic performances (Morillo et al. 2020).

Synthetic peptides, derived from the cloned molecules, have been prepared for cysticercosis diagnosis and employed in ELISA and WB. In some cases, the results were good, although the diagnostic properties of recombinant antigens were not improved. Based on the *Taenia* 8-kDa family of proteins, sTS14 and sTS18 peptides (TS14 and TS18 antigens) have been synthesized, showing excellent specificity but poor sensitivity (Greene et al. 2000). Moreover, nine of the 8-kDa family of proteins have been chemically synthesized (Ts18, Ts18 var1, Ts18 var3, Ts18 var4, Ts18 var6, TsRS2 var1 Ts14, Ts18 var8, and TsRS1) and evaluated by ELISA. Of these, TsRS1 (100% sensitivity and 100% specificity), when tested with cysticercosis-positive sera (previously reactive with the 8-kDa proteins) on WB, and Ts18 var1 and Ts18 var3 (97% sensitivity and 100% specificity) were theoretically selected for a future diagnostic antigen cocktail (Hancock et al. 2003). Recently, NC41 synthetic peptide was evaluated to diagnose NC, reaching high diagnostic performance (Ribeiro et al. 2019).

Peptides derived from *T. saginata* oncosphere molecules (Tovis1, Tovis5, HP6-3, TEG-1 y Cow-10) have also been tested for human, bovine, and porcine cysticercosis diagnosis, with results similar to the above (Fleury et al. 2003; Ferrer et al. 2003a, b, 2005b). Based on the primary sequence of an in silico structural model of the *T. saginata* 18-kDa surface/secreted oncospheral adhesion protein, an epitope region, designated as EP1, showed high diagnostic potential to detect bovine cysticercosis (Guimarães-Peixoto et al. 2018).

9.7.2.4 Molecular Diagnosis

In 2006, Almeida et al. demonstrated, for the first time, the presence of *T. solium* DNA in CSF from NCC patients, which has allowed the use of molecular techniques, PCRs, for NCC diagnosis. Conventional and real-time PCR protocols were developed, with excellent sensitivity (70–95%) for the identification of cases (Hernández et al. 2008; Michelet et al. 2011; Yera et al. 2011; Rottbeck et al.

2013). Recently, a novel quantitative PCR (based on a highly repetitive Tsol13 sequence) was designed, showing high sensitivity and specificity for the diagnosis of subarachnoid and ventricular NCC and to assess response to treatment (O'Connell et al. 2020). PCR systems have also been used to detect *T. solium* DNA in other samples such as brain biopsy (Ong et al. 2020) and blood and urine samples (Goyal et al. 2020). On the other hand, CSF next-generation sequencing-based pathogen analysis has been reported for successful diagnosis and follow-up of a patient with NCC (Liu et al. 2018).

Different PCR-based approaches have been described for bovine and porcine cysticercosis diagnosis (Hosseinzadeh et al. 2013; Figueiredo et al. 2019; Gauci et al. 2019; Waema et al. 2020).

Considering NCC complexity and diagnosis difficulties, identification of these diseases is possible by compiling laboratory diagnostic results and clinical/epidemiological data following Del Brutto's improved criteria (Del Brutto et al. 2017).

9.8 Treatment

9.8.1 Taeniasis

Niclosamide is the drug of choice, for the treatment of taeniasis, with an efficacy of up to 85% with a single dose. A single 2-gram oral is recommended for adults and children older than 6 years and 1 gram in children aged 2–6 years. It is not absorbable and thus nontoxic. It is effective against the adult form of the parasite (tapeworm), but not against the larval form (cysticercus) (PAHO/WHO 2019). Oral administration of *praziquantel* is effective against adult parasites (tapeworms) and larval forms (cysticerci) with an efficacy of up to 95% reported for the treatment of taeniasis. It has the disadvantage of being systemically absorbed and can thus cross the blood-brain barrier, with the consequent risk of causing neurological symptoms if latent NCC is present in the same individual. Regarding praziquantel posology, a single 2-gram oral is recommended for the treatment of taeniasis. *Albendazole*, a triple-dose albendazole (400 mg once a day for 3 consecutive days), is also effective for treating taeniasis. However, it is not clear if three consecutive doses of this drug lead to the same risks as *praziquantel* in patients with neurocysticercosis (PAHO/WHO 2019; WHO 2020).

9.8.2 Cysticercosis

NCC treatment is complex and should be tailored individually. Management of the disease may involve the use of cysticidal therapy, symptomatic therapy, and sometimes surgery; thus, it is recommended to adjust the treatment to the type of NCC. Treatment dosage and duration are highly diverse and depend on factors such as cyst

location, number, and developmental stage, surrounding inflammatory edema, acuteness and severity of clinical symptoms or signs, and host response. Treatment should always be done under medical supervision (White and Garcia 2018; WHO 2020).

9.8.2.1 Cysticidal Therapy

Praziquantel and *albendazole* are used for treating NCC. *Albendazole* is used at a dose of 15 mg/kg/day (maximum 800 mg). It is usually administered for 28 days, although shorter durations of 8–14 days have also been used. Side effects depend on dose and duration of therapy. *Praziquantel* is used at a dose of 50 mg/kg/day. Usual duration of therapy is 15 days. Side effects are dose-related, although uncommon (Singhi 2011). Praziquantel and albendazole have been used together with interesting results (Garcia et al. 2011; WHO 2020). These drugs are mainly employed for parenchymal viable cysts. Since its implementation, cysticidal therapy has been a matter of debate, regarding advantages of cyst destruction and real improvement of clinical outcome. Most publications report "reduction of the number of lesions" as the measure for anthelmintic drug effectiveness, which is misleading. Assessing cyst disappearance may possibly be a more appropriate approximation.

In summary, it is generally accepted, with few discrepancies, that both drugs are effective in destroying viable cysts, based on double-blind, placebo-controlled trials, comparing the effect of *albendazole* and *praziquantel*. However, their use in cases with enhancing lesions has been debated as these lesions are considered degenerating cysts, many of which resolve spontaneously (Carpio et al. 2008; Thussu et al. 2008; Chaurasia et al. 2010). Controversial results have also been obtained regarding the role of cysticidal therapy in the control of seizures secondary to NCC. Some authors found improved seizure control in adults with vesicular lesions, as well as of enhancing lesions (Garcia et al. 2004; Del Brutto et al. 2006), while others did not find any significant improvement and suggest that cyst degeneration and the subsequent inflammatory reaction increase seizure expression (Carpio et al. 2008; Abba et al. 2010; Garcia et al. 2017). In conclusion, cysticidal therapy seems to be effective in reducing the number of lesions, but further larger studies need to be done to determine its role in improving long-term seizure control.

Cysticidal drugs have also been found to be effective in the treatment of some extraparenchymal NCC cases and even for giant cysts (Proaño et al. 2001) although great caution is required in the management of this type of NCC. Cysticidal therapy should not be used in cases with markedly elevated intracranial pressure, in ophthalmic (intraocular) NCC, or in massive infections; steroids alone are used in these situations. Cysticidal therapy is of no use for calcified lesion(s). Single enhancing lesions and one to two viable parenchymal cysticerci may be treated with short courses of *albendazole* and corticosteroids. Multiple parenchymal lesions should be treated with a combination of corticosteroids, *albendazole*, and *praziquantel*. Ventricular cysticerci should be removed when possible, usually by minimally invasive

surgery. Subarachnoid cysticercosis often requires prolonged courses of antiparasitic and anti-inflammatory treatment (White and Garcia 2018).

There is no universally agreed single protocol for the treatment of NCC, and consensus guidelines recommend an individualized approach (Nash et al. 2006; Raibagkar and Berkowitz 2018).

9.8.2.2 Symptomatic Therapy

Seizures usually respond very well to first-line antiepileptic drugs (AEDs). Recurrence rate following AED withdrawal is low in single-lesion NCC cases. Moreover, recurrence of seizures after AED withdrawal is correlated with the presence of multiple lesions prior to initiation of cysticidal therapy and persistence or calcification of lesions after therapy (Talukdar et al. 2002; Goel et al. 2010; Singh and Sharma 2017; Raibagkar and Berkowitz 2018). Corticosteroids and combinations of anthelmintics and corticosteroid treatments reduce short-term incidence of seizures. Although AEDs are routinely employed in the treatment of seizures associated with NCC, there is no clear consensus regarding the choice and optimal duration of treatment with this type of medications. Long-term AED treatment is justified in people with calcific residues following involution of brain parenchymal cysticercosis (White and Garcia 2018).

9.8.2.3 Corticosteroids

Generally, oral corticosteroids are administrated few days before and with anticysticercal therapy to prevent any potential adverse reactions due to host inflammatory response. Oral prednisolone at 1–2 mg/kg is usually prescribed; intravenous dexamethasone may be used when there is concern of raised intracranial pressure. In cases with disseminated lesions and extensive cerebral edema, steroids may be required for a prolonged period (Singhi 2011; Singh and Sharma 2017).

9.8.2.4 Surgery

Surgical intervention is required in some cases, particularly in intraventricular and subarachnoid NCC. A ventriculoperitoneal shunt is needed for hydrocephalous; simultaneous use of steroids and albendazole and recurrent courses of steroids reduce the risk of frequent obstructions. Endoscopic removal of cysts is the least invasive approach being the procedure of choice. A safe, simple, and cost-effective endoscopic approach that allows minimally invasive removal of the fourth ventricle cysts and treatment of hydrocephalus without any morbidity has been described. Excision of giant cysts that fail to respond to medical therapy may be required (Goel et al. 2008; Suri et al. 2008; Singhi 2011; Sharma et al. 2019).

9.9 Prognosis

Striated muscle and subcutaneous cysticercosis have good prognosis. Ocular cysticercosis may lead to blindness if the eye parasite is not diagnosed in time (Sundar et al. 2010). Generally, NCC cases with single lesions have a good prognosis, seizures are usually well controlled, and the lesions disappear within 6 months in over 60% cases. Patients with multiple lesions and those with calcifications often experience recurrence of the seizures. Prognosis should be made with caution in cases of cysticercus encephalitis and extraparenchymal NCC (Singhi 2011). The benefits of antiparasitic treatment in parenchymal brain cysticercosis clearly outweigh the risks, and substantive evidence has been provided on the role of NCC as a cause of seizures and epilepsy. Antiparasitic therapy should be considered the primary option in the management of patients with live or degenerating brain NCC cysts (Garcia et al. 2017).

9.10 Prevention and Control

Cysticercosis is an NTD that occurs in communities with low socioeconomic conditions and poor sanitation-hygienic practices. To prevent, control, and finally eliminate *T. solium*, proper public health interventions with an approach spanning veterinary and human health and environmental sectors are required (WHO 2020). Globally, it can be prevented through improvements in health and education standards, treatment of *T. solium* carriers, improved pig rearing management, as well as treatment of infected animals (Flisser et al. 2003, 2004; Engels et al. 2003; González et al. 2003; Xiao et al. 2013; PAHO/WHO 2019; WHO 2020). The following should be taken into account when considering the eradication of cysticercosis: human tapeworm carriers are the definitive hosts and the sole source of infection for intermediate hosts, domestic animals are the main intermediate hosts, wild reservoirs are not important, and intervention tools for control are available (Gilman et al. 2012; PAHO/WHO 2019; WHO 2020; Mendlovic et al. 2021). However, reliable epidemiological data on the geographical distribution of *T. solium* taeniasis/cysticercosis in people and pigs are scarce. Suitable surveillance mechanisms should enable new cases of human or porcine cysticercosis to be recorded in order to help identify communities at high risk and focus on prevention and control measures in these areas (WHO 2020).

PAHO and WHO expert committees, as well as other initiatives (foundations and networks of specialized groups on international projects), have developed plans for the implementation of activities for the prevention and control of taeniasis and cysticercosis caused by *Taenia solium* (Ferrer et al. 2011; Fleury et al. 2013a; PAHO/WHO 2019; WHO 2020). Potential intervention measures should include the following:

- *Community health education, including hygiene and food safety.* Educational models for endemic rural areas, considering cultural influences and idiosyncrasies of the population. The models should include basic and adequate hygiene and sanitation measures, education on parasite biology and epidemiology, taeniasis/cysticercosis symptom appraisal, and modes of interrupting transmission, among other information.
- *Improved sanitation*. As supporting measures, improvement of basic sanitation, access to safe drinking water, sanitation, and hygiene. VIP latrines (ventilated pit latrines), septic tanks, and end open defecation are very helpful in keeping infected human excreta away from where people and pigs live to minimize the risk of infection.
- *Drug treatment of taeniasis carriers.* Human tapeworm carriers are the main proven risk factor for acquiring cysticercosis. Thus, finding and treating individuals infected with tapeworms should be the intervention of choice. Once a *Taenia* carrier is identified, careful treatment and follow-up should ensure the cure of the patient to interrupt transmission. So far, mass treatment programs to eliminate tapeworms with niclosamide or *praziquantel* (García et al. 2016; Braae et al. 2017a) have been successful, reducing disease transmission temporarily, but the effect has not been sustainable. A study with *T. solium* in endemic rural Tanzanian communities showed that school-based repeated mass drug administration (MDA) with *praziquantel*, and the tracking/treatment of taeniasis cases, significantly reduced the copro-Ag prevalence of taeniasis. Annual MDA was significantly better than a single MDA (Braae et al. 2017a, b).
- *Improved pig husbandry and improved meat inspection and processing of meat products*. Stop free-roaming pigs and slaughterhouse control are suggested as control measures. Keeping pigs in pigpens is essential. However, this option is opposed to the main reason of raising pigs in endemic regions, that is, they roam free and do not need to be fed by their owners. Development and establishment of illegal markets where potentially infected pork will be sold must also be avoided.
- *Interventions in pigs (anthelmintic treatment and pig vaccination).* There are several antiparasitic drugs that kill cysticerci in pigs. Oxfendazole, a very safe and effective benzimidazole anthelmintic drug, is the most recommended. A single oral dose of 30 mg/kg has been shown to kill cysticerci present in the muscles. Other antiparasitic drugs such as *praziquantel* and *albendazole* require multiple doses administered in short periods of time and may cause side effects (albendazole) (Gonzalez et al. 2012; Kabululu et al. 2020b). Progress made in vaccine technology makes pig vaccination a good alternative in the control of cysticercosis. Several intervention programs have already included the use of vaccines to interrupt parasite transmission, among other measures. There have been attempts to design vaccines based on different antigen sources, from crude extracts, recombinant antigens, and naked DNA peptides (Assana et al. 2013; Xiao et al. 2013; Monreal-Escalante et al. 2016; Kabululu et al. 2020b). Some of these studies are summarized below.

Pioneer vaccination studies in cattle were carried out with crude and E/S antigens from *T. saginata* and *T. hydatigena* (Rickard et al. 1981). Since then, trials with different parasite extracts prepared from *T. solium* oncospheres, and metacestodes, have been used in porcine vaccination trials. Different protection levels have been reported (Molinari et al. 1993a, 1997; Pathak and Gaur 1990; Verastegui et al. 2002). This type of assays has also been developed in *T. crassiceps* mouse model (Valdez et al. 1994; Sciutto et al. 1995), obtaining similar protection levels with *T. solium* and *T. crassiceps* antigenic extracts.

Recombinant antigens have also been used as vaccines. For the *T. saginata*/cattle system, most of these molecules were from surface and secreted components of the infective oncosphere (Benítez et al. 1996; Lightowlers et al. 1996; Bonay et al. 2002; Harrison et al. 2005). Antigens related to the taeniid 45-W protective gene family (Johnson et al. 1989; Lightowlers et al. 1996; Gauci and Lightowlers 2003; González et al. 2005), i.e., *T. saginata* TSA9, TSA18, and Tsag-HP6 recombinant proteins, were used in cattle immunization assays, with TSA18 and Tsag-HP6 yielding excellent results (Benítez et al. 1996; Lightowlers et al. 1996). It is interesting to note that all these purified recombinant proteins function as adhesion molecules, a property probably pertinent to their potential as vaccines, as is the case of the *T. saginata* HP6 molecule (Harrison and Parkhouse 1989; Benítez et al. 1996; Bonay et al. 2002; Harrison et al. 2005). Concurrently, in the *T. solium*/porcine system, genes and proteins from the oncosphere (Tsol18, Tsol45-1A, TSOL45-1B, TSOL16), and other sources [paramyosin, fatty acid-binding proteins (FABPs), KETc1, KETc4, KETc7, KETc11, KETc12], were cloned and tested in vaccination assays with TSOL18 yielding the best results (Manoutcharian et al. 1996; Vazquez-Talavera et al. 2001; Gauci et al. 2012).

Regarding peptides as vaccination tools, KETc1, KETc12, KETc7, GK1, GK2, and GK3 have been the most used (Manoutcharian et al. 2004; Toledo et al. 2001). Later, S3Pvac, a combination of KETc1, KETc12, KETc7, and GK1, was extensively employed in vaccination assays in *T. crassiceps*/mice, *T. pisiformis*/rabbit, and *T. solium*/porcine models. Protection with S3Pvac in pigs is 98.7% and showed therapeutics properties (de Aluja et al. 2005; Rassy et al. 2010; Sciutto et al. 2013a, b). A novel strategy for developing a multi-epitope low-cost vaccine was also explored. This approach combined the components (KETc1, KETc12, KETc7, and GK1) of the S3Pvac vaccine and the protective TSOL18 antigen expressed in a Helios2A polyprotein system. This protein arrangement was expressed in transgenic tobacco, carrots, and papaya cells. The plant-derived Helios2A vaccine was recognized by antibodies in the cerebral spinal fluid in NCC patients and elicited specific antibodies in BALB/c immunized mice (Monreal-Escalante et al. 2015, 2016; Fragoso et al. 2017; Rosales-Mendoza et al. 2018). Recently, the performance of *T. solium* cysticerci Cc48 mimotope (mCc48) and the corresponding synthetic peptide (sCc48) as the vaccine candidate in experimental murine cysticercosis was tested. The protection induced by the mimotope-based synthetic peptide sCc48 justifies further studies of this mimotope as a potential vaccine against cysticercosis (Manhani et al. 2020).

DNA vaccination has also been used in experimental studies; *T. saginata* and *T. solium* cDNAs (abovementioned) (KETc7, paramyosin, Tso18, others) have been employed with promising protection against cysticercosis (Manoutcharian et al. 1998; Cruz-Revilla et al. 2000; Rosas et al. 2002). In the case of TSOL18, DNA vaccination experiments yielded better results when an improved TSOL18 gene was developed through optimized codon usage (Wang et al. 2015).

Concerning trials with recombinant proteins, of all the tested molecules, the TSOL18 plasmid construction, expressed in *Escherichia coli* and purified, is the candidate with the best results, a protection of almost 100%. TSOL18 was developed by the University of Melbourne in Australia and is being used as a vaccine in control programs organized in different endemic regions in Mexico, Honduras, Peru, and Cameroon, among other countries, with good results (Flisser et al. 2004; Lightowlers 2006; Gauci et al. 2012; Assana et al. 2010, 2013; García et al. 2016; Okello et al. 2017; Kabululu et al. 2020b). The TSOL18 vaccine is a registered commercial vaccine for the control of porcine cysticercosis. It is produced in India by Indian Immunologicals Limited following Good Manufacturing Practice regulations and is commercialized under the name of Cysvax®.

The TSOL18 vaccine for swine cysticercosis associated with oxfendazole has provided a feasible method to control porcine cysticercosis that, combined with identification and treatment of intestinal tapeworm carriers, allows approaching taeniasis/cysticercosis control within the scope of *One Health*. *One Health* is a concept that recognizes that human health is closely related to animal and environmental health. Thus, identification of mechanisms of broad transmission of tapeworm eggs should also be assessed. It is widely accepted that integrated *One Health* interventions are more likely to achieve faster sustainable control of *T. solium*-related diseases (PAHO/WHO 2019; WHO 2020; Mendlovic et al. 2021).

References

Abba K, Ramaratnam S, Ranganathan LN (2010) Anthelmintics for people with neurocysticercosis. Cochrane Database Syst Rev 17(3):CD000215. https://doi.org/10.1002/14651858

Allan JC, Ávila G, García-Noval J et al (1990) Immunodiagnosis of taeniasis by coproantigen detection. Parasitology 101:473–477. https://doi.org/10.1017/s0031182000060686

Almeida CR, Ojopi EP, Nunes CM et al (2006) *Taenia solium* DNA is present in the cerebrospinal fluid of neurocysticercosis patients and can be used for diagnosis. Eur Arch Psychiatry Clin Neurosci 256:307–310. https://doi.org/10.1007/s00406-006-0612-3

de Aluja A, Villalobos N, Nava G et al (2005) Therapeutic capacity of the synthetic peptide-based vaccine against *Taenia solium* cysticercosis in pigs. Vaccine 23:4062–4069

Assana E, Kyngdon CT, Gauci CG et al (2010) Elimination of *Taenia solium* transmission to pigs in a field trial of the TSOL18 vaccine in Cameroon. Int J Parasitol 40:515–519. https://doi.org/10.1016/j.ijpara.2010.01.006

Assana E, Lightowlers MW, Zoli AP et al (2013) Taenia solium taeniosis/cysticercosis in Africa: risk factors, epidemiology and prospects for control using vaccination. Vet Parasitol 195:14–23

Atluri SR, Singhi P, Khandelwal et al (2009) Neurocysticercosis immunodiagnosis using *Taenia solium* cysticerci crude soluble extract, excretory secretory and lower molecular mass antigens

in serum and urine samples of Indian children. Acta Trop 110:22–27. https://doi.org/10.1016/j.actatropica.2008.12.004

Aung AK, Spelman DW (2016) Taenia solium taeniasis and cysticercosis in Southeast Asia. Am J Trop Med Hyg 94:947–954. https://doi.org/10.4269/ajtmh.15-0684

Ayala-Sulca E, Miranda-Ulloa E (2015) Evaluation of enzyme-linked immunoelectrotransfer blot test using purified native antigen mix from cysticercus fluid of *Taenia solium* for diagnosis of human cysticercosis. Rev Peru Med Exp Salud Publica 32:485–491

Benítez L, Gárate T, Harrison L et al (1996) Cloning and sequencing of the gene encoding the principal 18-kDa secreted antigen of activated oncospheres of *Taenia saginata*. Mol Bichem Parasitol 78:265–268

Bhattarai R, Carabin H, Proaño JV et al (2019) The monetary burden of cysticercosis in Mexico. PLoS Negl Trop Dis 13:e00007501. https://doi.org/10.1371/journal.pntd.0007501

Bonay P, González LM, Benítez L et al (2002) Genomic and functional characterisation of a secreted antigen of *Taenia saginata* oncospheres. Mol Bichem Parasitol 121:269–273

Bowles JR, McManus DP (1994) Genetic characterization of the *Asian Taenia*, a newly described taeniid cestode of humans. Am J Trop Med Hyg 50:33–44

Braae UC, Magnussen P, Ndawi B et al (2017a) Effect of repeated mass drug administration with praziquantel and track and treat of taeniosis cases on the prevalence of taeniosis in *Taenia solium* endemic rural communities of Tanzania. Acta Trop 165:246–251

Braae UC, Devleesschauwer B, Sithole F et al (2017b) Mapping occurrence of Taenia solium taeniosis/cysticercosis and areas at risk of porcine cysticercosis in Central America and the Caribbean basin. Parasit Vectors 10(1):424. https://doi.org/10.1186/s13071-017-2362-7

Brandt JR, Geerts S, De Deken R et al (1992) A monoclonal antibody-based ELISA for the detection of circulating excretory-secretory antigens in *Taenia saginata* cysticercosis. Int J Parasitol 22:471–477

Bruno E, Bartoloni A, Zammarchi L et al (2013) Epilepsy and neurocysticercosis in Latin America: A systematic review and meta-analysis. PLoS Negl Trop Dis 31:e2480

Bustos JA, Rodriguez S, Jimenez JA (2012) Cysticercosis Working Group in Peru. Detection of *Taenia solium* taeniasis coproantigen is an early indicator of treatment failure for taeniasis. Clin Vaccine Immunol 19:570–573

Cardona-Arias JA, Carrasquilla-Agudelo YE, Restrepo-Posada DC (2017) Validity of three methods for inmuno-diagnostic of neurocysticercosis: systematic review of the literature with meta-analysis 1960-2014. Rev Chil Infectol 34:33–44

Carmen-Orozco RP, Dávila-Villacorta DG, Cauna Y et al (2019) Blood-brain barrier disruption and angiogenesis in a rat model for neurocysticercosis. J Neurosci Res 97:137–148. https://doi.org/10.1002/jnr.24335

Carod JF, Dorny P (2020) Cysticercosis in Madagascar. J Infect Dev Ctries 14:931–942

Carpio A (2002) Neurocysticercosis: an update. Lancet Infec Dis 2:751–762

Carpio A, Kelvin EA, Bagiella E et al (2008) Effects of albendazole treatment on neurocysticercosis: a randomised controlled trial. J Neurol Neurosurg Psychiatry 79:1050–1055

Carpio A, Fleury A, Hauser WA (2013) Neurocysticercosis: five new things. Neurol Clin Pract 3:118–125

Carpio A, Fleury A, Romo ML et al (2016) New diagnostic criteria for neurocysticercosis: reliability and validity. Ann Neurol 80:434–442. https://doi.org/10.1002/ana.24732

Castillo Y, Rodriguez S, García HH et al (2009) Urine antigen detection for the diagnosis of human neurocysticercosis. Am J Trop Med Hyg 80:379–383

Chaurasia RN, Garg RK, Agarwall A et al (2010) Three day albendazole therapy in patients with a solitary cysticercus granuloma: a randomized double blind placebo controlled study. Southeast Asian J Trop Med Public Health 41:517–525

Chavarria A, Fleury A, Bobes RJ et al (2006) A depressed peripheral cellular immune response is related to symptomatic neurocysticercosis. Microbes Infect 8:1082–1089

Cho J, Jung BK, Lim H et al (2014) Four cases of *Taenia saginata* infection with an analysis of COX1 gene. Korean J Parasitol 52(1):79–83

Chung JY, Bahk YY, Huh S et al (1999) A recombinant 10 kDa protein of *Taenia solium* metacestodes specific to active neurocysticercosis. J Infect Dis 180:1307–1315

Coral-Almeida M, Gabriël S, Abatih EN, et al (2015) Taenia solium human cysticercosis: a systematic review of sero-epidemiological data from endemic zones around the world. PLoS Negl Trop Dis 9. https://doi.org/10.1371/journal.pntd.0003919

Coral-Almeida M, Henriquez-Trujillo AR, Asanza S et al (2020) Assessing the burden and spatial distribution of taenia solium human neurocysticercosis in Ecuador (2013–2017). PLoS Negl Trop Dis 14:1–16. https://doi.org/10.1371/journal.pntd.0008384

Correa D, Medina E (1999) Host-parasite immune relationship in *Taenia solium* taeniosis and cysticercosis. In: García HH, Martinez M (eds) *Taenia solium* Taeniasis/Cysticercosis. Editorial Universo, Perú, pp 83–96

Cortez MM, Rojas GC, Parkhouse RME (2018) The HP10 *Taenia* monoclonal antibody-based ELISA detects a similar protein in the vesicular fluid of *Taenia hydatigena*. Trop Anim Health Prod 50:697–700

Cortez MM, Rojas GC, Aguilar CM et al (2020) Seroepidemiological evidence for *Taenia solium* Taeniasis/Cysticercosis in three Venezuelan rural communities. J Helminthol 94:e179

Cruz-Revilla C, Rosas G, Fragoso G et al (2000) *Taenia crassiceps* cysticercosis: protective effect and immune response elicited by DNA immunization. J Parasitol 86:67–74

Del Brutto OH, Garcia HH (2013) Neurocysticercosis. Handb Clin Neurol 114:313–325

Del Brutto OH, Roos KL, Coffey CS et al (2006) Meta-analysis: Cysticidal drugs for neurocysticercosis: albendazole and praziquantel. Ann Intern Med 145:43–51

Del Brutto OH, Nash TE, White AC Jr et al (2017) Revised diagnostic criteria for neurocysticercosis. J Neurol Sci 372:202–210

Dermauw V, Carabin H, Cissé A et al (2018) Evaluating the recombinant T24H enzyme-linked immunoelectrotransfer blot assay for the diagnosis of neurocysticercosis in a panel of samples from a large community-based randomized control trial in 60 villages in Burkina Faso. Am J Trop Med Hyg. 98:565–569

Dorny P, Brandt J, Zoli A et al (2003) Immunodiagnostic tools for human and porcine cysticercosis. Acta Trop 87:79–86

Engels D, Urbani C, Belotto A et al (2003) The control of human (neuro)cysticercosis: Which way forward? Acta Trop 87:177–182

Eom KS, Rim HJ (1993) Morphologic descriptions of *Taenia asiatica* sp. Korean J Parasitol 31:1–6

Eom KS, Rim HJ, Jeon HK (2020) *Taenia asiatica*: Historical overview of taeniasis and cysticercosis with molecular characterization, Adv. Parasitol. 108:133–173. 1st ed. Elsevier Ltd

Espíndola NM, Vaz AJ, Pardini AX et al (2002) Excretory/secretory antigens (ES) from *in vitro* cultures of *Taenia crassiceps* cysticerci, and use of an anti-ES monoclonal antibody for antigen detection in samples of cerebrospinal fluid from patients with neurocysticercosis. Ann Trop Med Parasitol 96:361–368

Ferrer E, Benítez L, Foster-Cuevas M et al (2003a) *Taenia saginata* derived synthetic peptides with potential for the diagnosis of bovine cysticercosis. Vet Parasitol 111:83–94

Ferrer E, Moyano E, Benítez L et al (2003b) Cloning and characterization of *Taenia saginata* paramyosin cDNA. Parasitol Res 91:60–67

Ferrer E, González LM, Foster-Cuevas M et al (2005a) *Taenia solium*: characterization of a small heat shock protein (Tsol-sHSP35.6) and its possible relevance to the diagnosis and pathogenesis of neurocysticercosis. Exp Parasitol 110:1–11

Ferrer E, Cortéz MM, Cabrera Z et al (2005b) Oncospheral peptide-based ELISAs as potential seroepidemiological tools for *Taenia solium* cysticercosis/neurocysticercosis in Venezuela. Trans R Soc Trop Med Hyg 99:568–576

Ferrer E, González LM, Martínez-Escribano JA et al (2007a) Evaluation of recombinant HP6-Tsag, an 18 kDa *Taenia saginata* oncospheral adhesion protein, for the diagnosis of cysticercosis. Parasitol Res 101:517–525

Ferrer E, Bonay P, Foster M et al (2007b) Molecular cloning and characterisation of Ts8B1, Ts8B2 and Ts8B3, tree new members of the *Taenia solium* 8 kDa diagnostic antigen family. Mol Biochem Parasitol 152:90–100

Ferrer E, Martínez-Escribano JA, González Barderas ME et al (2009) Peptide epitopes of the *Taenia solium* antigen Ts8B2 are immunodominant in human and porcine cysticercosis. Mol Biochem Parasitol 168:168–171

Ferrer E, González LM, Gárate T, et al (2011) [Cysticercosis: an abandoned tropical disease. In: The fight against the diseases of poverty. Responsibility and necessity]. BBVA Foundation, Biblioteca Nueva Editorial. First edition, March, 2011. ISBN 978-84-92937-00-4. Chapter 3, Pages. 63–68

Ferrer E, Sánchez J, Milano A et al (2012) Diagnostic epitope variability within *Taenia solium* 8 kDa antigen family: implications for cysticercosis immunodetection. Exp Parasitol 130:78–85

Figueiredo BNS, Libório RA, Sato M et al (2019) Occurrence of bovine cysticercosis in two regions of the state of Tocantins-Brazil and the importance of pathogen identification. Pathogens 8:66

Fleury A, Beltran C, Ferrer E et al (2003) Application of synthetic peptides to the diagnosis of neurocysticercosis. Tropical Med Int Health 8:1124–1130

Fleury A, Hernández M, Avila M et al (2007) Detection of HP10 antigen in serum for diagnosis and follow-up of subarachnoidal and intraventricular human neurocysticercosis. J Neurol Neurosurg Psychiatry 78:970–974

Fleury A, Garcia E, Hernández M et al (2013a) Neurocysticercosis: HP10 antigen detection is useful for the follow-up of the severe patients. PLoS Negl Trop Dis 7:e2096

Fleury A, Sciutto E, de Aluja A et al (2013b) Chapter 5: Novel aspects on cysticercosis and neurocysticercosis: control of *Taenia solium* transmission of taeniosis and cysticercosis in endemic countries: the roles of continental networks of specialists and of local health authorities. Editorial InTech, 2013. ISBN: 980-953-307-458-5. Pages 93–112. https://doi.org/10.5772/51286

Fleury A, Sastre P, Sciutto E, et al. (2016) A lateral flow assay (LFA) for the rapid detection of extraparenchymal neurocysticercosis using cerebrospinal fluid. Exp Parasitol 27:S0014-4894(16)30277-6

Flisser A (2013) State of the art of *Taenia solium* as compared to *Taenia asiatica*. Korean J Parasitol 51:43–49. https://doi.org/10.3347/kjp.2013.51.1.43

Flisser A, Woodhouse E, Larralde C (1980) Human cysticercosis: antigens, antibodies and non-responders. Clin Exp Immunol 39:27–37

Flisser A, Sarti E, Lightowlers M et al (2003) Neurocysticercosis: regional status, epidemiology, impact and control measures in the Americas. Acta Trop 87:43–51

Flisser A, Gauci CG, Zoli A et al (2004) Induction of protection against porcine cysticercosis by vaccination with recombinant oncosphere antigens. Infect Immun 72:5292–5297

Flisser A, Avila G, Maravilla P et al (2010) *Taenia solium*: current understanding of laboratory animal models of taeniosis. Parasitology 137:347–357

Fogaça RL, Capelli-Peixoto J, Yamanaka IB et al (2014) Phage-displayed peptides as capture antigens in an innovative assay for *Taenia saginata*-infected cattle. Appl Microbiol Biotechnol 98:8887–8894

Fragoso G, Hernández M, Cervantes-Torres J et al (2017) Transgenic papaya: a useful platform for oral vaccines. Planta 245(5):1037–1048

Gabriël S, Mwape KE, Phiri IK et al (2018) *Taenia solium* control in Zambia: The potholed road to success. Parasite Epidemiol Control 4:e00082

Galán-Puchades MT, Fuentes MV (2013) *Taenia asiatica*: the most neglected human *Taenia* and the possibility of cysticercosis. Korean J Parasitol 51:51–54

García H, Del Brutto O (2003) Imaging findings in neurocysticercosis. Acta Trop 87:71–78

García HH, González AE, Gilman RH et al (2001) Short report: transient antibody response in *Taenia solium* infection in field conditions-a major contributor to high seroprevalence. Am J Trop Med Hyg 65:31–32

Garcia HH, Pretell EJ, Gilman RH, Martinez SM (2004) A trial of antiparasitic treatment to reduce the rate of seizures due to cerebral cysticercosis. N Engl J Med 350:249–258
Garcia HH, Gonzalez AE, Gilman RH (2011) Cysticercosis of the central nervous system: how should it be managed? Curr Opin Infect Dis 24:423–427
García HH, González AE, Tsang VC et al (2016) Elimination of *Taenia solium* transmission in Northern Peru. N Engl J Med 374:2335–2344
Garcia HH, Del Brutto OH, Cysticercosis Working Group in Peru (2017) Antiparasitic treatment of neurocysticercosis – The effect of cyst destruction in seizure evolution. Epilepsy Behav 76:158–162
Garvey BT, Moyano LM, Ayvar V et al (2018) Neurocysticercosis among people living near pigs heavily infected with cysticercosis in rural endemic Peru. Am J Trop Med Hyg 98:558–564
Gasser RB, Zhu X, Woods W (1999) Genotyping *Taenia* tapeworms by single-strand conformation polymorphism of mitochondrial DNA. Electrophoresis 20:2834–2837
Gauci C, Lightowlers MW (2003) Molecular cloning of genes encoding oncosphere proteins reveals conservation of modular protein structure in cestode antigens. Mol Biochem Parasitol 127:193–198
Gauci CG, Jayashi CM, Gonzalez AE et al (2012) Protection of pigs against *Taenia solium* cysticercosis by immunization with novel recombinant antigens. Vaccine 30:3824–3828
Gauci CG, Ayebazibwe C, Nsadha Z et al (2019) Accurate diagnosis of lesions suspected of being caused by *Taenia solium* in body organs of pigs with naturally acquired porcine cysticercosis. PLoS Negl Trop Dis 13:e0007408
Gilman RH, Gonzalez AE, Llanos-Zavalaga F et al (2012) Prevention and control of *Taenia solium* taeniasis/cysticercosis in Peru. Pathog Glob Health 106:312–318
Goel RK, Ahmad FU, Vellimana AK et al (2008) Endoscopic management of intraventricular neurocysticercosis. J Clin Neurosci 15:1096–1101
Goel D, Mittal M, Bansal KK et al (2010) Natural history of solitary cerebral cysticercosis cases after albendazole therapy: a longitudinal follow-up study from India. Acta Neurol Scand 121:204–208
Gonzales I, Rivera JT, Garcia HH (2016) Pathogenesis of Taenia solium taeniasis and cysticercosis. Parasite Immunol 38:136–146. https://doi.org/10.1111/pim.12307
González LM, Montero E, Harrison LJS et al (2000) Differential diagnosis of *Taenia saginata* and *Taenia solium* infection by PCR. J Clin Microbiol 38:737–744
González A, García HH, Gilman R et al (2003) Control of *Taenia solium*. Acta Trop 87:103–109
González A, Gauci C, Barber D et al (2005) Vaccination of pigs to control human neurocisticercosis. Am J Trop Med Hyg 72:837–839
González LM, Bailo B, Ferrer E et al (2010) Characterization of the *Taenia* spp HDP2 sequence and development of a novel PCR-based assay for discrimination of *Taenia saginata* from *Taenia asiatica*. Parasit Vectors 3:51
Gonzalez AE, Bustos JA, Jimenez JA et al (2012) Efficacy of diverse antiparasitic treatments for cysticercosis in the pig model. Am J Trop Med Hyg 87:292–296
Gottstein B, Zini D, Schantz PM (1987) Species-specific immunodiagnosis of *Taenia solium* cysticercosis by ELISA and immunobloting. Trop Med Parasitol 38:299–303
Gottstein B, Deplazes P, Tanner I et al (1991) Diagnostic identification of *Taenia saginata* with the polymerase chain reaction. Trans R Soc Trop Med Hyg 85:248–249
Goyal G, Phukan AC, Hussain M et al (2020) Identification of *Taenia solium* DNA by PCR in blood and urine samples from a tertiary care center in North India. J Neurol Sci 417:117057
Greene RM, Hancock K, Wilkins PP et al (2000) *Taenia solium*: molecular cloning and serologic evaluation of 14 and 18 kDa related, diagnostic antigens. J Parasitol 86:1001–1007
Guezala MC, Rodriguez S, Zamora H et al (2009) Development of a species-specific coproantigen ELISA for human *Taenia solium* taeniasis. Am J Trop Med Hyg 81:433–437
Guimarães-Peixoto RPM, Arruda Pinto PS, Rebouças Santos M et al (2018) Evaluation of a synthetic peptide from the *Taenia saginata* 18 kDa surface/secreted oncospheral adhesion protein for serological diagnosis of bovine cysticercosis. Acta Trop 164:463–468

Hamamoto Filho PT, Singh G, Winkler AS et al (2020) Could differences in infection pressure be involved in cysticercosis heterogeneity? Trends Parasitol 36:826–834. https://doi.org/10.1016/j.pt.2020.07.003

Hancock K, Broughel DE, Moura IN et al (2001) Sequence variation in the cytochrome oxidase I, internal transcribed spacer 1, and Ts14 diagnostic antigen sequences of *Taenia solium* isolates from South and Central America, India, and Asia. Int J Parasitol 31:1601–1617

Hancock K, Khan A, Williams FB et al (2003) Characterization of the 8-kilodalton antigens of *Taenia solium* metacestodes and evaluation of their use in an enzyme-linked immunosorbent assay for serodiagnosis. J Clin Microbiol 41:2577–2586

Hancock K, Pattabhi S, Greene RM et al (2004) Characterization and cloning of GP50, a *Taenia solium* antigen diagnostic for cysticercosis. Mol Biochem Parasitol 133:115–124

Hancock K, Pattabhi S, Whitfield FW et al (2006) Characterization and cloning of T24, a *Taenia solium* antigen diagnostic for cysticercosis. Mol Biochem Parasitol 147:109–117

Handa U, Garg S, Mohan H (2008) Fine needle aspiration in the diagnosis of subcutaneous cysticercosis. Diagn Cytopathol 36:183–187

Handali S, Klarman M, Gaspard AN et al (2010) Development and evaluation of a magnetic immunochromatographic test to detect *Taenia solium*, which causes taeniasis and neurocysticercosis in humans. Clin Vaccine Immunol 17:631–637

Harrison LJS, Parkhouse RME (1989) *Taenia saginata* and *Taenia solium:* reciprocal models. Acta Leiden 57:143–152

Harrison LJS, Joshua GW, Wright SH et al (1989) Specific detection of circulating surface/secreted glycoproteins of viable cysticerci in *Taenia saginata* cysticercosis. Parasite Immunol 11:351–370

Harrison LJS, Delgado J, Parkhouse RME (1990) Differential diagnosis of *Taenia saginata* and *Taenia solium* with DNA probes. Parasitology 100:459–461

Harrison L, Gárate T, Bryce D et al (2005) Ag-ELISA and PCR for monitoring the vaccination of cattle against *Taenia saginata* cysticercosis using an oncospheral adhesion protein (HP6) with surface and secreted localization. Trop Anim Health Prod 37:103–120

Hayunga EG, Sumner MP, Rhoads ML et al (1991) Development of a seroligic assay for cysticercosis, using an antigen isolated from *Taenia spp* cyst fluid. Am J Vet Res 52:462–470

Hernández M, Gonzalez LM, Fleury A et al (2008) Neurocysticercosis: detection of *Taenia solium* DNA in human cerebrospinal fluid using a semi-nested PCR based on HDP2. Ann Trop Med Parasitol 102:317–323

Hernández M, Astudillo OG, Diego G et al (2019) Immunodiagnosis of human neurocysticercosis: comparative performance of serum diagnostic tests in Mexico. Parasitol Res 118:2891–2899

Hernández-González A, Noh J, Perteguer MJ et al (2017) Comparison of T24H-his, GST-T24H and GST-Ts8B2 recombinant antigens in western blot, ELISA and multiplex bead-based assay for diagnosis of neurocysticercosis. Parasit Vectors 10:237

Herrador Z, Pérez-Molina JA, Henríquez Camacho CA et al (2020) Imported cysticercosis in Spain: a retrospective case series from the +REDIVI Collaborative Network. Travel Med Infect Dis 37:101683. https://doi.org/10.1016/j.tmaid.2020.101683

Herrick JA, Bustos JA, Clapham P et al (2020) Unique characteristics of epilepsy development in neurocysticercosis. Am J Trop Med Hyg 103:639–645. https://doi.org/10.4269/ajtmh.19-0485

Hosseinzadeh S, Setayesh A, Shekarforoush SS et al (2013) An epidemiological survey on the determination of *Taenia saginata* cysticercosis in Iran, using a PCR assay. Vet Rec 172:451

Hubert K, Andriantsimahavandy A, Michault A et al (1999) Serological diagnosis of human cysticercosis by use of recombinant antigens from *Taenia solium* cysticerci. Clin Diagn Lab Immunol 6:479–482

Ito A, Yamasaki H, Nakao M et al (2003) Multiple genotypes of *Taenia solium*-ramifications for diagnosis, treatment and control. Acta Trop 87:95–101

Jansen F, Dorny P, Berkvens D et al (2017) High prevalence of bovine cysticercosis found during evaluation of different post-mortem detection techniques in Belgian slaughterhouses. Vet Parasitol 244:1–6

Jansen F, Dorny P, Gabriël S et al (2018) Estimating prevalence and diagnostic test characteristics of bovine cysticercosis in Belgium in the absence of a 'gold standard' reference test using a Bayesian approach. Vet Parasitol 254:142–146

Jeon HK, Eom KS (2009) Immunoblot patterns of *Taenia asiatica* taeniasis. Korean J Parasitol 47: 73–77

Jeon HK, Eom KS (2013) Molecular approaches to *Taenia asiatica*. Korean J Parasitol 51:1–8

Jeon HK, Chai JY, Kong Y et al (2009) Differential diagnosis of *Taenia asiatica* using multiplex PCR. Exp Parasitol 121:151–156

Jeon HK, Yong TS, Sohn WM et al (2011) Molecular identification of *Taenia* tapeworms by Cox1 gene in Koh Kong, Cambodia. Korean J Parasitol 49:195–197

Jeon HK, Yong TS, Sohn WM et al (2013) Current status of human taeniasis in Lao People's Democratic Republic. Korean J Parasitol 51:259–263

Johnson KS, Harrison GB, Lightowlers MW et al (1989) Vaccination against ovine cysticercosis using a defined recombinant antigen. Nature 338:585–587

Kabululu ML, Johansen MV, Mlangwa JED et al (2020a) Performance of Ag-ELISA in the diagnosis of *Taenia solium* cysticercosis in naturally infected pigs in Tanzania. Parasit Vectors 13(1):534

Kabululu M, Ngowi HA, Mlangwa JED et al (2020b) TSOL18 vaccine and oxfendazole for control of *Taenia solium* cysticercosis in pigs: a field trial in endemic areas of Tanzania. PLoS Negl Trop Dis 14(10):e0008785

Kelvin EA, Carpio A, Bagiella E et al (2009) The association of host age and gender with inflammation around neurocysticercosis cysts. Ann Trop Med Parasitol 103:487–499

Lachuriya G, Garg RK, Jain A et al (2016) Toll-like receptor-4 polymorphisms and serum matrix metalloproteinase-9 in newly diagnosed patients with calcified neurocysticercosis and seizures. Med (United States) 95:1–7. https://doi.org/10.1097/MD.0000000000003288

Laclette JP, Shoemaker CB, Richter D et al (1992) Paramyosin inhibits complement C1. J Immunol 148:124–128

Landa A, Navarro L, Ochoa-Sánchez A, Jiménez L (2019) Taenia solium and Taenia crassiceps: MiRNomes of the larvae and effects of miR-10-5p and let-7-5p on murine peritoneal macrophages. Biosci Rep 39. https://doi.org/10.1042/BSR20190152

Laranjo-González M, Devleesschauwer B, Trevisan C et al (2017) Epidemiology of taeniosis/cysticercosis in Europe, a systematic review: Western Europe. Parasites and Vectors 10:349. https://doi.org/10.1186/s13071-017-2280-8

Larralde C, Sotelo J, Montoya RM et al (1990) Immunodiagnosis of human cysticercosis in cerebrospinal fluid. Antigens from murine *Taenia crassiceps* cysticerci effectively substitute those from porcine *Taenia solium*. Arch Pathol Lab Med 114:926–928

Lee C, Noh J, O'Neal SE et al (2019) Feasibility of a point-of-care test based on quantum dots with a mobile phone reader for detection of antibody responses. PLoS Negl Trop Dis 13:e0007746

León-Janampa N, Liendo R, Gilman RH et al (2019) Characterization of a novel cathepsin L-like protease from *Taenia solium* metacestodes for the immunodiagnosis of porcine cysticercosis. Vet Parasitol 267:9–16

Levine M, Calderon J, Wilkins PP et al (2004) Characterization, cloning, and expression of two diagnostic antigens for *Taenia solium* tapeworm infection. J Parasitol 90:631–638

Levine MZ, Lewis MM, Rodriquez S et al (2007) Development of an enzyme-linked immunoelectrotransfer blot (EITB) assay using two baculovirus expressed recombinant antigens for diagnosis of *Taenia solium* taeniasis. J Parasitol 93:409–417

Li T, Chen X, Wang H, Openshaw JJ, Zhong B, Felt SA, Ito A, Luby SP (2019) High prevalence of taeniasis and *Taenia solium* cysticercosis in children in western Sichuan. China Acta Trop 199: 105133

Lightowlers MW (2006) Cestode vaccines: origins, current status and future prospects. Parasitology;133 Suppl:S27–42

Lightowlers M, Rolfe R, Gauci C (1996) *Taenia saginata*: vaccination against cysticercosis in cattle with recombinant oncosphere antigens. Exp Parasitol 84:330–338

Liu P, Weng X, Zhou J et al (2018) Next generation sequencing based pathogen analysis in a patient with neurocysticercosis: a case report. BMC Infect Dis 18:113

Machnicka B, Dziemian E, Zwierz C (1996) Detection of *Taenia saginata* antigens in faeces by ELISA. Appl Parasitol 37:106–110

Madigubba S, Vishwanath K, Reddy G, Vemuganti G (2007) Changing trends in ocular cysticercosis over two decades: an analysis of 118 surgically excised cysts. Indian J Med Microbiol 25: 214. https://doi.org/10.4103/0255-0857.34761

Manhani MN, Tilelli CQ, Ribeiro VDS et al (2020) Mimotope-based antigens as potential vaccine candidates in experimental murine cysticercosis. Parasitology 147:1330–1337

Manoutcharian K, Rosas G, Hernandez M et al (1996) Cysticercosis: identification and cloning of protective recombinant antigens. J Parasitol 82:250–254

Manoutcharian K, Terrazas LI, Gevorkian G et al (1998) Protection against murine cysticercosis using cDNA expression library immunization. Immunol Lett 62:131–136

Manoutcharian K, Diaz-Orea A, Gevorkian G et al (2004) Recombinant bacteriophage-based multiepitope vaccine against *Taenia solium* pig cysticercosis. Vet Immunol Immunopathol 99:11–24

Martins-Melo FR, Ramos AN Jr, Cavalcanti MG et al (2017) Reprint of "Neurocysticercosis-related mortality in Brazil, 2000–2011: epidemiology of a neglected neurologic cause of death". Acta Trop 165:170–178. https://doi.org/10.1016/j.actatropica.2016.11.009

Mayta H, Talley A, Gilman RH et al (2000) Differentiating *Taenia solium* and *Taenia saginata* infections by simple hematoxylin-eosin staining and PCR-restriction enzyme analysis. J Clin Microbiol 38:133–137

Melki J, Koffi E, Boka M et al (2018) *Taenia solium* cysticercosis in West Africa: status update. Parasite 25. https://doi.org/10.1051/parasite/2018048

Mendlovic F, Fleury A, Flisser A (2021) Zoonotic *Taenia* infections with focus on cysticercosis due to *Taenia solium* in swine and humans. Res Vet Sci 134:69–77. https://doi.org/10.1016/j.rvsc.2020.11.015

Michelet L, Fleury A, Sciutto E et al (2011) Human neurocysticercosis: comparison of different diagnostic tests using cerebrospinal fluid. J Clin Microbiol 49:195–200

Molinari JL, Soto R, Tato P et al (1993a) Immunization against porcine cysticercosis in an endemic area in Mexico: a field and laboratory study. Am J Trop Med Hyg 49:502–512

Molinari JL, Tato P, Lara-Aguilera R et al (1993b) Effects of serum from neurocysticercosis patients on the structure and viability of *Taenia solium* oncospheres. J Parasitol 79:124–127

Molinari JL, Rodriguez D, Tato P et al (1997) Field trial for reducing porcine *Taenia solium* cysticercosis in Mexico by systematic vaccination of pig. Vet Parasitol 69:55–63

Molinari JL, García-Mendoza E, de la Garza Y et al (2002) Discrimination between active and inactive neurocysticercosis by metacestode excretory/secretory antigens of *Taenia solium* in an enzyme-linked immunosorbent assay. Am J Trop Med Hyg 66:777–781

Monreal-Escalante E, Bañuelos-Hernández B, Hernández M et al (2015) Expression of multiple *Taenia solium* immunogens in plant cells through a ribosomal skip mechanism. Mol Biotechnol 57:635–643

Monreal-Escalante E, Govea-Alonso DO, Hernández M et al (2016) Towards the development of an oral vaccine against porcine cysticercosis: expression of the protective HP6/TSOL18 antigen in transgenic carrots cells. Planta 243:675–685

Montenegro TC, Miranda EA, Gilman R (1996) Production of monoclonal antibodies for the identification of the eggs of *Taenia solium*. Ann Trop Med Parasitol 90:145–155

Montero E, González LM, Harrison LJ et al (2003) *Taenia solium* cDNA sequence encoding a putative immunodiagnostic antigen for human cysticercosis. J Chromatogr B Analyt Technol Biomed Life Sci 786:255–269

Morillo M, Noguera C, Gallego L et al (2020) Characterization and evaluation of three new recombinant antigens of *Taenia solium* for the immunodiagnosis of cysticercosis. Mol Biochem Parasitol 240:111321

Mwape KE, Phiri IK, Praet N et al (2013) The incidence of human cysticercosis in a rural community of Eastern Zambia. PLoS Negl Trop Dis 7:e2142
Mwape KE, Praet N, Benitez-Ortiz W et al (2011) Field evaluation of urine antigen detection for diagnosis of Taenia solium cysticercosis. Trans R Soc Trop Med Hyg 105:574–578
Náquira C (1999) *Taenia solium*: Biological cycle and characteristics. In: García HH, Martinez M (eds) *Taenia solium* Taeniasis/Cysticercosis. Editorial Universo, Perú, pp 7–14
Nash T (2012) Edema surrounding calcified intracranial cysticerci: clinical manifestations, natural history, and treatment. Pathog Glob Health 106:275–279
Nash TE, Singh G, White AC et al (2006) Treatment of neurocysticercosis: current status and future research needs. Neurology 67:1120–1127
Nash TE, Bustos JA, Garcia HH (2017) Disease centered around calcified *Taenia solium* Granuloma. Trends Parasitol 33:65–73. https://doi.org/10.1016/j.pt.2016.09.003
Nash TE, Ware JAM, Mahanty S (2018) Intraventricular neurocysticercosis: experience and long-term outcome from a tertiary referral center in the United States. Am J Trop Med Hyg 98:1755–1762. https://doi.org/10.4269/ajtmh.18-0085
Ng TF, Ko RC (1994) Serodiagnosis of cysticercosis: specificity of different antigens and enzyme-linked immunosorbent assays. Trans R Soc Trop Med Hyg 88:421–422
Ng-Nguyen D, Stevenson MA, Dorny P et al (2017) Comparison of a new multiplex real-time PCR with the Kato Katz thick smear and copro-antigen ELISA for the detection and differentiation of *Taenia* spp. in human stools. PLoS Negl Trop Dis 11(e0005743)
Nguekam JP, Zoli AP, Ongolo-Zogo P et al (2003) Follow-up of neurocysticercosis patients after treatment using an antigen detection ELISA. Parasite 10:65–68
Nguyen TTM, Dermauw V, Noh J et al (2020) Occurrence of *Taenia* species in pigs in slaughterhouses in Phu Tho province, northern Vietnam. J Helminthol 94:e201
Nkouawa A, Sako Y, Li T et al (2010) Evaluation of a loop-mediated isothermal amplification method using fecal specimens for differential detection of *Taenia* species from humans. J Clin Microbiol 48:3350–3352
Nkouawa A, Dschanou AR, Moyou-Somo R et al (2017) Seroprevalence and risk factors of human cysticercosis and taeniasis prevalence in a highly endemic area of epilepsy in Bangoua, west Cameroon. Acta Trop 165:116–120
Nunes DS, Gonzaga HT, Ribeiro VS et al (2017) Usefulness of gel filtration fraction as potential biomarker for neurocysticercosis in serum: towards a new diagnostic tool. Parasitology 144:426–435
O'Connell EM, Harrison S, Dahlstrom E et al (2020) A novel, highly sensitive quantitative polymerase chain reaction assay for the diagnosis of subarachnoid and ventricular neurocysticercosis and for assessing responses to treatment. Clin Infect Dis 70:1875–1881
Okello A, Ash A, Keokhamphet C et al (2014) Investigating a hyper-endemic focus of *Taenia solium* in northern Lao PDR. Parasit Vectors 7:134
Okello AL, Thomas L, Inthavong P et al (2017) Reprint of "Assessing the impact of a joint human-porcine intervention package for *Taenia solium* control: results of a pilot study from northern Lao PDR". Acta Trop 165:261–267
Oliveira HB, Machado GA, Mineo JR et al (2010) Taenia saginata metacestode antigenic fractions without affinity to concanavalin A are an important source of specific antigens for the diagnosis of human neurocysticercosis. Clin Vaccine Immunol 17:638–644
Ong SWX, Chavatte JM, Chia JWZ, et al (2020) Neurocysticercosis diagnosed by Taenia solium PCR on brain biopsy. Case Rep Infect Dis 8887647
Pan American Health Organization (PAHO/WHO) Operational guidelines for taeniasis and cysticercosis control activities caused by *Taenia solium*. Contribution to the control of Taenia solium in Latin America and the Caribbean. Washington, DC: PAHO; 2019. https://iris.paho.org/handle/10665.2/51660
Pal DK, Carpio A, Sander JW (2000) Neurocysticercosis and epilepsy in developing countries. J Neurol Neurosurg Psychiatry 68:137–143

Pardini AX, Vaz AJ, Dos Ramos ML et al (2001) Cysticercus antigens in cerebrospinal fluid samples from patients with neurocysticercosis. J Clin Microbiol 39:3368–3372
Paredes A, Sáenz P, Marzal MW et al (2016) Anti-*Taenia solium* monoclonal antibodies for the detection of parasite antigens in body fluids from patients with neurocysticercosis. Exp Parasitol 166:37–43
Parija SC, Rajesh Reddy S (2006) Co-agglutination test for cysticercus antigen detection in the serum for the diagnosis of cysticercosis. Trop Dr 36:144–147
Park SK, Yun DH, Chung JY et al (2000) The 10 kDa protein of *Taenia solium* metacestodes shows genus specific antigenicity. Korean J Parasitol 38:191–194
Parkhouse RME, Carpio A, Campoverde A et al (2018) Reciprocal contribution of clinical studies and the HP10 antigen ELISA for the diagnosis of extraparenchymal neurocysticercosis. Acta Trop 178:119–123
Parkhouse RME, Carpio A, Campoverde A et al (2019) A modified lateral flow assay, using serum, for the rapid identification of human and bovine cysticercosis in the absence of false positives. Trans R Soc Trop Med Hyg 113:101–104
Parkhouse RME, Rojas G, Aguilar CM et al (2020) Diagnosis of Taeniosis in rural Venezuelan communities: preliminary characterization of a *Taenia solium* specific monoclonal (VP-1) Coproantigen ELISA. Acta Trop 207:105445
Pathak KM, Gaur SMS (1990) Immunization of pigs with culture antigens of *Taenia solium*. Vet Parasitol 34:353–356
Pawlowski Z (2002) *Taenia solium:* basic biology and transmission. In: Singh G, Prabhakar S (eds) *Taenia solium* cysticercosis from basic to clinical science. CABInternational, London, pp 1–14
Pesantes MA, Moyano LM, Sommerville C (2020) Neurocysticercosis in Northern Peru: qualitative insights from men and women about living with seizures. PLoS Negl Trop Dis 14:1–16. https://doi.org/10.1371/journal.pntd.0008715
Pornruseetriratn S, Maipanich W, Sa-nguankiat S et al (2017) A simple and effective multiplex PCR technique for detecting human pathogenic Taenia eggs in houseflies. Southeast Asian J Trop Med Public Health 48:9–17
Porphyre V, Betson M, Rabezanahary H et al (2016) *Taenia solium* porcine cysticercosis in Madagascar: comparison of immuno-diagnostic techniques and estimation of the prevalence in pork carcasses traded in Antananarivo city. Vet Parasitol 219:77–83
Praet N, Verweij JJ, Mwape KE et al (2013) Bayesian modelling to estimate the test characteristics of coprology, coproantigen ELISA and a novel real-time PCR for the diagnosis of taeniasis. Tropical Med Int Health 18:608–614
Proaño JV, Madrazo I, Avelar F et al (2001) Medical treatment for neurocysticercosis characterized by giant subarachnoid cysts. N Engl J Med 345:879–885
Prodjinotho UF, Lema J, Lacorcia M et al (2020) Host immune responses during Taenia solium Neurocysticercosis infection and treatment. PLoS Negl Trop Dis 14:e0008005. https://doi.org/10.1371/journal.pntd.0008005
Qian MB, Xiao N, Li SZ et al (2020) Control of taeniasis and cysticercosis in China. Adv Parasitol 110:289–317. https://doi.org/10.1016/bs.apar.2020.04.005
Raibagkar P, Berkowitz AL (2018) The Many Faces of Neurocysticercosis. J Neurol Sci 390:75–76
Rassy D, Bobes RJ, Rosas G et al (2010) Characterization of S3Pvac anti-cysticercosis vaccine components: implications for the development of an anti-cestodiasis vaccine. PLoS One 5: e11287
Ribeiro Vda S, Nunes Dda S, Gonzaga HT et al (2014) Diethylaminoethyl (DEAE) binding fraction from Taenia solium metacestode improves the neurocysticercosis serodiagnosis. Parasitol Res 113:2569–2575
Ribeiro VDS, Gonzaga HT, Nunes DDS, el al (2019) Neurocysticercosis serodiagnosis: mimotope-based synthetic peptide as potential biomarker. Parasitol Res 118:1657–1660
Rickard MD, Arundel JH, Adolph AJ (1981) A preliminary field trial to evaluate the use of immunisation for the control of naturally acquired *Taenia saginata* infection in cattle. Res Vet Sci 30:104–148

Robertson LJ, Joshi H, Utaaker KS et al (2017) Changes in the seroprevalence of cysticercosis in suspected patients in Chandigarh, India between 1998 and 2014: analysis of 17 years of data. Epidemiol Infect 145:1159–1167. https://doi.org/10.1017/S0950268816003356

Rodríguez-Morales AJ, Yepes-Echeverri MC, Acevedo-Mendoza et al (2018) Mapping the residual incidence of taeniasis and cysticercosis in Colombia, 2009–2013, using geographical information systems: implications for public health and travel medicine. Travel Med Infect Dis 22:51–57. https://doi.org/10.1016/j.tmaid.2017.12.006

Rodríguez-Sosa M, Rivera-Montoya I, Espinoza A et al (2006) Acute cysticercosis favours rapid and more severe lesions caused by *Leishmania major* and *Leishmania mexicana* infection, a role for alternatively activated macrophages. Cell Immunol 242:61–71

Romo ML, Hernández M, Astudillo OG et al (2020) Diagnostic value of glycoprotein band patterns of three serologic enzyme-linked immunoelectrotransfer blot assays for neurocysticercosis. Parasitol Res 119:2521–2529

Rosales-Mendoza S, Monreal-Escalante E, González-Ortega O et al (2018) Transplastomic plants yield a multicomponent vaccine against cysticercosis. J Biotechnol 266:124–132

Rosas G, Fragoso G, Gárate T et al (2002) Protective immunity against *Taenia crassiceps* murine cysticercosis induced by DNA vaccination with a *Taenia saginata* tegument antigen. Microbes Infect 4:1417–1426

Rottbeck R, Nshimiyimana JF, Tugirimana P et al (2013) High prevalence of cysticercosis in people with epilepsy in southern Rwanda. PLoS Negl Trop Dis 7:e2558

Sahu PS, Parija SC, Narayan SK et al (2009) Kumar D. Evaluation of an IgG-ELISA strategy using *Taenia solium* metacestode somatic and excretory-secretory antigens for diagnosis of neurocysticercosis revealing biological stage of the larvae. Acta Trop 110:38–45

Sako Y, Nakao M, Ikejima T et al (2000) Molecular characterization and diagnostic value of *Taenia solium* low-molecular-weight antigens genes. J Clin Microbiol 38:4439–4444

Sako Y, Takayanagui OM, Odashima NS et al (2015) Comparative study of paired serum and cerebrospinal fluid samples from neurocysticercosis patients for the detection of specific antibody to Taenia solium immunodiagnostic antigen. Trop Med Health 43:171–176

Santosh T, Puneeta N, Patro MK et al (2019) Fine needle aspiration as a diagnostic tool in cysticercosis: a case series. J Med Case Rep 13(1):92

Sato MO, Sako Y, Nakao M et al (2006) Evaluation of purified *Taenia solium* glycoproteins and recombinant antigens in the serologic detection of human and swine cysticercosis. J Infect Dis 194:1783–1790

Sato MO, Sako Y, Nakao M et al (2011) A possible nuclear DNA marker to differentiate the two geographic genotypes of *Taenia solium* tapeworms. Parasitol Int 60:108–110

Sato MO, Sato M, Yanagida T, et al (2018) Taenia solium, Taenia saginata, Taenia asiatica, their hybrids and other helminthic infections occurring in a neglected tropical diseases' highly endemic area in Lao PDR. PLoS Negl Trop Dis 12. https://doi.org/10.1371/journal.pntd.0006260

Schantz P, Moore A, Muñoz J et al (1992) Neurocysticercosis in an Orthodox Jewish community in New York City. N Engl J Med 327:692–695

Sciutto E, de Aluja A, Fragoso G et al (1995) Immunization of pigs against *Taenia solium* cysticercosis: factors related to effective protection. Vet Parasitol 60:53–67

Sciutto E, Fragoso G, Hernández M et al (2013a) Development of the S3Pvac vaccine against porcine *Taenia solium* cysticercosis: a historical review. J Parasitol 99:686–692

Sciutto E, Fragoso G, Hernández M et al (2013b) Development of the S3Pvac vaccine against murine *Taenia crassiceps* cysticercosis: a historical review. J Parasitol 99:693–702

Sciutto E, Cárdenas G, Adalid-Peralta L et al (2013c) Human neurocysticercosis: Immunological features involved in the host's susceptibility to become infected and to develop disease. Microbes Infect 15:524–530. https://doi.org/10.1016/j.micinf.2013.03.007

Serpa JA, White AC (2012) Neurocysticercosis in the United States. Pathog Glob Health 106:256–260. https://doi.org/10.1179/2047773212Y.0000000028

Sharma BS, Sawarkar DP, Verma SK (2019) Endoscopic management of fourth ventricle neurocysticercosis: description of the new technique in a case series of 5 cases and review of the literature. World Neurosurg 122:e647–e654

Sikasunge CS, Phiri IK, Johansen MV et al (2008) Host-cell apoptosis in *Taenia solium*-induced brain granulomas in naturally infected pigs. Parasitology 135:1237–1242

da Silva GB, Nunes DS, de Sousa JEN et al (2017) Antigenic fractions from *Taenia crassiceps* metacestodes obtained by hydrophobicity for the immunodiagnosis of active and inactive forms of neurocysticercosis in human cerebrospinal fluid samples. Parasitol Int 66:134–138

Singh G, Sharma R (2017) Controversies in the treatment of seizures associated with neurocysticercosis. Epilepsy Behav 76:163–167

Singhi P (2011) Neurocysticercosis. Ther Adv Neurol Disord 4:67–81

Song SM, Yun HS, VanBik D et al (2019) Ten cases of *Taenia saginata* infection confirmed by analysis of the internal transcribed spacer 1 rDNA region in the Republic of Korea. Korean J Parasitol 57:417–422

Sotelo J (2011) Clinical manifestations, diagnosis, and treatment of neurocysticercosis. Curr Neurol Neurosci Rep 11:529–535

Spallone A, Woroch L, Sweeney K et al (2020) The burden of neurocysticercosis at a single New York hospital. J Pathog 2020:1–9. https://doi.org/10.1155/2020/8174240

Spolski RJ, Thomas PG, See EJ, Mooney KA, Kuhn RE (2002) Larval Taenia crassiceps secretes a protein with characteristics of murine interferon-gamma. Parasitol Res 88(5):431–438. https://doi.org/10.1007/s00436-002-0590-y

Sundar U, Chawla V, Lakkas Y et al (2010) Monocular blindness during therapy for cerebral neurocysticercosis. J Assoc Physicians India 58:570–572

Suri A, Goel RK, Ahmad FU et al (2008) Transventricular, transaqueductal scope-in-scope endoscopic excision of fourth ventricular neurocysticercosis: a series of 13 cases and a review. J Neurosurg Pediatr 1:35–39

Suzuki LA, Rossi CL (2011) Evaluation of two *Taenia solium* cysticercal antigenic preparations (vesicular fluid and a glycoprotein fraction with affinity for lentil lectin) for the immunodiagnosis of neurocysticercosis by enzyme-linked immunosorbent assay (ELISA). Arq Neuropsiquiatr 69:470–474

Suzuki LA, Arruda GC, Quagliato EM et al (2007) Evaluation of *Taenia solium* and *Taenia crassiceps* cysticercal antigens for immunodiagnosis of neurocysticercosis using ELISA on cerebrospinal fluid samples. Rev Soc Bras Med Trop 40:152–155

Talukdar B, Saxena A, Popli VK et al (2002) Neurocysticercosis in children: clinical characteristics and outcome. Ann Trop Paediatr 22:333–339

Terrazas CA, Sánchez-Muñoz F, Mejía-Domínguez AM et al (2011) Cestode antigens induce a tolerogenic-like phenotype and inhibit LPS inflammatory responses in human dendritic cells. J Biol Sci 7:1391–1400. https://doi.org/10.7150/ijbs.7.1391

Terrazas CA, Rodriguez-Sosa M, Terrazas LI (2012) Cestoda: Tapeworm infection. In: Lamb TJ (ed) Immunity to parasitic infection. Wiley, Hoboken, pp 307–322

Thussu A, Chattopadhyay A, Sawhney IM et al (2008) Albendazole therapy for single small enhancing CT lesions (SSECTL) in the brain in epilepsy. J Neurol Neurosurg Psychiatry 79: 272–275

Toenjes SA, Kuhn RE (2003) The initial immune response during experimental cysticercosis is of the mixed Th1/Th2 type. Parasitol Res 89:407–413

Toledo A, Fragoso G, Rosas G et al (2001) Two epitopes shared by *Taenia crassiceps* and *Taenia solium* confer protection against murine *T. crassiceps* cysticercosis along with a prominent T1 response. Infect Immun 69:1766–1773

Trevisan C, Devleesschauwer B, Schmidt V et al (2017) The societal cost of Taenia solium cysticercosis in Tanzania. Acta Trop 165:141–154. https://doi.org/10.1016/j.actatropica.2015.12.021

Trevisan C, Devleesschauwer B, Praet N et al (2018a) Assessment of the societal cost of Taenia solium in Angónia district, Mozambique. BMC Infect Dis 18:1–11. https://doi.org/10.1186/s12879-018-3030-z

Trevisan C, Sotiraki S, Laranjo-González M et al (2018b) Epidemiology of taeniosis/cysticercosis in Europe, a systematic review: Eastern Europe. Parasit Vectors 11(1):569. https://doi.org/10.1186/s13071-018-3153-5

Tripathy SK, Sen RK, Akkina N (2012) Role of ultrasonography and magnetic resonance imaging in the diagnosis of intramuscular cysticercosis. Skelet Radiol 41:1061–1066

Tsang V, Brand JA, Boyer AE (1989) An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). J Infect Dis 159:50–59

Ursini T, Di Giacomo R, Caldrer S et al (2020) Neurocysticercosis-related seizures in the post-partum period: two cases and a review of the literature. Lancet Infect Dis 20:e204–e214

Valdez F, Hernandez M, Govezensky T et al (1994) Immunization against *Taenia crassiceps* cysticercosis: identification of the most promising antigens in the induction of protective immunity. J Parasitol 80:931–936

Van Kerckhoven I, Vansteenkiste W, Claes M et al (1998) Improved detection of circulating antigen in cattle infected with *Taenia saginata* metacestodes. Vet Parasitol 76:269–274

Vargas-Calla A, Gomez-Puerta LA, Lopez MT et al (2019) Molecular characterization of the *Taenia solium* Tso31 antigen and homologous of other *Taenia* species from Peru. Parasitol Res 118:1307–1309

Vazquez-Talavera J, Solis C, Terrazas L et al (2001) Characterization and protective potential of the immune response to *Taenia solium* paramyosin in a murine model of cysticercosis. Infect Immun 69:5412–5416

Vega R, Piñero D, Ramanankandrasana B et al (2003) Population genetic structure of *Taenia solium* from Madagascar and Mexico: Implications for clinical profile diversity and immunological technology. Int J Parasitol 33:1479–1485. https://doi.org/10.1016/S0020-7519(03)00206-6

Verastegui M, Gilman R, Gonzalez A et al (2002) *Taenia solium* oncosphere antigens induce immunity in pigs against experimental cysticercosis. Vet Parasitol 108:49–62

Verma A, Nath Prasad K, Kumar Gupta R, et al (2010) Toll-like receptor 4 polymorphism and its association with symptomatic neurocysticercosis. 1219. https://doi.org/10.1086/656395

Villegas M, Sciutto E, Rosetti M, et al (2019) Association of TRAF1/C5 locus polymorphisms with epilepsy and clinical traits in Mexican patients with neurocysticercosis. Infect Immun 87. https://doi.org/10.1128/IAI.00347-19

Waema MW, Misinzo G, Kagira JM, et al (2020) DNA-detection based diagnostics for Taenia solium cysticercosis in Porcine. J Parasitol Res 5706981

Wang CY, Zhang HH, Ge LY (1992) A Mab-based ELISA for detecting circulating antigen in CSF of patients with neurocysticercosis. Hybridoma 11:825–827

Wang YY, Chang XL, Tao ZY et al (2015) Optimized codon usage enhances the expression and immunogenicity of DNA vaccine encoding *Taenia solium* oncosphere TSOL18 gene. Mol Med Rep 12:281–288

Wardrop NA, Thomas LF, Atkinson PM, el al (2015) The influence of socio-economic, behavioural and environmental factors on *Taenia* spp. Transmission in Western Kenya: Evidence from a cross-sectional survey in humans and pigs. PLoS Negl Trop Dis 9: e0004223

Webb C, Cabada MM (2017) Intestinal cestodes. Curr Opin Infect Dis 30:504–510

Weka RP, Kamani J, Cogan T et al (2019) Overview of Taenia solium cysticercosis in West Africa. Acta Trop 190:329–338. https://doi.org/10.1016/j.actatropica.2018.12.012

White AC Jr, Garcia HH (2018) Updates on the management of neurocysticercosis. Curr Opin Infect Dis 31:377–382

White AC Jr, Tato P, Molinari JL (1992) Host-parasite interactions in *Taenia solium* cysticercosis. Infect Agents Dis 1:185–193

WHO (2020) Taeniasis/cysticercosis fact sheet, (Accessed 11 January 2021), https://www.who.int/news-room/fact-sheets/detail/taeniasis-cysticercosis

Wilkins PP, Allan JC, Verastegui M et al (1999) Development of a serologic assay to detect *Taenia solium* taeniasis. Am J Trop Med Hyg 60:199–204

Wilson M, Bryan RT, Fried JA et al (1991) Clinical evaluation of the cysticercosis enzyme-linked immunoelectrotransfer blot in patients with neurocysticercosis. J Infect Dis 164:1007–1009

Xiao N, Yao JW, Ding W et al (2013) Priorities for research and control of cestode zoonoses in Asia. Infect Dis Poverty 2:16

Yamasaki H, Allan J, Sato M et al (2004) DNA differential diagnosis of taeniasis and cysticercosis by multiplex PCR. J Clin Microbiol 42:548–553

Yang HJ, Chung JY, Yun DH et al (1998) Immunoblot analysis of a 10 kDa antigen in cyst fluid of *Taenia solium* metacestodes. Parasite Immunol 20:483–488

Yera H, Dupont D, Houze S et al (2011) Confirmation and follow-up of neurocysticercosis by real-time PCR in cerebrospinal fluid samples of patients living in France. J Clin Microbiol 49:4338–4340

Zarlenga DS, McManus DP, Fan PC et al (1991) Characterization and detection of a newly described Asian taeniid using cloned ribosomal DNA fragments and sequence amplification by the polymerase chain reaction. Exp Parasitol 72:174–183

Chapter 10
Trichinellosis

Fabrizio Bruschi and Jean Dupouy-Camet

Abstract Trichinellosis is a worldwide zoonosis caused by the parasitic nematodes belonging to the *Trichinella* genus. This chapter describes the different aspects of epidemiology of infection, the life cycle of the parasite, and the host immune response to the different species of *Trichinella* in humans, as well as in the rodents which represent the most studied experimental model. The roles of antibodies, T cells, mast cells, eosinophils, and neutrophils in immune responses to this nematode are considered in experimental as well as in human infections. Immunopathological aspects of infection are also illustrated. Particular emphasis is given on the clinical diagnosis of trichinellosis which is difficult because of the lack of pathognomonic signs or symptoms. Therefore, anamnestic data are of great importance in diagnosing the infection. High eosinophilia and increased creatinine phosphokinase activity in the serum are the most frequently observed laboratory features, but only the finding of parasites in a muscle biopsy and the detection of specific circulating antibodies can confirm the diagnosis. The medical treatment includes anthelmintics (mebendazole or albendazole) and glucosteroids. A section is devoted to control measures, including a possible vaccine for which several molecules are under investigation.

10.1 Introduction

Trichinellosis is a worldwide zoonosis due to the nematode *Trichinella* and at the world level is mostly transmitted by pork from backyard pigs. It can be a serious disease, particularly in old persons, where severe complications such as myocarditis or encephalitis can lead to death. These parasites are widespread in wildlife on all

F. Bruschi (✉)
Department of Translational Research, N.T.M.S., Università di Pisa, Medical School, Pisa, Italy
e-mail: fabrizio.bruschi@unipi.it

J. Dupouy-Camet
Medical Faculty, University of Paris Cité, Paris, France

F. Bruschi (ed.), *Helminth Infections and their Impact on Global Public Health*,
https://doi.org/10.1007/978-3-031-00303-5_10

continents but Antarctica and in domestic pigs of many countries (Pozio and Murrell 2006). Infections occur in populations used to eat raw or undercooked meat and meat products of different animal origins (e.g., pork, horse, game).

10.2 The Agent

10.2.1 Species and Genotypes

At present, ten species and three genotypes are recognized in the genus *Trichinella*, namely, *Trichinella spiralis*, *T. nativa* and its related genotype *Trichinella* T6, T. *britovi* and its related genotype *Trichinella* T8, *T. pseudospiralis, T. murrelli* and its related genotype *Trichinella* T9, *T. nelsoni*, *T. papuae*, *T. zimbabwensis*, *T. patagoniensis*, and *T. chanchalensis* (Table 10.1). The parasites are perpetuated in life cycles with carnivorous and omnivorous animals representing the most important reservoir. All species can develop in mammals, but *T. pseudospiralis* can also develop in birds, and *T. papuae* and *T. zimbabwensis* also occur in some reptile species. A zoonotic parasite found in mammals, birds, and reptiles is quite unique in medical parasitology. No morphological differences exist between species and genotypes, and they are most reliably distinguished by biochemical or molecular analyses (Pozio and La Rosa 2003; Pozio 2007, 2020; Pozio and Zarlenga 2019; Zarlenga et al. 2020).

10.2.2 Parasite Cycle

The parasitic cycle (Fig. 10.1) can be divided into two phases, an intestinal (or enteral) phase and a systemic and muscular phase, which can coexist for a period lasting from a few days to weeks. Infection occurs after consumption of raw meat containing coiled larvae of half a mm long. After the gastric digestion of the infected meat, the larvae are released in the stomach; they take a snake-like appearance, penetrate the mucosa of the small intestine, and mature into adult worms (5 days after infection). The larval penetration of the intestinal mucosa causes modifications in the cells of the epithelium, specifically the brush border of villi, the *lamina propria*, and the smooth muscles of the jejunum. After mating in the intestine, adult females shed 100-μ-long newborn larvae (NBL) into the blood and lymphatic vessels. Then, these larvae migrate in the general circulation to find their definitive niche: the musculoskeletal fiber. The circulating larvae induce in their host a parasitic vasculitis. After penetrating the muscular fiber, the larvae take the control of the muscle fiber and, for most *Trichinella* species, induce the constitution of a collagen capsule surrounding the larva. The NBL will increase its volume by 600-fold within 2 weeks and become infective. The larvae will stay alive in the modified muscle fiber (called “nurse cell”) for months or years. Mature females

Table 10.1 *Trichinella* species and genotypes and human infections

Trichinella species or genotype	Distribution	Usual hosts	Human cases reported	Source of infection	Countries with reports in humans
T1, *T. spiralis*	Cosmopolitan	Swine, rats, carnivores	Yes +++	Pork	Argentina, Baltic countries, Chile, China, Croatia, Laos, Poland, Romania, Serbia
T2, *T. nativa*	Arctic or sub-arctic regions of the Holarctic zone	Terrestrial or marine carnivores,	Yes ++	Bear meat, walrus, dog	Nunavut, Nunavik, Russia, China
T3, *T. britovi*	Temperate areas of the Palearctic zone, North and West Africa	Carnivores and seldom swine	Yes ++	Wild boar meat, dog, jackal	Algeria, France, Poland, Spain, Turkey
T4[a], *T. pseudospiralis*	Cosmopolitan	Mammals and birds	Yes +	Wild boar	France, Thailand
T5, *T. murrelli*	Temperate areas of the Nearctic zone	Carnivores	Yes +	Horse bear	France, USA
T6	Arctic or sub-arctic regions of Canada and the USA	Terrestrial or marine carnivores	Yes +	Bear cougar	Canada, USA
T7, *T. nelsoni*	Ethiopic region	Carnivores	?		
T8	South Africa	Carnivores	?		
T9	Japan	Carnivores	?		Japan
T 10[a] *T. papuae*	Southeast Asia	Mammals and reptiles	Yes +	Soft-shelled turtles, wild boar	Thailand, Taiwan, Korea, Cambodia
T11[a], *T. zimbabwensis*	East Africa	Mammals and reptiles	?		
T12, *T. patagoniensis*	Argentina	Carnivores	?		
T13, *T. chanchalensis*	Yukon, Canada	Wolverine	?		

[a]Nonencapsulated species

release NBL for 3–4 weeks; although this estimate was based only on experimental data from pigs, it has been confirmed by the observation of a *Trichinella* female containing embryos on a duodenal section of a person infected 3–4 weeks earlier and presenting with fever, myalgia, and high eosinophilia (Dupouy-Camet and Murrell 2007). The females then die or are expelled by smooth muscle hypercontractility elicited by the immune response.

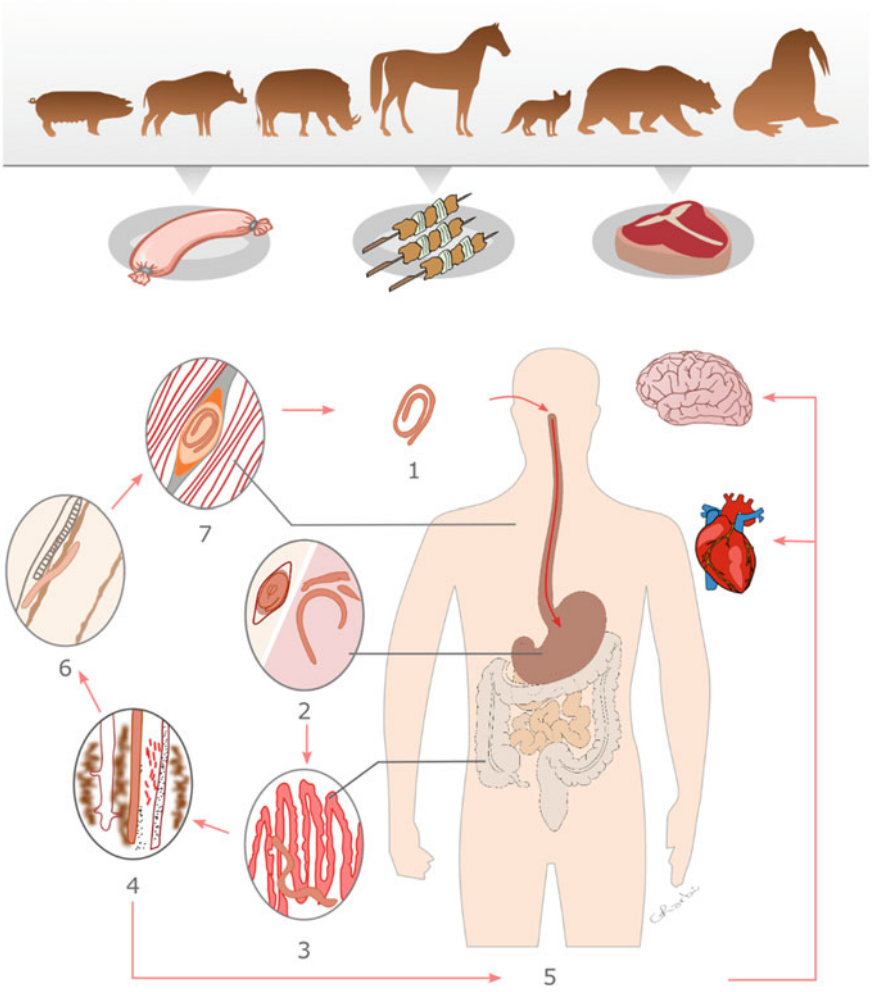

Fig. 10.1 Transmission and biological cycle of *Trichinella* in humans. Infection occurs after consumption of raw meat from various mammals containing muscular larvae (1). After gastric digestion of the meat, larvae are released in the stomach (2) and penetrate the mucosa of the small intestine and mature into adult worms (3). After mating in the intestine, adult females shed newborn larvae (NBL) into the blood and lymphatic vessels (4, 5). Then, these larvae migrate in the general circulation to musculoskeletal fibers (6). The circulating larvae induce in their host a parasitic vasculitis particularly harmful for the heart and brain. After penetrating the muscular fiber, larvae of most *Trichinella* species induce the constitution of a collagen capsule (7). Adapted from De Bruyne et al. (2006) by M. Gharbi

10.3 Epidemiology of Infection

10.3.1 Past Situation

Evaluating the worldwide prevalence of the disease is difficult because the definitive localization of the larvae in the muscles precludes simple parasitological surveys as parasite identification requires muscle biopsy. Extensive surveys have been based on the examination of diaphragms from cadavers, but there are no recent data from such studies. In the 1940s, 12,000 necropsies carried out in the USA showed that about one in six Americans were infected (Stoll 1947). Serological surveys are possible but have several drawbacks: they are expensive; antibody titers fall quite rapidly though patients still harbor the parasite and cross-reaction can occur, requiring the use of expensive western blots (Robert et al. 1996; De-la-Rosa et al. 1995). In rural Chile (Contreras et al. 1994), a correlation was observed between the serologic prevalence of trichinellosis (1.5%) and necropsy positives (2%). In rural Mexico (De-la-Rosa et al. 1998), a higher prevalence of antibodies was found in females (2.36%) than in males (0.35%). Crompton (1999), in his paper entitled "How much human helminthiasis is there in the world?", curiously considers trichinellosis as a "localized infection, similarly to capilliariasis and anisakiasis." This observation contrasts with Stoll's famous 1947 paper, "This wormy world," in which he stated that at least 21 million North Americans, one million South Americans, and five million Europeans were infected by *Trichinella*. This review did not mention the possible occurrence of the disease in Asia. For a review paper published in 2000, Dupouy-Camet assessed the global distribution of trichinellosis by scrutinizing the MEDLINE database (1965–1999) using the following query: (*Trichinella* or trichinosis) and name of the considered country. Titles and abstracts were analyzed to estimate the worldwide distribution of trichinellosis in humans and animals. These data confirmed that *Trichinella* had a worldwide distribution. It was not reported in desert zones, and data were missing for the northern parts of South America (Brazil, Venezuela, Colombia, etc.). Some regions of Africa and Madagascar had not been investigated. At that time, from a public health point of view, the situation appeared particularly worrisome in Argentina, Croatia, Yugoslavia, Russia, Romania, Latvia, Lithuania, and China. At that time, as many as 11 million people might be infected worldwide and more than 10,000 cases of human trichinellosis had been reported by the International Commission on trichinellosis since 1995 up to June 1997 (Dupouy-Camet 2000).

10.3.2 Present Situation

Trichinellosis certainly remains an important zoonotic disease on a global basis. In an extensive review of published cases, Murrell and Pozio (2011) analyzed outbreak report data for 1986–2009. Searches of 6 international databases yielded 494 reports.

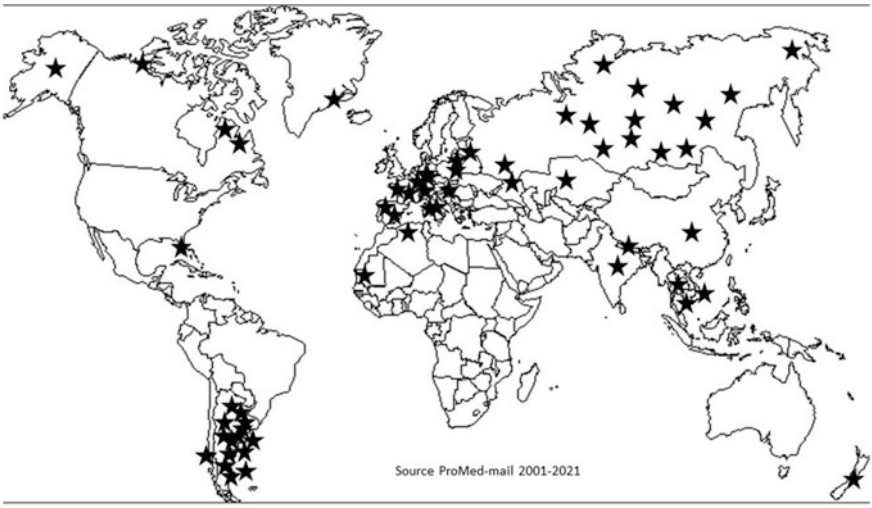

Fig. 10.2 Geographical locations of trichinellosis outbreaks reported in ProMED mail (2001–2021). The disease is obviously underreported in China

After applying strict criteria for relevance and reliability, they selected 261 reports for data extraction. From 1986 through 2009, there were 65,818 cases and 42 deaths reported from 41 countries. The World Health Organization in the European Region accounted for 87% of cases; 50% of those occurred in Romania, mainly during 1990–1999. Incidence in the region ranged from 1.1 to 8.5 cases per 100,000 population. Trichinellosis affected primarily adults (median age 33.1 years) and about equally affected men (51%) and women. Pork was the major source of infection; wild game sources were also frequently reported. The global burden of trichinellosis was assessed using the disability-adjusted life year metric (Devleesschauwer et al. 2015). The global number of disability-adjusted life years due to trichinellosis was estimated to be 76 per billion persons per year. The authors of the study therefore considered that the global burden of trichinellosis was much lower than that of other foodborne parasitic diseases and is in sharp contrast to the high budget allocated to prevent the disease in many industrialized countries. An analysis of all reports made on the ProMED mail organization from 2001 to 2020 yielded 72 outbreaks involving more than 2765 cases and 24 deaths from 27 countries (see Fig. 10.2 and 10.3) The source of infection was pork (from pigs or wild boars) for 91.3% of cases, bear meat for 5.7%, and meats from canids, badger, or walrus for 3% (see Fig. 10.4). Of course, these reports were early warnings not fully analyzed, reporting unusual vectors, imported cases, or severe and lethal outbreak, and data from China are not reported through this media (Dupouy-Camet, unpublished). Usual sources of infection for humans are detailed in Table 10.2; the most frequent source being pork from domestic or wild pigs harboring *T. spiralis*, *T. britovi*, and sometimes *T. pseudospiralis*. Meats from wild carnivores (bears, dogs, badgers, etc.) are a source of small outbreaks among hunters (Schellenberg

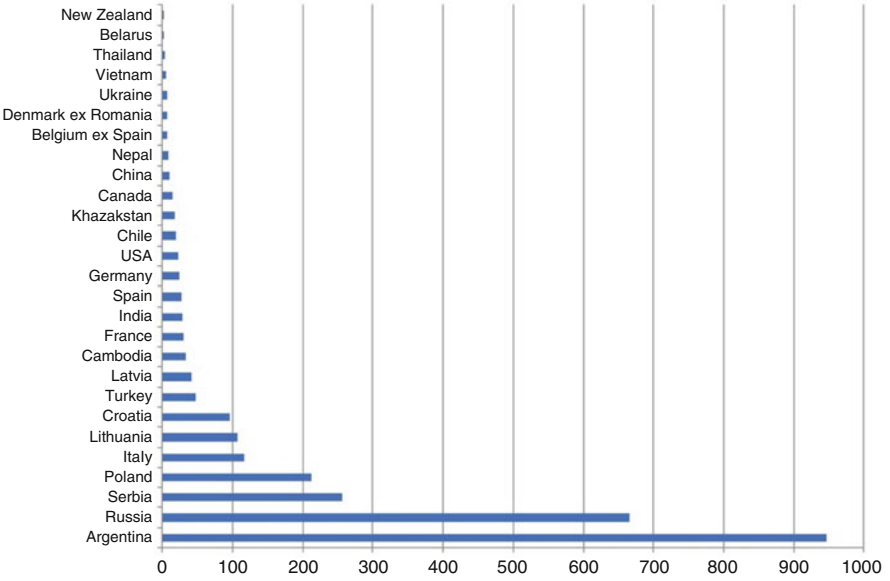

Fig. 10.3 Number of cases reported in ProMED mail (2001–2021) according to the different countries. The disease is obviously underreported in China

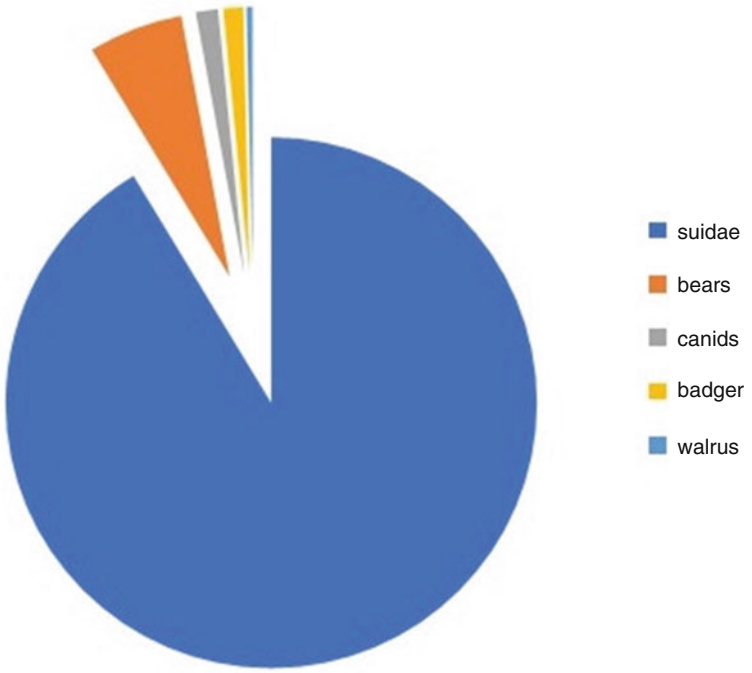

Fig. 10.4 Source of trichinellosis outbreaks reported in ProMED mail (1998–2013)

Table 10.2 Unusual sources of trichinellosis in humans

Unusual host	Countries	Number of cases	Species	Ref
Badger	Russia, Korea	+	*T. spiralis*, other?	Sohn et al. (2000), Suzdaltsev et al. (1999)
Beaver	Russia	Suspected	*?*	Bronstein and Lukashev (2019)
Cougar	USA	Sporadic	*T. nativa*, T6	Dworkin et al. (1996)
Dog	Slovakia, China	++	*T. britovi, T. spiralis, T. nativa*	Dubinský et al. (2001), Cui and Wang (2001)
Fox	Italy	Sporadic	*T. britovi*?	Pozio et al. (1987)
Horse	Italy, France	3339 cases between 1975 and 2000	*T. spiralis, T. britovi, T. murrelli*	Boireau et al. (2000)
Jackal	Algeria	Sporadic	*T. britovi*	Nezri et al. (2006)
Mutton	China	++	Not identified	Wang et al. (2007)
Polar bear	Greenland, Siberia	Sporadic	*T. nativa*	Dupouy-Camet et al. (2016, 2017)
Turtle	Taiwan, Korea, Thailand	Sporadic	*T. papuae*	Khamboonruang (1991), Lo et al. (2009), SR Lee et al. (2013)
Walrus	Nunavik, Nunavut	++	*T. nativa*	MacLean et al. (1989), Serhir et al. (2001)
Warthog	Senegal, Ethiopia	Sporadic	*T. britovi*?	Kefenie and Bero (1992), Dupouy-Camet et al. (2009)

et al. 2003; Rostami et al. 2017) and their associated social groups (friends, relatives, etc.), and horsemeat has been implicated in a number of larger outbreaks in France and Italy where this meat is consumed raw or rare (Ancelle 1998; Boireau et al. 2000). A list of unusual vectors of human trichinellosis is also given in Table 10.2. It is not possible to give here details on the epidemiology in every country of the world, but the interested reader will find details in the excellent papers of Pozio et al. (Pozio and Murrell 2006; Pozio 2007; Murrell and Pozio 2011). The latest data on trichinellosis in Europe can be found in the European Union One Health 2019 Zoonoses Report (EFSA/ECDC 2021). "In 2019, fewer human food-borne cases ($n = 44$) were reported to EFSA (food-borne outbreaks database) than confirmed sporadic cases ($n = 96$) reported to TESSy managed by ECDC. In 2019, five *Trichinella* outbreaks were reported in Bulgaria, Croatia, Italy and Romania, and two outbreaks in Serbia. All outbreaks were reported with strong-evidence and associated with pig meat and products. Around 218 million pigs were tested for *Trichinella* in 2019, out of about 246 million reared pigs in the European Union, with only 219 positive animals, about 0.89 per million reared pigs. All positive findings were from pigs not raised under controlled housing conditions. Spain accounted for the highest number of positive domestic pigs ($n = 113$) followed by Romania ($n = 79$) Poland ($n = 22$), Croatia ($n = 3$), Bulgaria ($n = 1$) and France

($n = 1$). In addition to domestic pigs, hunted wild boar are an important source of trichinellosis infections for humans. However, the prevalence of *Trichinella* spp. infections in this animal species has declined over the years due to the increased control for these pathogens. From 2012 to 2016, the prevalence of infection was reduced threefold (from 0.13% in 2012 to 0.05% in 2016) but increased up to 0.09% in 2018 in the hunted wild boar population."

10.3.3 An Emerging or Re-emerging Disease?

Is trichinellosis emerging or re-emerging, or is it the diagnosis of this disease which is emerging? The first obvious cause of this apparent emergence of trichinellosis could be better reporting to an improved public health system of a disease formerly misdiagnosed as influenza. The critical situations observed in the former Yugoslavia, Romania, and Russia have led to a public health breakdown and a disorganization of veterinary controls. The development of international travel explains the acquisition of the disease (from warthogs in Africa (Dupouy-Camet et al. 2009) or bears, including polar bears (Greenland, Alaska, or northern Canada) by individual or small groups of travelers or hunters (Ancelle et al. 2005; Houzé et al. 2009; Dupouy-Camet et al. 2016, 2017). Isolated cases reported in travelers from countries where the usual habit is to consume raw meats are good indicators of the epidemiology of the disease in some countries. Imported cases are most likely to occur in developed countries and may reveal a high transmission in some countries where the disease is or had become unknown (e.g., Senegal, Laos, etc.). In addition, consumers from countries where the habit of eating raw meat is common will certainly be at higher risk, particularly if they are backpackers, adventure travelers, or hunters of exotic animals. Acquiring trichinellosis while travelling abroad is not a new phenomenon, as McAuley et al. (1991) reviewing "*Trichinella* infection in travelers" in the USA from 1975 to 1989 reported 26 cases after pork consumption while travelling in Central America. In France, since 1975, 67 cases were contracted abroad, while 2497 cases were contracted in the country and were mostly related to 8 outbreaks due to horsemeat consumption (each involving from 7 to 642 cases). Implementation of radical preventive measures in 1998 (education of technicians, quality control, and lab accreditation) has prevented the occurrence of new horsemeat-related outbreaks. Since then, 28 imported cases represented 37% of all cases reported to the National Reference Center, with a mean annual incidence of 2 cases. Between 1975 and 1998, 40 imported cases represented only 1.5% of all identified cases but with a comparable mean annual incidence of 1.6 cases (Dupouy-camet, unpublished). Incidence of imported cases could even have decreased as numbers of international travelers increased during that period. Imported cases diagnosed in France in the period 75–95 were acquired in Egypt, Turkey, and Algeria from pork or wild boar meat; since 1995, most cases were acquired in Laos, West Africa, and Canada from pork, warthog, or bear meat. During the last decade, occasional cases were imported in European countries from East European

countries (Poland, Romania, former Yugoslavia) where trichinellosis re-emerged after social upheavals of the 1990s (Angheben et al. 2008; Nöckler et al. 2007; Milne et al. 2001). In the beginning of 2017, an outbreak with a common source was observed in France and Serbia. Three cases were exposed in Serbia and brought back to France pork delicatessen which they shared with relatives and friends. Around 47 individuals were exposed to the parasitized meat, and 20 cases were reported in both countries (Barruet et al. 2020). Cases were also imported in Asia after turtle consumption (Lo et al. 2009; Lee et al. 2013) and after travelling to a seaside resort in a neighboring island of Singapore (Kurup et al. 2000). Therefore, travelers should be informed about the risks of eating raw meat (pork and pork products, game or reptile meat) and should be discouraged from illegally importing potentially infected meat which could introduce the parasite in *Trichinella*-free areas. Modifications of food habits could also explain the emergence of the parasitosis. High-class restaurants are increasingly serving barely cooked dishes while boasting the freshness of their ingredients. Also, new fashionable culinary habits such as "nouvelle cuisine" are leading consumers to eat undercooked meat. In addition, there is sometimes a kind of social pressure to eat raw meat as this consumption is associated with hunting practice and virility and as new and fashionable diets are more and more popular. Conversely, trichinellosis is practically never reported in Muslim countries or in Jewish communities, due to the proscription of domestic and wild pork. The emergence in France and Italy of horsemeat-related outbreaks of trichinellosis in the last 25 years of the twentieth century was particularly difficult to explain and illustrates the difficulties of prevention, even in industrialized countries (Ancelle 1998; Boireau et al. 2000). In both countries, horsemeat has been consumed for years without any apparent cases of trichinellosis, but between 1975 and 2000, 13 outbreaks were reported, despite veterinary controls implemented in 1985 (5 outbreaks from 1975 to 1985, 8 since 1986). An insufficient amount of meat examined, professional mistakes and fraud, and the absence of quality controls (Forbes and Gajadhar 1999) would explain the failure of veterinary control. The fact that herbivorous animals such as horses are carriers of *Trichinella* spp. implies that these animals are fed intentionally with meat or accidentally with hay containing pieces of rodents, as we have personally observed (Dupouy-Camet et al. 1994). Globalization of international trade is also a risk factor: many countries where trichinellosis is endemic among wildlife (e.g., in North America) or domestic animals (e.g., in Eastern Europe) are exporting animals for consumption. It is believed that all horses responsible for the French and Italian outbreaks were imported either from North America (USA, Canada, Mexico) or Eastern Europe (former Yugoslavia, Poland). In China, the foci of human and swine trichinellosis were located along railway lines, suggesting that transportation of live pigs and pork increased the risk of transmission (Wang et al. 1998). Ecological modifications can result in an increase of wild game. Reforestation, the increase in fallow land, and the reduction in the number of farms have led to an increase in wild boar populations in Europe and have contributed to the maintenance of sylvatic trichinellosis (Pozio et al. 1996). For example, in France, a 14-fold increase in populations of wild boars has been observed in the past 35 years: 36,429 wild boars were killed in 1973–1974,

compared to 526,709 in 2011–2012 (Office National de la Chasse). A 44% increase in forest surface was observed in France from 1912 to 1990. Modern pig farming (indoor housing with rodent barriers and commercial diets) has made pork-related trichinellosis rare in the industrialized countries. Nevertheless, the recent development of traditional extensive outdoor breeding could facilitate the transmission of *Trichinella* from wildlife to pigs. In some countries, there is no apparent emergence of trichinellosis. This is the case of the USA where a trichinellosis surveillance program was implemented 50 years ago and the annual number of cases of human trichinellosis has declined from 400 (10–15 deaths) in 1947 to 5 cases in 2007 (Moorhead et al. 1999; Kennedy et al. 2009).

10.4 The Host Response to *Trichinella*

The host immune response to the parasite is regulated on a genetic basis and genes involved belong either to the major histocompatibility complex (MHC) loci or to non-MHC regions (Bell 1998).

The different stages of the parasite express different antigens (Parkhouse and Ortega-Pierres 1984), being able to induce resistance to reinfection. In Table 10.3 it is clearly shown, according to Wakelin, that antigens derived from different stages elicit distinct host immune response mechanisms, depending on the infection phase (Wakelin 1993).

10.4.1 Immune Response at Intestinal Level

The mechanisms regulating the intestinal immune response to a primary infection in humans are not well clarified. In outbreaks involving the Inuit population in the Canadian Arctic, a prolonged diarrhea was observed (Viallet et al. 1986; MacLean et al. 1989), suggesting the persistence of the adult worms in the intestine of people probably repeatedly exposed to infection. Several studies have elucidated the fine mechanisms by which *Trichinella* adult worm expulsion from the intestine occurs, and three concepts are well established: (1) the parasites undergo a rapid expulsion by immunological mechanisms, (2) the immune response is directed against stage-specific antigens, and (3) the inflammatory response plays a crucial role in the parasite's expulsion process and is regulated by T-helper (Th) cells (Bell 1998). As regards this last issue, parasite-specific T CD4 + cells (Grencis et al. 1985; Riedlinger et al. 1986), locally generated in the first 2–4 days of infection (Korenaga et al. 1989), migrate to Peyer's patches and to the mesenteric nodes and finally to the tissues (Bell 1998). Cytokine produced by the two Th subsets, Th1 and Th2 (Mucida and Cheroutre 2010), is involved in the adult worm expulsion process, a complex phenomenon involving both humoral- and cellular-mediated responses along with mast cells, eosinophils, goblet, and Paneth cells (Kamal et al. 2001). After a very

Table 10.3 Scheme of the stages involved in the life cycle of *Trichinella spiralis* and the immune and inflammatory responses initiated in the infected mouse host (modified from Wakelin 1993)

Parasite life cycle	Host response
Entry of infective muscle larvae Invasion of enterocytes Release of stichosome antigen Exposure to surface antigen	Uptake, processing, and recognition of antigens, initial Th1 response
Maturation of adults Release of newborn larvae (NBL) Exposure to adult and NBL antigens	**Antibody response** **Mast cell response** **Gut inflammation begins**
Expulsion of adult worms Migration of NBL	**Antibody response** **Mast cell response** Acute inflammation **Shift to Th2 response**
Invasion of muscles Nurse cell formation Release of stichocyte antigen Formation of capsule, when present	**Antibody response** **Eosinophilia** Gut inflammation subsides **Inflammation in muscle** **Consolidated Th2 response**

Processes described also in humans are written in bold characters

early type 0 or type 1 cytokine response (Grencis et al. 1987; Ramaswamy et al. 1996), a switch to type 2 response was observed (Ishikawa et al. 1998), according to experimental results. Unfortunately, no information is available regarding early human infection. The Th2 response is essential to control infection at the intestinal level, by producing both IL-4 and IL-13 (this latter produced also by natural killer cells (McDermott et al. 2005; Lawrence et al. 1998) through STAT-6 activation (Urban 2000). IL-4 regulates the production of specific IgE (Finkelman et al. 1986), which transfers intestinal immunity (Ahmad et al. 1991), and stimulates the uptake and transport of IgE in the intestine (Ramaswamy et al. 1994). Contrasting results were obtained as regards IL-9 (Khan et al. 2003). The lack of IL-10 impairs a fruitful intestinal response to the parasite (Helmby and Grencis 2003). IL-12 counteracts the effects of Th2 response, delaying worm expulsion and increasing worm burden at muscle level in an IFNγ-independent way (Helmby and Grencis 2003a). IL-17 and IL-23 (involved in the activation of Th17 cells) increase in the intestine of *T. spiralis* experimentally infected animals, and in vitro studies show that IL-17 can induce a smooth muscle hypercontractility (Fu et al. 2009). A dual role is played by IL-18, depending on the cytokine environment, mediating either Th1 or Th2 responses. The development of mastocytosis is inhibited by exogenous IL-18 in infected animals, and IL-18$^{-/-}$ mice expel the adult worms of *T. spiralis* more rapidly than nongenetically modified mice do (Helmby and Grencis 2002).

Trichinella infection induces in the thymus the production (T-Bet independent) of a population of what we may call natural Th1 cells. These cells are expanded by IL-4 and contain preformed IFN-γ mRNA which allows the rapid production of the

cytokine after stimulation (Kannan et al. 2017). We don't know yet which role these cells might have in the anti-*Trichinella* response.

10.4.2 Cells Involved in the Intestinal Response

An in vitro model of epithelial invasion by *Trichinella* was set up (Man Warren et al. 1997), with the aim to study the fine mechanisms of host-parasite relations, at intestinal level (McVay et al. 2000; Li et al. 1998).

10.4.2.1 Mucosal Mast Cells and Goblet Cells

Primary *Trichinella* infection in the mouse is characterized by increased number of mucosal mast cells (originated by bone marrow) which accumulate in the small intestine (Alizadeh and Wakelin 1982) and secretion of mucosal mast cell protease I (mmcpI); both are in temporal correlation with worm expulsion (Huntley et al. 1990), with differences dependent on mouse strains (Tuohy et al. 1990).

Mastocytosis and activation of mucosal mast cells were also observed in patients (Gustowska et al. 1983). These cells are produced and differentiated under the control of several cytokines produced by T cells (Garside et al. 1992) as well as that of the transcription molecule STAT-6 (Finkelman et al. 1986). Mast cells play a crucial role in worm expulsion (Suzuki et al. 2008). Bone marrow restoration of W/Wv mice (naturally deficient in mast cells because of the mutation of the *c-kit*, a tyrosine kinase receptor for the stem cell factor) recovered their ability to mount a worm expulsion (Ha et al. 1983). Furthermore, treatment of infected animals with anti-*c-kit* (Donaldson et al. 1996) antibodies or anti-stem cell factor (Faulkner et al. 1997) suppressed this function, demonstrating unequivocally that mast cells are essential for adult worm expulsion from the intestine. After activation, mast cells in some species (rodents and sheep) release their granule content and in particular the granule chymases (chymotrypsin-like serine proteases) during the worm expulsion process (Miller 1996; Knight et al. 2000). These proteases make the gut wall more permeable to antibodies, arriving easily to the parasite sites (Scudamore et al. 1995, 1998). Mast cells play an important role not only in the innate immune function during the acute phase of parasitic infections but also later in chronic immune responses (Shin et al. 2008). Due to *T. spiralis*-induced mast cell hyperplasia and activation, after a second challenge infection, rats expel almost the totality of *T. spiralis* L1 larvae from the intestine in a noticeably short time (few hours) (rapid expulsion) (Bell 1998). This occurs only in rats but not in mice which however expel a secondary infection in an accelerated way (Bell 1992). If rats are infected bypassing the intestinal phase, mast cell accumulation does not occur, but if animals receive an oral challenge, the animals can again mount a rapid expulsion (Blum et al. 2009).

Intestinal infection by *Trichinella* is associated with an increase in the number of bi-potent mast cell/erythrocyte precursor cells in the intestine. This increase resulted associated with an early anemia observed prior to NBL shedding by the female adult worms (Inclan-Rico et al. 2020).

Mast cells participate in the innate immunity since degranulation may also occur in an IgE-independent way as was shown along with increasing the expression of IL-4 and TNF-$\propto$ while depressing that of IFN-γ and IL-10, in a rat mast cell line, incubated with TSL-1 antigens (Arizmendi et al. 2001; Niborski et al. 2004). These can bind to the surface of mast cells, but the receptor is not yet identified (Niborski et al. 2004) and triggers histamine secretion from rat unsensitized mast cell, with no detectable changes in intracellular Ca^{++} (Arizmendi-Puga et al. 2006). These effects on mast cells are not induced by NBL antigens (Yépez-Mulia et al. 2009).

In a primary infection, worm expulsion from the intestine is also associated with an increased number of intestinal goblet cells which produce gel-forming mucin (particularly Muc2) (Alizadeh and Wakelin 1982; Khan et al. 2001). Goblet cell hyperplasia is predominantly under the control of type 2 cytokines, such as IL-4 and overall IL-13, but also IL-22 plays a role, according to studies carried out in mice experimentally infected with either *Nippostrongylus brasiliensis* or *Trichuris muris* (Turner et al. 2013).

The adult worms are not irreversibly damaged by host immunity from either primary or challenge infections; in fact, if taken just prior to expulsion from the intestine, they fully recover if surgically transplanted into new naïve host intestine where there is no inflammation (Kennedy and Bruce 1981).

10.4.2.2 Eosinophils at the Intestinal Level

Intestinal infection caused by *T. spiralis* is followed by an upregulation of IL-5 expression, inflammation sustained mainly by eosinophils, and hypercontractility of intestinal muscle cells, responsible for worm expulsion. All these processes are significantly reduced in IL-5-deficient mice during a primary infection (Vallance et al. 1999), but not in response to a secondary infection (Vallance et al. 2000). Different from peritoneal eosinophils, intestinal eosinophils from infected rats do not kill NBL in vitro in an antibody-dependent cellular cytotoxicity (ADCC) system, unless activated by IL-5 (Lee 1991).

10.4.2.3 Inflammatory Response at Intestinal Level

Enteritis renders the habitat hostile to the parasite, thus facilitating worm expulsion, regardless the age and the viability of the parasite (Bell 1998). Myeloid rather than lymphoid cells are involved in the worm expulsion, as shown in chimeric mice selectively expressing the receptor α for IL-4 (IL4Rα) on BM - or non-BM-derived cells (Urban et al. 2001). Mice W/Wv deficient in mast cells (Ha et al. 1983) received BM cells from wild-type animals or from mice KO for TNFα or for IL-4, before

infection with *T. spiralis*. Those receiving cells from KO for TNFα or for IL-4 mice cleared worms more slowly and experienced a reduced enteritis, different from W/Wv mice reconstituted with normal BM. Furthermore, mast cell responses were reduced in both types of KO mice, suggesting that autocrine production of TNFα and IL-4 by mast cells is involved in the protective Th2 response as well as in inflammation associated with worm expulsion (Ierna et al. 2008). The pro-inflammatory cytokine TNF-α is not responsible for expulsion; in fact, mice deficient in the corresponding receptor can still clear parasites despite the decreased enteritis, indicating that pathology is not required for protection (Lawrence et al. 1998). In transgenic mice for the transmembrane TNFα (tmTNFα) isoform (expressing only the non-cleavable TNFα), it was observed that soluble TNFα, but not the tmTNFα, is involved in protection against the parasite, mediated by Th2 responses; however, both forms are responsible for villous atrophy and crypt hyperplasia caused by *Trichinella spiralis* at intestinal level. Furthermore, it was also shown that TNFα is required for the induction of Th2 immune responses typical of infections with intestinal helminths (Ierna et al. 2009). KO mice for nitric oxide synthase II (NOSII), infected with *T. spiralis*, show a low Th2-associated cytokine expression (IL-4, IL-5) and humoral response (IgG1, IgE), mastocytosis, and fluid accumulation in the intestine, compared to wild-type animals but no change in worm expulsion in comparison to the heterozygotes, despite their lower intestinal pathology, excluding the nitric oxide (NO) involvement in parasite expulsion, although this molecule can mediate the parasitic infection-related enteritis (Lawrence et al. 2000).

An association between elevated TGF-β production and activation driven by dendritic cells along with increased numbers of Th17 cells and IL-17 and worm expulsion as well as hypercontractility of the small intestinal muscle has been observed (Steel et al. 2019). T-regulatory cells and IL-17 are also involved in the regulation of early phases of weight loss which follows intestinal infection (Steel et al. 2019).

10.4.3 Immune Response at Muscle Level

Trichinella is the only helminth which has a special relation with the skeletal muscle; in fact, it is unique in possessing an intracellular localization (Pozio 2007). The different species of *Trichinella* cause various grades of inflammatory response around the nurse cell-parasite complex, depending on the ability to be or not to be surrounded by a collagen capsule (Bruschi et al. 2009); this is probably due to the respective biological feature (Shupe and Stewart 1991). The persistence of muscle infection by *Trichinella* is the result of a fine relationship with host immune response which is mainly characterized by a Th2 phenotype (Li and Ko 2001). Furthermore, increased levels of parasite-specific IgG1 and IgE during the chronic infection confirm such polarization of immune response (Beiting et al. 2004, 2007; Fabre et al. 2009a). Host immune response to *Trichinella*, at muscle level, is partially regulated by the enteric phase of infection; in orally infected animals, in fact the

myositis is higher compared to that observed in animals infected by intravenous injection of NBL, thus bypassing the intestine (Fabre et al. 2009a, b). The presence of parasites in the muscle fibers elicits, as already said, a strong inflammatory response which is not able, however, to eliminate the parasite but unfortunately causes a myositis, responsible for the typical clinical signs of the parenteral phase of infection. Attention of researchers had been focused in the past on the type of infiltrating cells, encapsulation process, and mechanisms of muscle pathology mainly in infections caused by *T. spiralis* (Fabre et al. 2009b; Bruschi and Chiumiento 2011) and in a few studies those by *T. pseudospiralis* (Stewart 1995; Li and Ko 2001; Bruschi et al. 2009). In humans, cell-mediated immunity was studied during the muscle phase during infections by *T. spiralis* or *T. britovi* (Gomez-Morales et al. 2002), showing that up to 14 months p.i. peripheral blood mononuclear cells in response to parasite antigens express and produce a type 2 cytokine pattern (IL-4, IL-5, IL-6, IL-10), irrespective of *Trichinella* species.

While studying patients involved in a trichinellosis outbreak caused by *T. britovi*, *T. spiralis* E/S antigen, extensively cross-reacting, specific T-cell clones were obtained, showing that a mixed Th1-Th2 response characterize the first months of infection (Della Bella et al. 2017).

10.4.3.1 The Role of Eosinophils

These cells during the parenteral phase of infection have a *janus* role, depending on how many times the host is exposed to parasitic antigens. In fact, if in a primary infection they protect the parasite, promoting survival (inhibiting the classical activation of macrophages, acting as regulatory cells) and growth of the L_1 larvae (stimulating the glucose uptake, through the STAT1 inhibition), in secondary infections, they protect the host (as effector cells) together with antibodies, thus limiting the arrival of NBL to the skeletal muscle cell (Huang and Appleton 2016).

10.5 Immunopathology

10.5.1 Enteral Phase of Infection

During experimental infection with nematodes, a pronounced hyperplasia (mastocytosis) and activation of mucosal mast cells occur in both experimental infected animals and patients (Ha et al. 1983; Gustowska et al. 1983; Woodbury et al. 1984; Tuohy et al. 1990; Lawrence et al. 2004).

10.5.2 Parenteral Phase of Infection

During this phase of infection, allergic manifestations occur, which are caused by activation of sensitized mast cell, induced by parasitic antigens in an IgE-dependent way (Watanabe et al. 2005). The clinical manifestation which derives from this phenomenon is represented by facial and periorbital edema (Dupouy-Camet and Bruschi 2007). Blood and tissue eosinophilia are characteristic of this phase (see later). A major question about the role of eosinophils in parenteral phase is whether they are protective or not against *Trichinella* (Bruschi et al. 2008). It has been documented how eosinophils could support parasite growth and survival by promoting accumulation of Th2 cells and preventing induction of NOS II in macrophage and neutrophil NO-mediated killing (Fabre et al. 2009a; Gebreselassie et al. 2012). The parenteral or muscular phase is characterized by inflammatory and allergic responses to the invasion of the skeletal muscle cells by the migrating larvae.

The damage induced by the parasite in the skeletal muscle cell may be direct or indirect which means it is induced by the infiltration of inflammatory cells, primarily eosinophils, and later through immunopathological processes (Pratesi et al. 2006). A correlation was observed between the eosinophil levels and those of serum muscle enzymes and between eosinophil levels and myalgic score in patients infected by *T. britovi*, suggesting a clear relationship between eosinophil levels and tissue damage and pain (Ferraccioli et al. 1988).

10.5.3 Heart and CNS Involvement

Neurotrichinellosis, better than neurotrichinosis (Bruschi et al. 2013), represents the major complication of trichinellosis in humans, and it is caused mainly by vasculitis and granulomatous inflammatory reactions. The NBL tend to wander, causing tissue damage before reentering the bloodstream, or remain trapped and destroyed by the following granulomatous reaction (Katz et al. 1989). Neural cells may also be damaged by eosinophil degranulation products such as eosinophil-derived neurotoxin (EDN) and major basic protein (MBP) (Durack et al. 1979; Mawhorter and Kazura 1993). Myocarditis is triggered initially by invasion of the migrating larvae and then by immunopathological processes such as activated eosinophil infiltration and mast cell degranulation, according to experimental results in rats (Paolocci et al. 1998) and histopathological observations (Bruschi et al. 2008). The mechanisms responsible for eosinophilia in trichinellosis as well as in other helminthic infections are not yet fully elucidated. As already said, IL-5 plays a crucial role, but probably other factors can also be involved (Bruschi et al. 2008), and the role of IgE in the induction of eosinophilia is controversial (Watanabe et al. 2005). It was shown that sera of patients in late period of trichinellosis recognized several proteins present in human heart ventricle, not recognized by normal sera. Tested against rat or human heart ventricle wall, a high proportion of sera (42%) recognized a protein of 68 kDa.

However, the frequency of such reactivity did not change significantly between patients with or without cardiac involvement. The reactivity against the 68-kDa antigen of heart ventricle wall as well as that against the 27- and 41-kDa skeletal muscle antigens was organ-specific, in fact they were not observed against other organs such as the kidney, placenta, and spleen (Pratesi et al. 2006).

10.6 Clinical Manifestations

The clinical aspects of trichinellosis have been extensively reviewed (Dupouy-Camet and Bruschi 2007; Gottstein et al. 2009; Dupouy-Camet et al. 2020).

10.6.1 Acute Phase

In most persons, the acute stage begins with the sudden appearance of general discomfort and severe headaches, an increase in fever and chills, and an excessive sweating. These signs can appear after an incubation ranging from less than a week to 2 weeks or more after the consumption of the parasitize meat. The major syndrome of the acute stage consists of persistent fever, facial edema (characteristically periorbital), muscle pain, and severe asthenia, lasting for several weeks. Transient dizziness and nausea can also occur. Though less common, diarrhea and conjunctival and subungual hemorrhages are also observed. This is the stage during which the adults and the migrating larvae provoke the signs and symptoms of the disease. The clinical typical signs (intestinal signs, fever, facial -edema, and myalgia) must be searched for. The most common intestinal signs and symptoms are diarrhea (from loose stools to as many as 10 to 15 stools per day, frequently containing mucus but free of blood) and abdominal pain. These signs and symptoms usually precede fever and myalgia by 3–4 days, and they disappear in less than 1 week. It has been observed that the shorter the duration between infection and the appearance of diarrhea and fever, the longer the duration of both fever and facial edema (Dupouy-Camet et al. 1988). Fever is one of the earliest and most common signs of trichinellosis. Body temperature increases rapidly, usually stabilizing at 39 °C to 40 °C. The fever usually lasts from 8 to 10 days, although it can persist for up to 3 weeks when the disease is severe. Symmetrical periorbital and facial edema are very typical signs of trichinellosis, although their intensity varies depending upon the intensity of the reaction to the infection. It usually vanishes rapidly following treatment (i.e., within 5 d.p.i. to 7 d.p.i), particularly when glucocorticosteroids are used. In the severe form of trichinellosis, edema extends to the upper and lower extremities. Myalgia and muscle pain affect various muscle groups, and its intensity is related to the severity of the disease. It most frequently affects the muscles of the cervix, trunk, and upper and lower extremities; it also affects the masseters, although less frequently. The pain usually appears upon exertion,

although most persons with severe trichinellosis or phlebitis associated with trichinellosis also experience myalgia at rest. Some persons with severe disease become disabled with a profound muscle weakness because of pronounced angiomyositis-type lesions and neuromuscular disturbances. The restriction of movement due to pain associated with exertion leads to contractures of the upper and lower limbs, nuchal pseudorigidity, and occasionally trismus. Severe myalgia generally lasts for 2–3 weeks. Other signs such as conjunctival and subungual hemorrhagic lesions are caused by vasculitis, the leading pathological process of trichinellosis. In addition, maculopapular rash (after the onset of muscular pain) and formication have been reported for a small proportion of persons.

10.6.2 Complications

Complications usually develop within the first 2 weeks. They are observed mainly in severe cases, but they have also been reported in moderate cases, when treatment was not proper or prompt, particularly in the elderly. A positive correlation has been reported between age and the frequency and severity of complications (Dupouy-Camet et al. 1985). Encephalitis and myocarditis, which are both life-threatening, are often simultaneously present (Fourestié et al. 1993).

10.6.2.1 Cardiovascular Complications

Cardiovascular complications can occur in moderate or severe cases of trichinellosis, usually later in the infection (i.e., between the third and fourth week p.i.) (Compton et al. 1993; Lazarevic et al. 1999; Puljiz et al. 2005; Dupouy-Camet and Bruschi 2007). Myocarditis develops in 5% to 20% of all infected persons. The symptoms include pain in the heart region, tachycardia, and electrocardiogram (ECG) abnormalities. The ECG disorder most frequently observed are nonspecific ventricular repolarization disturbances (with ST-T wave changes), followed by bundle-branch conduction disturbances and sinus tachycardia. Other ECG disorders recorded, during various phases of the infection, are sinus bradycardia, right bundle-branch block, supraventricular and ventricular extrasystoles, low-voltage QRS complexes in standard limb leads, first-degree atrioventricular block, and atrial fibrillation. Although ECG abnormalities appear to be a common feature of trichinellosis, especially during the invasive phase of the disease, they are rarely associated with a poor prognosis. A transient, nonspecific, ventricular repolarization disturbance is the abnormality most observed. High levels of troponin have been observed in patients with myocarditis (personal observation). The persistence of the ECG abnormalities, even if other signs and symptoms of trichinellosis have already subsided, usually reflects hypokalemia. Echography can identify myocardium functional anomalies (segmentary hypokinesis or ventricular dilation). Another cardiovascular complication is thromboembolic disease, specifically deep thrombophlebitis,

intraventricular thrombi, and/or pulmonary embolism, all of which can lead to death (Dalcin et al. 2017). Sudden death may result from embolism of the pulmonary artery or from paroxysmal tachycardia. Echography can identify pericardial effusion or a transitory intracavitary thrombus.

10.6.2.2 Neurological Complications

Neurological complications include a variety of signs and symptoms (Ellrodt et al. 1987; Ryczak et al. 1987; Fourestié et al. 1993; Dupouy-Camet and Bruschi 2007; Bruschi et al. 2013) and could be less frequent if the infected person is treated early. Persons with severe disease can show consciousness disorders or excessive excitement and frequently somnolence and apathy; some of the persons with these symptoms show signs of meningitis or encephalopathy. Dizziness, nausea, and tinnitus are transient. Anisocoria, facial nerve paresis, and Babinski's reflexes have also been observed in severe cases. Brain damage, which is usually observed within a few days after the onset of fever, can result in diffuse encephalopathy or focal signs such as disorientation, memory disturbances, frontal syndrome, behavioral disturbances, transient hemiparesis or hemiplegia, oculomotor dysfunction, aphasia, and cerebellar syndrome. Small hypodensities are seen with the CT scan or magnetic resonance (MRI) (Feydy et al. 1996; De Graef et al. 2000; Gelal et al. 2005; McDonald et al. 2014; Rosca and Simu 2018). CT scan can find nodular multifocal hypodensities, sometimes bilateral and cortical or under-cortical topography or within the hemispherical white substance. After injection of contrast medium, cortical lesions and, much more rarely, those of the white substance are enhanced. This enhancement translates the ischemic nature of the first, while the seconds are rather regarded as being of granulomatous origin. The imaging by MRI confirms these aspects. These images are not specific. There is no narrow correlation of radiological signs and clinical signs and symptoms. Most CT scan or MRI brain abnormalities disappear in 4–8 weeks p.i. as well as the clinical signs and symptoms. Decreased muscular strength and tendon reflexes, dysphagia, and trismus usually occur at the beginning of the disease and may persist for a long period of time.

10.6.2.3 Other Complications

Ocular lesions appear during the acute stage of the disease and result from disturbances in microcirculation. The typical traits are edema and vascular lesions within the conjunctiva, the uvea, the retina, and, in some cases, the optic nerve. An intense invasion of muscles of the ocular bulb provokes pain when moving the eyeballs, muscle paralysis, diplopia, or a disturbed accommodation. Dyspnea is relatively common and is caused primarily by parasite invasion and subsequent inflammation of respiratory muscles such as the diaphragm. Respiratory complications are uncommon. They can occur during both early and late stages of trichinellosis. They consist of pneumonia, obstructive bronchitis, or Löffler-type infiltrates or ventilatory

failures (Compton et al. 1993). Following glucocorticosteroid treatment, the respiratory disturbances regress within a few days. Digestive complications occur during the acute stage of infection, and they consist of massive protein exudation leading to hypoalbuminemia and localized edemas, acute intestinal necrosis, or prolonged diarrhea. In some outbreaks (Dupouy-Camet and Bruschi 2007), edema of limbs was reported in 6% to 8% of infected persons. A particular syndrome has been described in persons regularly eating infected meat (i.e., Inuit populations). In these cases, clinics are dominated by a chronic diarrhea, probably due to the strong intestinal immune reaction which leads to a rapid expulsion of adult worms from the intestine, thus preventing the muscular phase (Viallet et al. 1986; MacLean et al. 1989).

10.6.3 Clinical Forms

10.6.3.1 Severity and Infective Dose

The severity of trichinellosis depends on a number of variables which are often interrelated, including the infecting dose (i.e., the number of larvae ingested); the frequency of consumption of infected meat; how the meat was cooked or treated (e.g., whether it was raw or rare or whether it had been smoked or salted); the amount of alcohol consumed at the time of meat consumption, given that alcohol could increase the resistance to the infection (Pawlowski 1983); the *Trichinella* species involved (as already said, the fertility of females differs by species); and individual susceptibility in relation to ethnic factors as well as gender, age, and the level of immune competence of the host (reviewed in Dupouy-Camet and Bruschi 2007). There are no precise data defining the minimal infective dose able to exert clinical trichinellosis in an individual person. Murrell and Bruschi (1994), quoting Piekarski (1954), reported that 70 live larvae were sufficient to provoke clinical disease, but mathematical models have estimated this infective dose to be lower (Teunis et al. 2012). It is also assumed that meat containing at least one larva per gram is necessary to induce a clinical infection in man (Zimmermann 1983), which could correspond to an infective dose of approximately 150 larvae for the usual consumer (assuming a meat consumption of 150 g). On the other hand, an infection is clinically patent in humans when the number of larvae per gram (Ipg) of muscle biopsy is around ten and severe when the number of Ipg of muscle biopsy is above 100 (Pawlowski 1983). From these data and from the theoretical number of NBL shed by *T. spiralis* females (around 1000/female), the minimum infective dose could be estimated around 100 and 300 larvae. An intake of more than 1000 to 3000 larvae could lead to a severe disease. The length of the incubation period depends upon the same variables as disease severity. Furthermore, it has been observed that for the more severe forms of trichinellosis, the incubation period is generally shorter, lasting approximately 1 week in the severe form, 2 weeks in the moderately severe form, and at least 3–4 weeks in the benign and abortive forms.

10.6.3.2 Species and Genotypes

Although clinical differences have been observed among persons infected with different species of *Trichinella* (Bruschi and Murrell 2002), it has not been possible to attribute these differences to the species of the pathogen because the number of infecting larvae ingested by each person was generally unknown. However, *T. spiralis* infections could be more severe than those caused by *T. britovi*, and this could be since *T. britovi* females are less prolific (Pozio et al. 1993). The nonencapsulated *T. pseudospiralis* seems to provoke signs and symptoms that last longer (Jongwutiwes et al. 1998; Ranque et al. 2000). In a recent outbreak, serologically attributed to *T. pseudospiralis*, occurred in Italy, myalgia was observed in almost all patients, whereas periorbital edema, a typical sign of trichinellosis, was observed only in 13.3% (Gomez-Morales et al. 2021).

10.6.3.3 Pregnancy and Childhood

In pregnant women, trichinellosis can cause abortion or premature delivery (Ancelle et al. 1988). Although the underlying mechanisms have not been clarified, these complications could be due to modified production of choriogonadotropin, progesterone, or cytokines (Kociecka 1988). The existence of congenital trichinellosis has not been clearly established; however, most women infected during their pregnancy have delivered healthy babies (Kociecka 2000; Taybouavone et al. 2009; Bruschi and Carlier 2013). In children, the signs and symptoms of trichinellosis are the same as those found in adults, although myalgia and diarrhea are less frequent, the clinical signs and symptoms are less pronounced and regress more quickly, and the frequency of complications is lower. The clinical picture is milder possibly because of lower infecting doses and a less intense allergic reaction to the larvae invasion (Dupouy-Camet and Bruschi 2007).

10.7 Diagnosis

10.7.1 Nonspecific Laboratory Signs

10.7.1.1 Eosinophilia

Blood eosinophilia is a typical response to tissue-dwelling parasites such as *Trichinella*, depending on parasite (e.g., inoculum size) and host (Th2 response, genetic background) factors (Bruschi et al. 2008). The key role for the induction of eosinophilia is exerted by the cytokine IL-5, produced by Th2 cells. This stimulates the production and differentiation of this granulocyte population in the BM but also prevents their apoptosis (reviewed in Bruschi et al. 2008).

Eosinophilia has been observed in practically every case of trichinellosis, with few exceptions. It appears early, before the development of the general syndrome of clinical signs and symptoms, and it increases between the second and the fifth week of infection. Eosinophilia occurs in various degrees: low (< 1000/μl or 1G/l), moderate (1000–3000 /μl or 1–3 G/l), and high (> 3000/μl or 3G/l); up to 19,000 cells per μl have been reported (Dupouy-Camet and Bruschi 2007). It regresses slowly and can remain at lower levels for a period of several weeks to 3 months. The level of eosinophilia is correlated with the degree of myalgia (Ferraccioli et al. 1988) and is significantly higher in persons with neurological complications (Fourestié et al. 1993). During the acute stage of infection, a massive decrease of eosinophils in persons with severe trichinellosis can be considered as a predictor of a severe outcome.

In a recent outbreak, attributed serologically to *T. pseudospiralis*, which involved 30 individuals defined as cases, according to the ECDC criteria (out of 52, potentially exposed to the parasite), eosinophilia (median value, 9170 cells/μl with a range between 510 and 23,330 cells/μl) was observed in all the evaluated patients, different from serum CPK levels which was increased only in 36,7% of patients (Gomez-Morales et al. 2021).

10.7.1.2 Muscle Enzymes

The levels of all muscle enzymes increase in serum during trichinellosis: CPK, LDH, aldolase, and, occasionally, aspartate aminotransferase (AspAT). Increased muscle enzyme levels are found in 75–90% of infected persons. The increase, which is severalfold, occurs between the second and the fifth week of infection (Capo and Despommier 1996). No correlation has been found between increased CPK and the severity of infection, although a correlation has been found with the intensity of muscular pain (Ferraccioli et al. 1988).

10.7.1.3 Immunoglobulin Level Increase

Trichinellosis like other helminth infections is characterized by increased levels in serum immunoglobulins (hypergammaglobulinemia), mainly of IgE and IgG1 isotypes, as a consequence of the Th2 cell skewing. Excess production of these immunoglobulins is in part due to a polyclonal activation induced by parasite antigens, which characterizes helminth infections (reviewed in Watanabe et al. 2005), and in part to the humoral specific response against the parasite. It was longly debated the possible protective role of parasite-specific IgE against *Trichinella*; in fact, the results in experimental models are contradictory (Watanabe et al. 2005). In humans, it is not clear whether IgE are protective for the host, but certainly they mediate allergic reactions, typical of the parenteral phase of infection (Watanabe et al. 2005). During trichinellosis, patients undergo immunoglobulin level increase particularly in total IgE as it occurs in many other helminthic infections. However, this increase in total IgE levels is not a consistent phenomenon, and it is not possible

to exclude trichinellosis because of its absence. A low correlation between total and specific IgE has been observed for both *T. spiralis* and *T. britovi* infections, suggesting that higher production of IgE is due to a polyclonal activation, rather than to an effective host defense process (Watanabe et al. 2005). Clinical observations suggest that *Trichinella*-specific IgE are responsible for allergic manifestations typical of the clinical picture of trichinellosis, such as cutaneous rash or edemas (Watanabe et al. 2005).

10.7.1.4 Matrix Metalloproteinases

It has been suggested that the serum levels of matrix metalloproteinase (MMP)-9, but not MMP-2, might represent a reliable marker of systemic inflammation in trichinellosis patients; in fact, these proteins resulted higher in those with diarrhea, facial edemas, and myalgia (Bruschi et al. 2016).

10.7.2 Immunodiagnosis

10.7.2.1 Antigens to Be Used in the Serological Methods

The choice of the appropriate antigens represents the major challenge in the setting up of a test. Different antigens can be used for serological diagnosis. Cryo-sections of infected muscles or isolated larvae (muscle larva cuticle antigen) were used in the past for indirect immunofluorescence (IIF). For ELISA, the first antigen used was crude antigen prepared from muscle larvae.

In asymptomatic individuals, the tyvelose (3–6-dideoxy hexose) antigen was able to detect infection (Owen et al. 2001). Antibody directed against this antigen was found even after 15 years after a *T. britovi* outbreak (Piergili-Fioretti et al. 2005). The specificity is high (Bruschi et al. 2001; Owen et al. 2001), with some exceptions (Dea-Ayuela et al. 2001).

Nowadays, an excretory/secretory antigen (E/S antigen) produced in vitro after no more than 18 hours of culture of the muscle larvae is the most frequently used since it guarantees high specificity. Gomez-Morales et al. (2008) standardized and validated the procedure to prepare it. The antigenic composition is quite similar among all *Trichinella* species and genotypes; thus, the antigen prepared with one species, genotype, or strain can be used to detect specific antibodies in people infected with any species.

10.7.2.2 Antibody Response

The humoral immune response leads to the production of parasite-specific antibodies which have a great diagnostic value. At the onset of clinical signs, however,

antibodies are not easily detectable, appearing with a distinct time sequence, depending on the isotype (reviewed in Dupouy-Camet and Bruschi 2007).

During the first days of the febrile phase, it occurs frequently to have negative serological results; for this reason, it is advisable to repeat the exam a few days later. The concentration of antibodies increases during the following 2–3 weeks, particularly in severe cases. The persistence of IgG antibodies may last for many years after infection, even in benign or asymptomatic cases (Harms et al. 1993). Serology is helpful greatly in diagnosis, not in prognosis; in fact, antibody levels do not correlate with the severity or the clinical course of the disease in humans (Murrell and Bruschi 1994). Seroconversion usually occurs between the second and fifth week of infection, and the latency period inversely correlated with the infective dose. Serum may remain positive up to 1 year or more (19 years has been reported) after the end of the acute phase of infection (Pozio et al. 1993). In human infections caused *T. britovi*, seroconversion has been documented up to 2 months postinfection (Pozio et al. 1993). Serological testing performed in a large outbreak of human trichinellosis due to *T. nativa* revealed a positivity rate of 45% and 87% at 3–4 and 10–11 weeks postinfection among confirmed cases. However, seroconversion from confirmed cases in convalescent samples occurred in only 55% (Schellenberg et al. 2003). It is useful to perform an evaluation of the antibody concentration in infected persons every 3 months, allowing to follow up the effects of chemotherapy. In patients involved in an outbreak caused by *T. britovi*, it was shown that circulating antibodies disappeared (in about one-half of patients) within 6 months and all persons became seronegative within 3 years (Pozio et al. 1993).

A global proteomic analysis was recently carried out to compare the difference of immunologically recognized protein profiles among encapsulated (*T. spiralis*) and nonencapsulated (*T. pseudospiralis* and *T. papuae*) species (Somboonpatarakun et al. 2018).

10.7.2.3 Serological Techniques

As in many other parasitological infections, screening serological techniques are represented by IIF and ELISA.

Many kits for ELISA are commercially available with sensitivities ranging from 80 to 90% and specificities from 70 to 97%. Only few do not give false-positive results due to cross-reaction with other parasitic antigens (e.g., visceral *larva migrans* and *Loa loa* (Dupouy-Camet and Bruschi 2007) or inflammatory proteins (autoimmune diseases). Using either a crude larval extract or ES antigens, absolute sensitivity (100%) has been reached in humans infected with *T. spiralis* by ELISA (measuring IgG) (van Knapen et al. 1982; Bruschi et al. 2001). This high sensitivity rate, observed 50 days after infection, declined to about 80% after 2 years. This does not occur when specific IgM are searched; in fact, they were found even 15 years after infection (Pinelli et al. 2007).

The study of the humoral response against stage-specific antigens has not improved the diagnosis; however, the detection of NBL-specific IgA has resulted promising, especially in the early phase of infection when more than 80% of infected

persons resulted positive after 3 weeks of infection (Mendez-Loredo et al. 2001). However, further experience on this synthetic antigen is required. A capture ELISA (cELISA) was set up using TSL-1 antigens immobilized with specific monoclonal antibodies, and it gave 100% specificity and sensitivity at the patent stage of infection (Escalante et al. 2004). A GalNAcβ1–4(Fucα1–3) GlcNAc-R (LDNF) was identified as a glycan antigen that is recognized by antibodies from *Trichinella*-infected individuals. An ELISA-based test using a glycan represented by five LDNF molecules coupled to bovine serum albumin gave a high sensitivity (96%) and 67% of specificity (Aranzamendi et al. 2011).

Western blot (WB) can discriminate efficiently patients with trichinellosis from patients with other helminth infections (Yera et al. 2003), although possible cross-reactions may occur in schistosomiasis (Dupouy-Camet, unpublished data). This technique can be used as a primary or, as already stated, confirmatory test, and results obtained with E/S antigens are quite specific and useful for follow-up studies. It is possible to detect antibodies earlier in the course of the disease by WB than by ELISA or IIF (Yera et al. 2003). The presence of antibodies specific for the TSL-1 antigen family (40–70 kDa in the reduced form) should be considered diagnostic. WB has been used to study the reactivity against the purified 45-kDa glycoprotein to evaluate IgG subclasses (IgG4) (Pinelli et al. 2004, 2007). In a study performed on 150 patients with trichinellosis and 300 individuals with a positive ELISA, only sera from persons with a confirmed trichinellosis, according to Dupouy-Camet and Bruschi's algorithm (2007), reacted with a three-band-specific pattern ranging from 48 to 72 kDa in a WB assay performed with a validated E/S antigen. A distinctive pattern of 53–72 kDa for recognizing *Trichinella* spp. infections in humans by WB was defined, obtaining a sensitivity and a specificity of 100%. Even sera with a high O.D. obtained in ELISA, but falsely positive (without trichinellosis), did not react with this pattern. It should be mentioned, however, that 84% and 71% of the sera from the same patients also reacted with 104–111 kDa and 38–42 kDa proteins, respectively, and less represented also other proteins (Gomez-Morales et al. 2012). The obtained results should be evaluated on the basis of a cutoff value which is obtained by calculating the mean ± 2 or 3 standard deviations of optical densities obtained with a panel of at least 100–200 sera, considered representative of the human population for which the test will be used. Different factors can influence the background of a serological test, among them the human genotype, food habits, and environmental characteristics. This preliminary evaluation should be done for either commercial kits or in-*house* developed tests. It is also important that the cutoff value be confirmed, every time the antigen, reagents, or materials (e.g., type of ELISA plate) are modified or changed (Bruschi et al. 2019).

Recently, by means of WB, it was possible to identify the *Trichinella* species responsible of an outbreak (*T. pseudospiralis*), in consideration of the characteristic recognition pattern obtained (Gomez-Morales et al. 2021).

A bead assay to detect and quantify total IgG or IgG4 *Trichinella* spp. antibodies in human serum using *T. spiralis* E/S antigens was set up and validated, with a possibility to be used in multiplex applications. Sensitivity and specificity were

93.6% and 94.3% for total IgG and 89.2% and 99.2% for IgG4, respectively, with a similar performance as that of *Trichinella* E/S ELISA. The *Trichinella* spp. bead assay shows promise as a method to detect trichinellosis (Kahsay et al. 2021).

10.7.3 Muscle Biopsy

It allows the parasitological diagnosis. Muscle biopsy should be collected, preferably from the deltoid muscle, although any skeletal muscle could be used. At least 0.2–0.5 g of muscle tissue (less than a pea size) should be collected, paying attention to avoid fat or skin. One part of the muscle biopsy should be weighed and stored without any fixative, avoiding dehydration; the other part should be processed for histological examination. The sensitivity of the parasitological diagnosis depends on the amount of muscle sample tested and the number of larvae per gram (lpg). In fact, in paucilarval infection, it may give false-negative results.

10.7.3.1 Trichinelloscopy

It is widely used in diagnosis because it detects *Trichinella* larvae, defines the intensity of infection (i.e., the number of lpg of examined tissue), and allows the collection of individual larvae, extremely useful to identify the parasite at the level of species or genotype. The number of lpg is correlated with the severity of infection which is very severe when approximately 1000 lpg are present, according to Pawlowski (1983). This technique, like all techniques for parasitological diagnosis, is also useful for diagnosing sporadic cases of the infection and doubtful cases (e.g., atypical clinical course, the absence of circulating antibodies, as occurs in immunosuppressed persons, and retrospective analysis of persons) and, frequently, for purposes of compensation claims. To perform trichinelloscopy, small muscle samples (no larger than a grain) are compressed between two thick slides held together with two screws and examined under a trichinelloscope or a dissection microscope at a magnification of 30–40 X, or between two microscopy slides, and examined under a light microscope at a magnification of 50–100 X. The larvae are easier to detect when the muscle biopsy is performed in the late stage of infection, which is characterized by a fully developed nurse cell. However, trichinelloscopy may fail when the larval density is low or for not yet encapsulated larvae or larvae from nonencapsulated species, resulting in false-negative results. This method should not be used for the veterinary control of the trichinellosis in animals.

10.7.3.2 Artificial Digestion

Digestion of muscle samples using 1% pepsin and HCl digestion fluid is very useful to achieve a very useful parameter for the follow-up of the patient, i.e., the number of

lpg of muscle tissue; furthermore, after digestion, it is possible to isolate larvae for the following molecular identification. A critical point is represented by the period of infection; in fact, if the muscle biopsy is taken too early after infection, the larvae are not yet resistant to artificial digestion and are destroyed. Only muscle larvae from muscle biopsies collected 2–3 weeks p.i. are not destroyed by artificial digestion. Particular attention should be paid when a nonencapsulated species is suspected to be the etiological agent since too long digestion might destroy the parasites. The procedure consists of cutting the muscular biopsy in small pieces and incubating at 41 °C for 30 minutes in a small beaker containing 2 ml of water with 5 g/L of pepsin (2000 FIP-U/g and 5.5 mL/L of pure HCl for 100 mg of muscle tissue). At the end of digestion, larvae are collected and counted. The sensitivity of this method depends on the amount of muscle sample tested. To increase the number of parasites, it is also possible to feed mice with the isolated larvae.

10.7.3.3 Histology

By histological analysis of muscle tissue, it is possible to reveal fragments of larvae at various stages of development, the presence of the collagen capsule (for encapsulated species) or what remains of a destroyed capsule, the presence of muscle cell basophilic transformation, and the type and composition of inflammatory cell infiltrates, mainly eosinophils among the muscle fibers (myositis). The basophilic transformation of muscle cells represents a valuable diagnostic criterion of *Trichinella* invasion even when no larvae have been detected. Histological examinations may reveal also fatty metamorphosis, hyaline or hydropic degeneration, or both increased vascularity and small hemorrhages (Weatherly 1983; Gutierrez 1990). The histological observation is more sensitive than trichinelloscopy, especially in the early stage of muscle invasion, when young larvae are still small and not easily distinguishable from the muscle fibers (Wranicz et al. 1998).

10.7.3.4 Molecular Analysis

Molecular analysis is particularly useful in clinical parasitology to type *Trichinell*a isolates. By DNA amplification of various targets, it is possible to type samples containing as few as a single larva, by means of a multiplex polymerase chain reaction (PCR) (Zarlenga et al. 1999) or of sequencing the conserved 5S rRNA gene (Rombout et al. 2001; De Bruyne et al. 2005). Partial DNA sequence data were generated from the internal transcribed spacers ITS1 and ITS2 and from the expansion segment V region of the rRNA repeat from different *Trichinella* species and genotypes (Zarlenga et al. 1999). This multiplex PCR is a sensitive, inexpensive, and rapid molecular approach that can unequivocally identify a single larva at the species and genotype levels (Pozio and La Rosa 2003). A PCR amplification of the mitochondrial large subunit ribosomal RNA (lsu-RNA) gene was coupled with a pyrosequencing technique to distinguish among *T. spiralis*, *T. pseudospiralis*,

T. papuae, and *T. zimbabwensis* to analyze larvae either from infected mouse muscles or as single larvae (Sadaow et al. 2013).

10.7.4 Differential Diagnosis

Isolated cases can be mistaken for autoimmune disease or other infectious diseases. For example, persons with high fever and myalgia are often misdiagnosed with flu, particularly in winter. Protracted diarrhea is often attributed to salmonellosis, shigellosis, or other infections of the alimentary tract. Eosinophilia combined with myalgia and an inflammatory response should be differentiated from eosinophilia-myalgia syndromes, such as toxic oil syndrome, tryptophan intake, and eosinophilic fasciitis. Eosinophilia combined with fever should be differentiated from tissular parasitosis such as fascioliasis, toxocarosis, or invasive schistosomiasis. Parasitic myositis can be due to other parasites such as *Taenia solium* or exceptionally to *Haycocknema perplexum* (Basuroy et al. 2008) or *Sarcocystis* sp. (Esposito et al. 2012). Periorbital or facial edema with fever should be differentiated from glomerulonephritis, serum sickness, and allergic reactions to drugs or allergens, polymyositis, dermatomyositis, and periarteritis nodosa (Nuzzolo-Shihadeh et al. 2020). Intense headaches and stiff neck with confusion, drowsiness, irritability, and neurological symptoms should be differentiated from infectious meningitis and encephalitis. Hemorrhages of the conjunctiva or hemorrhagic skin petechiae associated with fever should be differentiated from leptospirosis, bacterial endocarditis, and typhus exanthematicus. Persons without periorbital edema but with high fever and neurological symptoms may be misdiagnosed with typhoid fever. Table 10.4 gives an algorithm which can help for diagnosis (Dupouy-Camet and Bruschi 2007).

Table 10.4 Algorithm for diagnosing the probability of being infected with acute *Trichinella* in humans

Group A	Group B	Group C	Group D
Fever Eyelid and/or facial edema Myalgia	Diarrhea Neurological signs Cardiological signs Conjunctivitis Subungual hemorrhages Cutaneous rash	Eosinophilia (> 1.G/l) and/or increased total IgE Increased levels of muscular enzymes	Positive serology (with a highly specific test) Seroconversion Positive muscular biopsy
The diagnosis is: Very unlikely: one A or one B or one C Suspected: one A or two B and one C Probable: three As and one C Highly probable: three As and two Cs Confirmed: three As, two Cs, and one D, any of groups A or B and one C and one D			

10.8 Treatment, Evolution, and Prognosis

10.8.1 Specific Treatment

There are "areas of uncertainty in the management of human trichinellosis" because there have been very few prospective, controlled clinical trials of treatment for this infection (Watt and Silachamroon 2004). However, on an empiric basis, most experts recommend the association of diffusible anthelmintics and corticosteroids (Dupouy-Camet et al. 2002; Dupouy-Camet and Bruschi 2007). Only three comparative studies have been made to evaluate the efficacy of the different anthelmintics for trichinellosis (ref). The principal anthelmintics used for trichinellosis are mebendazole (Vermox^{R}, Janssen) and albendazole (Zentel^{R}, GlaxoSmithKline). To eliminate adult worms from the intestinal lumen, thus preventing production of NBLs and muscle invasion, and the development of trichinellosis, anthelmintics must be used during the period of intestinal invasion (i.e., less than 1 week after infection). However, this is rarely possible, and treatment is usually started at the beginning of larval development in muscle cells. Since it has not been clearly established how long the adult females survive and produce NBL in the human intestine, it is recommended that anthelmintics should be administered to all persons with trichinellosis during the 4–6 weeks following infection.

10.8.1.1 Mebendazole

Mebendazole was shown to prevent the occurrence of clinical disease when given to persons 48 h after consumption of meat highly infected with *Trichinella* (Kociecka et al. 1996). The later the treatment is prescribed, the higher the probability that the infected person will harbor viable larvae in their muscles for years, with possible persistent myalgia. Several studies have reported that mebendazole is effective against trichinellosis (Kociecka et al. 1996). Mebendazole, an anthelmintic benzimidazole, is poorly absorbed in the intestinal lumen. Mebendazole is available in tablets (100 mg) or as a suspension (30-ml bottle at a concentration of 100 mg/5 ml) and should be administered at a daily dose of 5 mg per kg body weight (administered in two doses) (e.g., in adults, two tablets twice daily) for 10 to 15 days. The whole treatment cycle may be repeated after 5 days. In some countries (e.g., Germany and Italy), higher doses are recommended (20 mg/kg/day to 25 mg/kg/day administered in three doses for 10 to 14 days). However, compared to lower doses, this dose has been more frequently associated with adverse effects, such as allergic reactions, increased liver enzymes values, alopecia, and bone marrow depression. The efficacy of mebendazole against larvae in muscle tissues depends on the time between infection and treatment and could be dose-dependent. For example, when using a cumulative dose of 7.5 g to 15 g of mebendazole for 10 to 13 days started 1 month after infection, the treatment failed to kill muscle larvae (Pozio 2001).

10.8.1.2 Albendazole

Albendazole, an anthelmintic benzimidazole carbamate, is absorbed in the intestinal lumen relatively quickly. When the drug is administered with a fatty meal, a twofold to fourfold increase in plasma concentration is observed, although large intraindividual and interindividual variability in the plasma concentration has been reported (Lange et al. 1988). Albendazole is well tolerated in persons with trichinellosis (Fourestié et al. 1988; Kociecka 1988; Cabié et al. 1996; Watt et al. 2000). Albendazole is available in tablets (200 mg) or as a suspension (20-ml bottle at a concentration of 100 mg/5 ml). In adults, it should be used at a daily dose of 800 mg/day (15 mg/kg/day) administered in two doses, for 10 to 15 days; in children over 2 years of age, the drug is given at 10 mg per kg body weight. For severe infection, the treatment may be repeated after 5 days. Blood cell counts and liver function should be regularly monitored.

10.8.2 Nonspecific Treatment

Though no valid controlled studies have been performed, glucocorticosteroids are used by most physicians to treat the signs and symptoms of type I hypersensitivity. They must always be used in combination with anthelmintics and never alone since they could increase the larval burden by delaying the intestinal worm expulsion. Shimoni et al. (2007) showed that a short course of prednisone was safe and alleviated symptoms. Glucocorticosteroids could also be used to treat acute vasculitis and myositis; in this case, they could also help to prevent complications by inhibiting eosinophil activation, degranulation, and consequent cytotoxicity for endothelium (Fourestié et al. 1993). Moreover, dexamethasone administered with albendazole has been reported to increase the serum levels of albendazole sulfoxide by about 50% (Jung et al. 1990). The most used glucocorticosteroid is prednisolone, which is available in tablets of 1 mg or 5 mg and is administered at a dosage of 30 mg per day to 60 mg per day, in multiple doses, for 10 to 14 days.

10.8.3 Pregnancy and Childhood

Since mebendazole is teratogenic in rats, it is contraindicated in pregnant women and in children less than 2 years of age. However, a recent study showed that mebendazole therapy during pregnancy (but at 200 mg/day for 3 days) was not associated with a significant risk for major congenital defects when administered during the second and third trimesters, but not during the first trimester (De Silva et al. 1999). Thus, during pregnancy, especially in the first trimester, mebendazole should be used only when the infection is severe, and treatment must begin no later

than 1–3 weeks from infection, because at the recommended dose for pregnant women, it is not effective after this period. Albendazole is contraindicated in pregnant women although offspring of pregnant women accidentally receiving albendazole at high dosages did not show any damage at birth (Kociecka 1988; Horton 1993; Auer et al. 1994; Bradley and Horton 2001). Therefore, during pregnancy, hospitalization is compulsory for symptomatic forms. Only anthelmintics that are poorly absorbed by the intestinal lumen should be used (i.e., pyrantel at 10 mg/kg body weight for 1–3 days), although the efficacy of these drugs has not been evaluated in humans or is doubtful. However, for severe infection, mebendazole could be administered under the physician's control and responsibility. Children should be treated by administration of anthelmintics (albendazole or mebendazole) if older than 2 years of age; the use of these drugs in younger children is, in principle, contraindicated, but trichinellosis is not frequent at that age. Glucocorticosteroids (e.g., prednisolone) will be prescribed if necessary. Regarding treatment in children, the use of mebendazole has been given before the age of 2 years in situations where it was deemed necessary (Dupouy-Camet and Bruschi 2007).

10.8.4 Evolution and Prognosis

The evolution of the disease is usually simple but depends on the severity of the disease. A severe disease will have a complicated evolution. Complicated evolutions are seen after an important infective dose, ins elderly patients and in patients with associated debilitating factors.

10.8.4.1 Lethality

Death is rare. Only 42 deaths occurred worldwide in the period 1986–2009, which accounted for 5377 cases (Murrell and Pozio 2011). In September 2017, a severe trichinellosis outbreak occurred in Cambodia after persons consumed raw wild pig meat; 33 persons were infected, and 8 died (Caron et al. 2020). In the survey performed in ProMED posts between 2001 and 2021, 24 deaths were reported for 2765 cases of trichinellosis. No details are unfortunately given in these shorts posts, but we can imagine that deaths were due to thromboembolic disease in elderly patients as reported during two horsemeat outbreak reported in France where 5 deaths were reported among 1000 patients (Ancelle et al. 1988). One death due to trichinellosis was reported in the European Union 2019 Zoonoses Report resulting in a European Union case fatality of 4.2% (EFSA/ECDC 2021). Correctly managed, patients with severe trichinellosis should avoid death.

10.8.4.2 Chronic Trichinellosis

The convalescent stage of trichinellosis begins when the adult females cease to release migrating larvae and the already established larvae have completed their development in the muscle cells. The transition to this stage is characterized by the progressive disappearance of the signs and symptoms of the disease and by the return of laboratory parameters to normal values. This stage usually begins between the sixth and the eighth week p.i., and infected persons could still have a severe asthenia for several weeks and chronic muscular pain for up to 6 months. Most persons will then become asymptomatic, though live larvae will persist in their muscles for years. Whether or not a chronic form of trichinellosis exists is still under debate, and chronic trichinellosis could be difficult to distinguish from sequelae of the acute phase. However, its existence is supported by reports of persons who complain of chronic pain and a feeling of general discomfort and who show signs of paranoia and a syndrome of persecution, months or even years after the acute stage. Persistent formication, numbness, and excessive sweating have been observed more frequently in persons who have had severe trichinellosis (Pielok 2001). Impaired muscle strength, conjunctivitis, impaired coordination, and IgG antibodies have been reported in some persons up to 10 years postinfection (p.i.) (Harms et al. 1993), whereas live larvae in muscles were detected without clinical signs and symptoms up to 39 years p.i. (Fröscher et al. 1988). Electromyographic disturbances can be observed for several years after the acute stage (i.e., in persons considered to be chronically infected), usually in persons who had not been adequately treated in the early period of invasion (Kociecka et al. 2001). Five (two of which were treated at onset of infection) out of 13 patients re-evaluated 15 years after a *T. britovi* infection still presented EMG changes (Piergili-Fioretti et al. 2005). The existence of a chronic form is supported by the presence of IgG antibodies in the serum, of bioelectric muscle disturbances, and of inflammatory cells in the muscles, all due to the chronic presence of live larvae. Moreover, this syndrome can also result from unnoticed brain localizations during the acute phase of the disease. For the treatment of sequelae and of chronic trichinellosis, anthelmintics are useless; on the contrary, glucocorticosteroids or nonsteroidal anti-inflammatory drugs prescribed for short periods can lead to some transient improvement of myalgia. Physiotherapy and psychotherapy could certainly alleviate muscular and neurological sequelae.

10.9 Perspectives of Control

Control of trichinellosis in humans is mainly based on animal inspection at slaughterhouse as well as on well cooking meat before consumption (Gamble et al. 2000, 2007; Nöckler and Kapel 2007). The prevention of trichinellosis in humans is based on three main approaches: (i) education of the consumer about the risk of

consumption of raw or semi-raw meat and meat products from both domestic (e.g., pigs, horses, and dogs) and sylvatic (e.g., wild boars, bears, walruses, cougars, badgers, foxes, jackals, armadillos, crocodiles, and monitor lizards) animals that can be carriers of *Trichinella* parasites if they are not properly tested for *Trichinella* larvae upon meat inspection; (ii) farming of pigs (the most important source of *Trichinella* infection for humans) in modern, industrialized, indoor pigsties under strict veterinary control and use of certified feedstuff (see below); and (iii) control of all susceptible animals (both domestic and sylvatic) by a standardized artificial digestion method at slaughtering or after hunting (Gottstein et al. 2009). In many countries, individual control of pig carcasses at meat inspection is mandatory but incurs high costs in relation to absence of positive carcasses from pigs reared under controlled housing. Therefore, for some authors, *Trichinella* testing of pigs under controlled housing is not adding any value to protect human health (Franssen et al. 2017). Alban and Petersen (2016) summarized particularly well the methods of prevention of *Trichinella* in pig industry: "Today, the majority of pigs are raised in industrialized pig production systems with a high level of biosecurity resulting in a negligible risk of *Trichinella*. Carcass testing therefore seems less relevant if the aim of testing is to protect public health. However, traditional pig production (including backyard production) still occurs, and organic pig production is on the increase in some areas, suggesting a continued need for carcass testing from such compartments. . . . This has necessitated an adaption of the legislation regarding *Trichinella* surveillance and control, in order to ensure and document public health while also allowing trade of livestock and meat without unnecessary restrictions. This is reflected in the recent development of the international legislation regarding *Trichinella* and associated control obligations for trade in pork as described in the EU legislation and by OIE/FAO/Codex. A common element in the adapted legislation, standards and guidelines is that if a high level of biosecurity can be demonstrated on a pig farm, then the farm belongs to the negligible-risk compartment and there is no need for carcass testing. Maintaining a negligible-risk compartment involves compliance checks for biosecurity requirements, for example through regular audit visits to the farms. . . . For farms that do not belong to a negligible-risk compartment an auditing of biosecurity does not make sense in the traditional way. For these farms, all pigs should be tested for *Trichinella* in order to ensure food safety and to undertake early-warning surveillance of a potentially high-risk sub--population." In addition, during the last years, the organic or free-ranging pig production systems are growing in popularity and are increasing the risk of *Trichinella*; all pigs from these farms should be tested (Papatsiros et al. 2020). Finally, accreditation and quality control should be implemented for veterinary laboratories in charge of detecting *Trichinella* larvae in pork, horsemeat, and game meat (Forbes et al. 2005). Vaccine development for this zoonosis is mainly focused on veterinary medicine. Unlike rodents, the typical experimental host, pigs do not develop strong intestinal immunity (Murrell 1985); hence, muscle larval L1 stage stichocyte antigens are insufficient for a vaccine. The antigens from the NBL have proved highly effective in pigs, and a first-generation vaccine has been developed (Marti et al. 1987). The major problem in the vaccine development for helminth

parasites is represented by the complexity of these organisms, which is obviously much higher in comparison with bacteria and viruses (Meeusen and Piedrafita 2003). Different strategies during the years have been employed with the aim to obtain a certain level of protection against *Trichinella* spp., using total crude extracts of larvae (McGuire et al. 2002), recombinant proteins (Sun et al. 1994) such as the heat-shock protein 70 (Wang et al. 2009) or paramyosin (Yang et al. 2010a), synthetic peptides such as that derived from the 43-kDa glycoprotein (Robinson et al. 1995; McGuire et al. 2002), phage display (Gu et al. 2008), and DNA (Wang et al. 2006), even delivered by attenuated *Salmonella typhimurium* (Yang et al. 2010b) or *Salmonella enterica* by intranasal route (Pompa-Mera et al. 2011). Reader interested to this topic may find further information in Zhang et al. (2018). We may conclude that if in pigs the utility of a reliable vaccine is certain, in humans, it has no sense.

10.10 Conclusions

If, at the world level, pigs are the main vectors of the parasitosis, their role in each country will depend on the mode of breeding. In big, controlled plants with rodent and garbage control programs, the incidence of the disease is minimal. Social upheavals are most of the time key factors for the emergence of these outbreaks as control at farm level and veterinary inspection at slaughterhouses are abandoned as it was observed in Eastern Europe during the 1990s. Any physician who observes a case of trichinellosis should alert public health and veterinary authorities so that other cases and the source of infection can be identified and so that treatment can be started as soon as possible. Although it has not been clearly proven by case-control studies, early treatment with anthelmintics and glucocorticosteroids must be used to alleviate the general syndrome of the disease, to prevent complications, and to reduce persistent muscular pain. Anthelmintics are effective in the intestinal stages of the parasite and should be prescribed in all occurrences, although efficacy against muscle larvae decreases as the time between infection and treatment increases.

At the world level, a group of scientists (biologists, physicians, vets) is regularly convening during international conferences devoted to *Trichinella* and trichinellosis. They are part of the International Commission on Trichinellosis which was founded in 1960 (Dupouy-Camet et al. 2020). The Commission emits recommendations on the management and control of the parasitosis, e.g., genotyping of *Trichinella larvae*, detection of the parasite in food, quality assurance in digestion testing programs, and use of serological tests in humans and animals (Bruschi et al. 2019; Gajadhar et al. 2019; Gamble et al. 2019; Nöckler et al. 2019; Pozio and Zarlenga 2019).

References

Ahmad A, Wang CH, Bell RG (1991) A role for IgE in intestinal immunity. Expression of rapid expulsion of *Trichinella spiralis* in rats transfused with IgE and thoracic duct lymphocytes. J Immunol 146:3563–3570

Alizadeh H, Wakelin D (1982) Comparison of rapid expulsion of *Trichinella spiralis* in mice and rats. Int J Parasitol 12(1):65–73

Ancelle T (1998) History of trichinellosis outbreaks linked to horsemeat consumption 1975–1998. Eur Secur 3:86–89

Ancelle T, Dupouy-Camet J, Bougnoux M-E, Fourestie V, Petit H, Mougeot G, Nozais J-P, Lapierre J (1988) Two outbreaks of trichinosis caused by horsemeat in France in 1985. Am J Epidemiol 127(6):1302–1311. https://doi.org/10.1093/oxfordjournals.aje.a114923

Alban L, Petersen JV (2016) Ensuring a negligible risk of Trichinella in pig farming from a control perspective. Vet Parasitol 231:137–144. https://doi.org/10.1016/j.vetpar.2016.07.014

Ancelle T, De Bruyne A, Poisson D, Dupouy-Camet J (2005) Outbreak of trichinellosis due to consumption of bear meat from Canada, France, September 2005. Euro Surveill 10(10): E051013.3

Angheben A, Mascarello M, Zavarise G et al. (2008) Outbreak of imported trichinellosis in Verona, Italy, January 2008. Euro Surveill 13(22), pii: 18891

Aranzamendi C, Tefsen B, Jansen M et al (2011) Glycan microarray profiling of parasite infection sera identifies the LDNF glycan as a potential antigen for serodiagnosis of trichinellosis. Exp Parasitol 129:221–226

Arizmendi N, Yepez-Mulia L, Cedillo-Rivera R et al (2001) Interleukin mRNA changes in mast cells stimulated by TSL-1 antigens. Parasite 8:S114–S116

Arizmendi-Puga NG, Enciso JA, Ortega-Pierres G et al (2006) *Trichinella spiralis*: histamine secretion induced by TSL-1 antigens from unsensitized mast cells. Exp Parasitol 114:67–76

Auer H, Kollaritsch H, Jüptner J et al (1994) Albendazole and pregnancy. Appl Parasitol 35:146–147

Barruet R, Devez A, Dupouy-Camet J et al (2020) A common source for a trichinellosis outbreak reported in France and Serbia in 2017. Euro Surveill 25:1900527

Basuroy R, Pennisi R, Robertson T et al (2008) Parasitic myositis in tropical Australia. Med J Aust 188:254–256

Beiting DP, Bliss SK, Schlafer DH et al (2004) Interleukin-10 limits local and body cavity inflammation during infection with muscle-stage *Trichinella spiralis*. Infect Immun 72:3129–3137

Beiting DP, Gagliardo LF, Hesse M et al (2007) Coordinated control of immunity to muscle stage *Trichinella spiralis* by IL-10, regulatory T cells, and TGF-beta. J Immunol 178:1039–1047

Bell RG (1992) *Trichinella spiralis*: evidence that mice do not express rapid expulsion. Exp Parasitol 74:417–430

Bell RG (1998) The generation and expression of immunity to *Trichinella spiralis* in laboratory rodents. Adv Parasitol 41:149–217

Blum LK, Thrasher SM, Gagliardo LF et al (2009) Expulsion of secondary *Trichinella spiralis* infection in rats occurs independently of mucosal mast cell release of mast cell protease II. J Immunol 183:5816–5822

Boireau P, Vallée I, Roman T et al (2000) *Trichinella* in horses: a low frequency infection with high human risk. Vet Parasitol 93:309–320

Bradley M, Horton J (2001) Assessing the risk of benzimidazole during pregnancy. Trans Roy Soc Trop Med Hyg 95:72–73

Bronstein AM, Lukashev AN (2019) Possible case of trichinellosis associated with beaver (*Castor fiber*) meat. J Helminthol 93:372–374

Bruschi F, Carlier Y (2013) Does congenital trichinellosis exist? Acta Trop 125:122

Bruschi F, Chiumiento L (2011) *Trichinella* inflammatory myopathy: host or parasite strategy? Parasite Vectors 4:42

Bruschi F, Murrell KD (2002) New aspects of human trichinellosis: the impact of new *Trichinella* species. Postgrad Med J 78:15–22
Bruschi F, Moretti A, Wassom D et al (2001) The use of a synthetic antigen for the serological diagnosis of human trichinellosis. Parasite 8:S141–S143
Bruschi F, Korenaga M, Watanabe N (2008) Eosinophils and *Trichinella* infection: toxic for the parasite and the host? Trends Parasitol 24:462–467
Bruschi F, Marucci G, Pozio E et al (2009) Evaluation of inflammatory responses against muscle larvae of different *Trichinella* species by an image analysis system. Vet Parasitol 159:258–262
Bruschi F, Brunetti E, Pozio E (2013) Neurotrichinellosis. In: Garcia HH, Tanowitz HB, Del Brutto OH (Eds) Neuroparasitology and tropical neurology. Handb Clin Neurol. Elsevier, Edimburgh, 114 3rd series:243–9
Bruschi F, D'Amato C, Piaggi S, Bianchi C, Castagna B, Paolicchi A, Pinto B (2016) Matrix metalloproteinase (MMP)-9: a realiable marker for inflammation in early human trichinellosis. Vet Parasitol 231:132–136. https://doi.org/10.1016/j.vetpar.2016.04.011
Bruschi F, Gómez-Morales MA, Hill DE (2019) International commission on Trichinellosis: recommendations on the use of serological tests for the detection of Trichinella infection in animals and humans. Food Waterborne Parasitol 14:e00032
Cabié A, Bouchaud O, Houzé S et al (1996) Albendazole versus thiabendazole as therapy for trichinosis: a retrospective study. Clin Infect Dis 22:1033–1035
Capo V, Despommier D (1996) Clinical aspects of infection with *Trichinella* spp. Clin Microbiol Rev 9:47–54
Caron Y, Bory S, Pluot M et al (2020) Human outbreak of trichinellosis caused by *Trichinella papuae* Nematodes, Central Kampong Thom Province. Cambodia Emerg Infect Dis 26:1759–1766
Compton SJ, Celum CL, Lee C et al (1993) Trichinosis with ventilatory failure and persistent myocarditis. Clin Infect Dis 16:500–504
Contreras MC, Schenone H, Sandoval L et al (1994) Epidemiology of trichinosis in Chile, prevalence study by immunodiagnostic reactions. Bol Chil Parasitol 49:73–75
Crompton DWT (1999) How much helminthiasis is there in the world? J Parasitol 85:397–403
Cui J, Wang ZQ (2001) Outbreaks of human trichinellosis caused by consumption of dog meat in China. Parasite 8(2 Suppl):S74–S77
Dalcin D, Zarlenga DS, Larter NC et al (2017) *Trichinella nativa* outbreak with rare thrombotic complications associated with meat from a black bear hunted in Northern Ontario. Clin Infect Dis 64(10):1367–1373
De Bruyne A, Yera H, Le Guerhier F et al (2005) Simple species identification of *Trichinella* isolates by amplification and sequencing of the 5S ribosomal DNA intergenic spacer region. Vet Parasitol 132:57–61
De Bruyne A, Vallée I, Ancelle T et al (2006) Trichinelloses. EMC – Mal Infect 23:1–19
De Graef M, Smadja P, Benis J et al (2000) Neurotrichinosis: a case report with MRI evaluation. J Radiol (Paris) 81:817–819
Dea-Ayuela MA, Romarís F, Ubeira FM et al (2001) Possible presence of common tyvelose-containing glycans in Trichinella L1 larvae and embryonated eggs of several nematodes. Parasite J 8(2 Suppl):S120–S122
Della Bella C, Benagiano M, De Gennaro M, Gomez-Morales MA, Ludovisi A, D'Elios S, Luchi S, Pozio E, D'Elios MM, Bruschi F (2017) T-cell clones in human trichinellosis: evidence for a mixed Th1/Th2 response. Parasite Immunol 39(3):e12412. https://doi.org/10.1111/pim.12412
De-la-Rosa JL, Alcantara P, Correa D (1995) Investigation of cross-reactions against *Trichinella spiralis* antigens by enzyme-linked immunosorbent assay and enzyme-linked immunoelectrotransfer blot assay in patients with various diseases. Clin Diagn Lab Immunol 2:122–124
De-la-Rosa JL, Aranda JG, Padilla E et al (1998) Prevalence and risk factors associated with serum antibodies against *Trichinella spiralis*. Int J Parasitol 28:317–321

Devleesschauwer B, Praet N, Speybroeck N et al (2015) The low global burden of trichinellosis: evidence and implications. Int J Parasitol 45:95–99
Donaldson LE, Schmitt E, Huntley JF et al (1996) A critical role for stem cell factor and c-kit in host protective immunity to an intestinal helminth. Int Immunol 8:559–567
Dubinský P, Stefancíková A, Kinceková J et al (2001) Trichinellosis in the Slovak Republic. Parasite 8(2 Suppl):S100–S102
Dupouy-Camet J (2000) Trichinellosis: a worldwide zoonosis. Vet Parasitol 93:191–200
Dupouy-Camet J, Bruschi F (2007) Management and diagnosis of human trichinellosis. In: Dupouy-Camet J, Murrell KD (Eds.) FAO/WHO/OIE guidelines for the surveillance, management, prevention and control of trichinellosis, World Organisation for Animal Health Press Paris (France), pp. 37–69
Dupouy-Camet J, Murrell KD (2007) FAO/WHO/OIE Guidelines for the surveillance, management, prevention and control of trichinellosis. World Organisation for Animal Health Press Paris (France), p. 108
Dupouy-Camet J, van Knapen F, Ancelle T et al (1988) Study of specific immunoglobulins (total, IgG, IgM, IgA, IgE) in indirect immunofluorescence and ELISA in 40 patients with trichinosis followed over a 9-month period. Pathol Biol 36:803–807
Dupouy-Camet J, Soule C, Ancelle T (1994) Recent news on trichinellosis: a new outbreak due to horsemeat in France in 1993. Parasite 1:99–103
Dupouy-Camet J, Kociecka W, Bruschi F et al (2002) Opinion on the diagnosis and treatment of human trichinellosis. Expert Opin Pharmacother 3:1117–1130
Dupouy-Camet J, Lecam S, Talabani H et al (2009) Trichinellosis acquired in Senegal from warthog ham, March 2009. Euro Surveill 14:19220
Dupouy-Camet J, Yera H, Dahane N et al (2016) A cluster of three cases of trichinellosis linked to bear meat consumption in the Arctic. J Travel Med 13:23
Dupouy-Camet J, Bourée P, Yera H (2017) *Trichinella* and polar bears: a limited risk for humans. J Helminthol 91:440–446
Dupouy-Camet J, Kapel CMO, Gołąb E et al (2020) Early days of the International Commission on Trichinellosis (1958–1972). Ann Parasitol 66:259–263
Durack DT, Sumi SM, Klebanoff SJ (1979) Neurotoxicity of human eosinophils. Proc Natl Acad Sci USA 76:1443–1447
Dworkin MS, Gamble HR, Zarlenga DS et al (1996) Outbreak of trichinellosis associated with eating cougar jerky. J Infect Dis 1743:663–666
EFSA/ECDC. The European Union One Health 2019 Zoonoses Report. EFSA J 2021;19:6406
Ellrodt A, Halfon P, Le Bras P, Halimi P, Bouree P, Desi M, Caquet R (1987) Multifocal central nervous system lesions in three patients with trichinosis. Arch Neurol 44(4):432–434. https://doi.org/10.1001/archneur.1987.00520160064016
Escalante M, Romarís F, Rodríguez M et al (2004) Evaluation of *Trichinella spiralis* Larva Group 1 antigens for serodiagnosis of human trichinellosis. J Clin Microbiol 42:4060–4066
Esposito DH, Freedman DO, Neumayr A et al. (2012) Ongoing outbreak of an acute muscular Sarcocystis-like illness among travellers returning from Tioman Island, Malaysia, 2011–2012. Euro Surveill 17, pii:20310
Fabre V, Beiting DP, Bliss SK et al (2009a) Eosinophil deficiency compromises parasite survival in chronic nematode infection. J Immunol 182:1577–1583
Fabre MV, Beiting DP, Bliss SK et al (2009b) Immunity to *Trichinella spiralis* muscle infection. Vet Parasitol 159:245–248
Faulkner H, Humphries N, Renauld JC et al (1997) Interleukin-9 is involved in host protective immunity to intestinal nematode infection. Eur J Immunol 27:2536–2540
Ferraccioli G, Mercadanti M, Salaffi F et al (1988) Prospective rheumatological study of muscle and joint symptoms during *Trichinella nelsoni* infection. Quart J Med 69:973–984
Feydy A, Touze E, Miaux Y et al (1996) MRI in a case of neurotrichinosis. Neuroradiology 38: S80–S82

Finkelman FD, Katona IM, Urban JF et al (1986) Suppression of in vivo polyclonal IgE responses by monoclonal antibody to the lymphokine B-cell stimulatory factor 1. Proc Natl Acad Sci USA 83:9675–9678

Forbes LB, Gajadhar AA (1999) A validated *Trichinella* digestion assay and an associated sampling and quality assurance system for use in testing pork and horse meat. J Food Prot 62:1308–1313

Forbes LB, Scandrett WB, Gajadhar AA (2005) A program to accredit laboratories for reliable testing of pork and horse meat for *Trichinella*. Vet Parasitol 132(1–2):173–177

Fourestié V, Bougnoux ME, Ancelle T et al (1988) Randomized trial of albendazole versus thiabendazole plus flubendazole during an outbreak of human trichinellosis. Parasitol Res 75: 36–41

Fourestié V, Douceron H, Brugieres P et al (1993) Neurotrichinosis. A cerebrovascular disease associated with myocardial injury and hypereosinophilia. Brain 116:603–616

Franssen F, Swart A, van der Giessen J, Havelaar A, Takumi K (2017) Parasite to patient: a quantitative risk model for *Trichinella* spp. in pork and wild boar meat. Int J Food Microbiol 241:262–275

Fröscher W, Gullotta F, Saathoff M et al (1988) Chronic trichinosis. Clinical, bioptic, serological and electromyographic observations. Eur Neurol 28:221–226

Fu Y, Wang W, Tong J et al (2009) Th17: a new participant in gut dysfunction in mice infected with *Trichinella spiralis*. Mediat Inflamm 2009:517052

Gajadhar AA, Noeckler K, Boireau P et al (2019) International Commission on Trichinellosis: recommendations for quality assurance in digestion testing programs for *Trichinella*. Food Waterborne Parasitol 16:e00059

Gamble HR, Bessonov AS, Cuperlovic K et al (2000) International commission on Trichinellosis: recommendations on methods for the control of Trichinella in domestic and wild animals intended for human consumption. Vet Parasitol 93:393–408

Gamble HR, Boireau P, Nöckler K et al. (2007) Prevention of *Trichinella* infection in the domestic pig. In: Dupouy-Camet J, Murrell KD (Eds.), FAO/WHO/OIE guidelines for the surveillance, management, prevention and control of trichinellosis. World Organisation for Animal Health Press Paris (France), pp. 101–107

Gamble HR, Alban L, Hill D et al (2019) International commission on Trichinellosis: recommendations on pre-harvest control of *Trichinella* in food animals. Food Waterborne Parasitol 15: e00039

Garside P, Grencis RK, Mowat AM (1992) T lymphocyte dependent enteropathy in murine *Trichinella spiralis* infection. Parasite Immunol 14:217–225

Gebreselassie NG, Moorhead AR, Fabre V et al (2012) Eosinophils preserve parasitic nematode larvae by regulating local immunity. J Immunol 188:417–425

Gelal F, Kumral E, Vidinli BD et al (2005) Diffusion-weighted and conventional MR imaging in neurotrichinosis. Acta Radiol 46:196–199

Gomez-Morales MA, Mele R, Sanchez M et al (2002) Increased CD8+-T cell expression and a Type 2 cytokine pattern during the musculature phase of *Trichinella* infection in humans. Infect Immunity 70:233–239

Gomez-Morales MA, Ludovisi A, Amati M et al (2008) Validation of an enzyme-linked immunosorbent assay for diagnosis of human trichinellosis. Clin Vaccine Immunol 15:1723–1729

Gomez-Morales MA, Ludovisi A, Amati M et al (2012) A distinctive Western blot pattern to recognize *Trichinella* infections in humans and pigs. Int J Parasitol 42:1017–1023

Gomez-Morales MA, Mazzarello G, Bondi E et al (2021) Second outbreak of *Trichinella pseudospiralis* in Europe: clinical patterns, epidemiological investigation and identification of the etiological agent based on the western blot patterns of the patients' serum. Zoonoses Public Health 68:29–37

Gottstein B, Pozio E, Nöckler K (2009) Epidemiology, diagnosis, treatment, and control of trichinellosis. Clin Microbiol Rev 22:127–145

Grencis RK, Riedlinger J, Wakelin D (1985) L3T4-positive T lymphoblasts are responsible for transfer of immunity to *Trichinella spiralis* in mice. Immunology 56:213–218

Grencis RK, Riedlinger J, Wakelin D (1987) Lymphokine production by T cells generated during infection with *Trichinella spiralis*. Int Arch Allergy Appl Immunol 83:92–95

Gu Y, Li J, Zhu X et al (2008) *Trichinella spiralis*: characterization of phage-displayed specific epitopes and their protective immunity in BALB/c mice. Exp Parasitol 118:66–74

Gustowska L, Ruitenberg EJ, Elgersma A et al (1983) Increase of mucosal mast cells in the jejunum of patients infected with *Trichinella spiralis*. Int Arch Allergy Appl Immunol 71:304–308

Gutierrez Y (1990) Trichinelloidea: *Trichuris*, *Trichinella* and *Capillaria*-diocto-phymatoidea: dioctophyme. In: Gutierrez Y (Ed.) Diagnostic pathology of parasitic infections with clinical correlations. Lea and Febiger, Philadelphia, pp. 336–350

Ha TY, Reed ND, Crowle PK (1983) Delayed expulsion of adult *Trichinella spiralis* by mast cell-deficient W/Wv mice. Infect Immun 41:445–447

Harms G, Binz P, Feldmeier H et al (1993) Trichinosis: a prospective controlled study of patients ten years after acute infection. Clin Infect Dis 17:637–643

Helmby H, Grencis RK (2002) IL-18 regulates intestinal mastocytosis and Th2 cytokine production independently of IFN-gamma during *Trichinella spiralis* infection. J Immunol 169:2553–2560

Helmby H, Grencis RK (2003) Contrasting roles for IL-10 in protective immunity to different life cycle stages of intestinal nematode parasites. Eur J Immunol 33:2382–2390

Helmby H, Grencis RK (2003a) IFN-gamma-Independent Effects of IL-12 During Intestinal Nematode Infection. J Immunol 171:3691–3696

Horton J (1993) The use of antiprotozoan and anthelmintic drugs during pregnancy and contraindications. J Infect 26:104–105

Houzé S, Ancelle T, Matra R et al (2009) Trichinellosis acquired in Nunavut, Canada in September 2009: meat from grizzly bear suspected. J Eur Surveill 14(44) pii: 19383

Huang L, Appleton JA (2016) Eosinophils in Helminth infection: defenders and dupes. Trends Parasitol 32(10):798–807

Huntley JF, Gooden C, Newlands GF et al (1990) Distribution of intestinal mast cell proteinase in blood and tissues of normal and *Trichinella*-infected mice. Parasite Immunol 12:85–95

Ierna MX, Scales HE, Saunders KL et al (2008) Mast cell production of IL-4 and TNF may be required for protective and pathological responses in gastrointestinal helminth infection. Mucosal Immunol 1:147–155

Ierna MX, Scales HE, Mueller C et al (2009) Trans-membrane tumor necrosis factor alpha is required for enteropathy and is sufficient to promote parasite expulsion in gastrointestinal helminth infection. Infect Immun 77:3879–3885

Inclan-Rico JM, Hernandez CM, Henry EK et al (2020) *Trichinella spiralis*-induced mastocytosis and erythropoiesis are simultaneously supported by a bipotent mast cell/erythrocyte precursor cell. PLoS Pathog 16(5):e1008579

Ishikawa N, Goyal PK, Mahida YR et al (1998) Early cytokine responses during intestinal parasitic infections. Immunology 93:257–263

Jongwutiwes S, Chantachum N, Kraivichian et al (1998) First outbreak of human trichinellosis caused by *Trichinella pseudospiralis*. Clin Infect Dis 26:111–115

Jung H, Hurtado M, Medina MT, Sanchez M, Sotelo J (1990) Dexamethasone increases plasma levels of albendazole. J Neurol 237:279–280

Kahsay R, Gómez-Morales MA, Rivera HN, McAuliffe I, Pozio E, Handali S (2021) A bead-based assay for the detection of antibodies against *Trichinella* spp. infection in humans. Am J Trop Med Hyg 104:1858–1862

Kamal M, Wakelin D, Ouellette AJ et al (2001) Mucosal T cells regulate Paneth and intermediate cell numbers in the small intestine of *T. spiralis*-infected mice. Clin Exp Immunol 126:117–125

Kannan AK, Mohinta S, Huang W, Huang L, Koylass N, Appleton JA, August A (2017) T-Bet independent development of IFNγ secreting natural T helper 1 cell population in the absence of Itk. Sci Rep 7:45935

Katz M, Despommier DD, Gwadz RW (1989) In: Parasitic Diseases Katz M, Despommier DD, Gwadz RW (eds) Trichinella spiralis. Springer, New York, p 28

Kefenie H, Bero G (1992) Trichinosis from wild boar meat in Gojjam, north-west Ethiopia. Trop Geogr Med 44:278–280

Kennedy MW, Bruce RG (1981) Reversibility of the effects of the host immune response on the intestinal phase of *Trichinella spiralis* in the mouse, following transplantation to a new host. Parasitology 82:39–48

Kennedy ED, Hall RL, Montgomery SP et al (2009) Centers for Disease Control and Prevention (CDC). Trichinellosis surveillance - United States, 2002–2007. MMWR Surveill Summ 58:1–7

Khamboonruang C (1991) The present status of trichinellosis in Thailand. Southeast Asian J Trop Med Public Health 22(Suppl):312–315

Khan WI, Blennerhasset P, Ma C, Matthaei KI, Collins SM (2001) Stat6 dependent goblet cell hyperplasia during intestinal nematode infection. Parasite Immunol 23(1):39–42

Khan WI, Richard RM, Akiho H et al (2003) Modulation of intestinal muscle contraction by interleukin-9 (IL-9) or IL-9 neutralization: correlation with worm expulsion in murine nematode infections. Infect Immun 71:2430–2438

van Knapen F, Franchimont JH, Verdonk AR et al (1982) Detection of specific immunoglobulins (IgG IgM, IgA, IgE) and total IgE levels in human trichinosis by means of the enzyme-linked immunosorbent assay (ELISA). Am J Trop Med Hyg 31:973–976

Knight PA, Wright SH, Lawrence CE et al (2000) Delayed expulsion of the nematode *Trichinella spiralis* in mice lacking the mucosal mast cell-specific granule chymase, mouse mast cell protease-1. J Exp Med 192:1849–1856

Kociecka W (1988) Trichinosis. In: MacLoad C (ed) Parasitic infections in pregnancy and the newborn. Oxford University Press, Oxford, pp 216–226

Kociecka W (2000) Trichinellosis: human disease, diagnosis and treatment. Vet Parasitol 93:365–383

Kociecka W, Gustowska L, Mrozewicz B (1996) Effect of early prophylactic therapy in patients infected with *T. spiralis*. In: Ortega-Pierres G, Gamble R, van Knapen F, Wakelin D (Eds.) Trichinellosis. Proceedings of the 9th international conference on trichinellosis. CINVESTAV, Mexico, D.F., (Mexico), pp. 635–641

Kociecka W, Bombicki K, Pielok L et al (2001) New aspects of clinical pathology and electrophysiological muscle disturbances in patients with history of trichinellosis. Parasite 8:S173–S175

Korenaga M, Wang CH, Bell RG et al (1989) Intestinal immunity to *Trichinella spiralis* is a property of OX8- OX22- T-helper cells that are generated in the intestine. Immunology 66:588–594

Kurup A, Yew WS, San LM et al (2000) Outbreak of suspected trichinosis among travelers returning from a neighboring island. J Travel Med 7(4):189–193

Lange H, Eggers R, Bircher J (1988) Increased systemic availability of albendazole when taken with a fatty meal. Europ J Clin Pharmacol 34:315–317

Lawrence CE, Paterson JC, Higgins LM et al (1998) IL-4-regulated enteropathy in an intestinal nematode infection. Eur J Immunol 28:2672–2684

Lawrence CE, Paterson JC, Wei X et al (2000) Nitric oxide mediates intestinal pathology but not immune expulsion during *Trichinella spiralis* infection in mice. J Immunol 164:4229–4234

Lawrence CE, Paterson YY, Wright SH et al (2004) Mouse mast cell protease-1 is required for the enteropathy induced by gastrointestinal helminth infection in the mouse. Gastroenterology 127: 155–165

Lazarevic AM, Neskovic AN, Goronja M et al (1999) Low incidence of cardiac abnormalities in treated trichinosis: a prospective study of 62 patients from a single-source outbreak. Am J Med 107:18–23

Lee TD (1991) Helminthotoxic responses of intestinal eosinophils to *Trichinella spiralis* newborn larvae. Infect Immun 59:4405–4411

Lee SR, Yoo SH, Kim HS et al (2013) Trichinosis caused by ingestion of raw soft-shelled turtle meat in Korea. Korean J Parasitol 51:219–221

Li CKF, Ko RC (2001) Inflammatory response during the muscle phase of *Trichinella spiralis* and *Trichinella pseudospiralis* infections. Parasitol Res 87:708–714
Li CFK, Seth R, Gray T et al (1998) Production of proinflammatory cytokines and inflammatory mediators in human intestinal epithelial cells after invasion by *Trichinella spiralis*. Infect Immun 66:2200–2206
Lo YC, Hung CC, Lai CS et al (2009) Human trichinosis after consumption of soft-shelled turtles, Taiwan. Emerg Infect Dis 15:2056–2058
MacLean JD, Viallet J, Law C, Staudt M (1989) Trichinosis in the Canadian Arctic: report of five outbreaks and a new clinical syndrome. J Infect Dis 160:513–520
Man Warren T, Gagliardo L, Geyer J et al (1997) Invasion of intestinal epithelia in vitro by the parasitic nematode *Trichinella spiralis*. Infect Immun 65:4806–4812
Marti HP, Murrell KD, Gamble HR (1987) *Trichinella spiralis*: Immunization of pigs with newborn larval antigens. Exp Parasitol 63:68–73
Mawhorter ST, Kazura JW (1993) Trichinosis of the central nervous system. Semin Neurol 13:148–149
McAuley JB, Michelson MK, Schantz PM (1991) *Trichinella* infection in travelers. J Infect Dis 164(5):1013–1016
McDermott JR, Humphreys NE, Forman SP et al (2005) Intraepithelial NK cell-derived IL-13 induces intestinal pathology associated with nematode infection. J Immunol 175:3207–3213
McDonald CM, Tai P, Krings T (2014) Pearls & Oysters: a rare case of neurotrichinosis with MRI. Neurology 82(4):e30–e32
McGuire C, Chan WC, Wakelin D (2002) Nasal immunization with homogenate and peptide antigens induces protective immunity against *Trichinella spiralis*. Infect Immun 70:7149–7152
McVay CS, Bracken P, Gagliardo LF et al (2000) Antibodies to tyvelose exhibit multiple modes of interference with the epithelial niche of *Trichinella spiralis*. Infect Immun 68:1912–1918
Meeusen EN, Piedrafita D (2003) Exploiting natural immunity to helminth parasites for the development of veterinary vaccines. Int J Parasitol 33:1285–1290
Mendez-Loredo B, Martinez Y, Zamora R et al (2001) Class specific antibody responses to newborn larva antigens during *Trichinella spiralis* human infection. Parasite 8:S152–S157
Miller HR (1996) Mucosal mast cells and the allergic response against nematode parasites. Vet Immunol Immunopathol 54:331–336
Milne LM, Bhagani S, Bannister BA et al (2001) Trichinellosis acquired in the United Kingdom. Epidemiol Infect 127(2):359–363
Moorhead A, Grunenwald PE, Dietz VJ et al (1999) Trichinellosis in the United States, 1991–1996: declining but not gone. Am J Trop Med Hyg 60(1):66–69
Mucida D, Cheroutre H (2010) The many face-lifts of CD4 T helper cells. Adv Immunol 107:139–152
Murrell KD (1985) *Trichinella spiralis*: acquired immunity in swine. Exp Parasitol 59:347–354
Murrell KD, Bruschi F (1994) Clinical trichinellosis. In: Sun T (ed) Progress in clinical parasitology. CRC Press, Boca Raton, pp 117–150
Murrell KD, Pozio E (2011) Worldwide occurrence and impact of human trichinellosis, 1986–2009. Emerg Infect Dis 17:2194–2202
Nezri M, Ruer J, De Bruyne A et al (2006) First report of a human case of trichinellosis due to *Trichinella britovi* after jackal (*Canis aureus*) meat consumption in Algeria. Bull Soc Pathol Exot 99:94–95
Niborski V, Vallée I, Fonseca-Linan R et al (2004) *Trichinella spiralis*: Stimulation of mast cells by TSL-1antigens trigger cytokine mRNA expression and release of IL-4and TNF through an Ig-independent pathway. Exp Parasitol 108:101–108
Nöckler K, Kapel CMO (2007) Detection and surveillance for *Trichinella*: meat inspection and hygiene, and legislation. In: Dupouy-Camet J, Murrell KD (eds) FAO/WHO/OIE guidelines for the surveillance, management, prevention and control of trichinellosis. World Organisation for Animal Health Press, Paris, pp 71–85

Nöckler K, Wichmann-Schauer H, Hiller P et al (2007) Trichinellosis outbreak in Bavaria caused by cured sausage from Romania, January 2007. Euro Surveill 12(8):E070823.2
Nöckler K, Pozio E, van der Giessen J et al (2019) International Commission on Trichinellosis: recommendations on post-harvest control of *Trichinella* in food animals. Food Waterborne Parasitol 14:e00041
Nuzzolo-Shihadeh L, Camacho-Ortiz A, Villarreal-Salinas D et al (2020) Human trichinosis mimicking polymyositis. Int J Infect Dis 92:19–20
Owen IL, Pozio E, Tamburrini A et al (2001) Focus of human trichinellosis in Papua New Guinea. Am J Trop Med Hyg 65:553–557
Paolocci N, Sironi M, Bettini M et al (1998) Immunopathological mechanisms underlying the time course of *Trichinella spiralis* cardiomyopathy in rats. Virchows Arch 432:261–266
Papatsiros V, Athanasiou LV, Stougiou D, Christodoulopoulos G, Boutsini S (2020) Trichinella britovi as a risk factor for alternative pig production systems in Greece and Europe. Vet Res Forum: Int Q J 11:199–205
Parkhouse RME, Ortega-Pierres G (1984) Stage-specific antigens of *Trichinella spiralis*. Parasitology 88(Pt 4):623–630
Pawlowski ZS (1983) Clinical aspects in man. In: Campbell WC (ed) *Trichinella* and trichinosis. Plenum Press, New York and London, pp 367–401
Piekarski G (1954) Lehrbuch der Parasitologie. Springer, Berlin
Pielok L (2001) Clinical analysis and evaluation of selected laboratory parameters in patients examined in distant periods after trichinellosis. Wiad Parazytol 47(2):185–209
Piergili-Fioretti D, Castagna B, Frongillo RF et al (2005) Re-evaluation of patients involved in a trichinellosis outbreak caused by *Trichinella britovi* 15 years after infection. Vet Parasitol 132: 119–123
Pinelli E, Mommers M, Homan W et al (2004) Imported human trichinellosis: sequential IgG4 antibody response to *Trichinella spiralis*. Eur J Clin Microbiol Infect Dis 23:57–60
Pinelli E, Mommers M, Kortbeek LM et al (2007) Specific IgG4 response directed against the 45-kDa glycoprotein in trichinellosis: a re-evaluation of patients 15 years after infection. Eur J Clin Microbiol Infect Dis 26:641–645
Pompa-Mera EN, Yépez-Mulia L, Ocaña-Mondragón A et al (2011) *Trichinella spiralis*: Intranasal immunization with attenuated *Salmonella enterica* carrying a gp43 antigen-derived 30mer epitope elicits protection in BALB/c mice. Exp Parasitol 129:393–401
Pozio E (2001) New patterns of *Trichinella* infections. Vet Parasitol 98:133–148
Pozio E (2007) Taxonomy, biology and epidemiology of *Trichinella* parasites. In: Dupouy-Camet J, Murrell KD (Eds.) FAO/WHO/OIE Guidelines for the surveillance, management, prevention and control of trichinellosis. World Organisation for Animal Health Press, Paris (France), pp. 1–36
Pozio E (2020) Scientific achievements of the last 60 years: from a single to a multispecies concept of the genus Trichinella. Vet Parasitol:109042. Advance online publication
Pozio E, La Rosa G (2003) PCR-derived methods for the identification of *Trichinella* parasites from animal and human samples. Methods Mol Biol 216:299–309
Pozio E, Murrell KD (2006) Systematics and epidemiology of *Trichinella*. Adv Parasitol 63:367–439
Pozio E, Zarlenga D (2019) International Commission on Trichinellosis: Recommendations for genotyping *Trichinella* muscle stage larvae. Food Waterborne Parasitol 15:e00033
Pozio E, Rossi P, Amati M (1987) Epidemiology of trichinosis in Italy: correlation between the wild cycle and man. Ann Parasitol Hum Comp 62:456–461
Pozio E, Varese P, Gomez-Morales MA et al (1993) Comparison of human trichinellosis caused by *Trichinella spiralis* and by *Trichinella britovi*. Am J Trop Med Hyg 48:568–575
Pozio E, La Rosa G, Serrano FJ et al (1996) Environmental and human influence on the ecology of *Trichinella spiralis* and *Trichinella britovi* in Western Europe. Parasitology 113:527–533
Pratesi F, Bongiorni F, Kociecka W et al (2006) Heart and skeletal muscle specific antigens recognized by trichinellosis patient sera. Parasite Immunol 28:447–451

Puljiz I, Beus A, Kuzman I et al (2005) Electrocardiographic changes and myocarditis in trichinellosis: a retrospective study of 154 patients. Ann Trop Med Parasitol 99:403–411

Ramaswamy K, Hakimi J, Bell RG (1994) Evidence for an interleukin 4-inducible immunoglobulin E uptake and transport mechanism in the intestine. J Exp Med 180:1793–1803

Ramaswamy K, Negrao-Correa D, Bell RG (1996) Local intestinal immune responses to infections with *Trichinella spiralis*. Real-time, continuous assay of cytokines in the intestinal (afferent) and efferent thoracic duct lymph of rats. J Immunol 156:4328–4337

Ranque S, Faugère B, Pozio E et al (2000) *Trichinella pseudospiralis* outbreak in France. Emerg Infect Dis 6(5):543–547

Riedlinger J, Grencis RK, Wakelin D (1986) Antigen-specific T-cell lines transfer protective immunity against *Trichinella spiralis* in vivo. Immunology 58:57–61

Robert F, Well B, Kassis N et al (1996) Investigation of immunofluorescence cross-reaction against *Trichinella spiralis* by Western blot (Immunoblot) analysis. Clin Diagn Lab Immunol 3:575–577

Robinson K, Bellaby T, Chan WC et al (1995) High levels of protection induced by a40-mer synthetic peptide vaccine against the intestinal nematode parasite *Trichinella spiralis*. Immunology 86:495–498

Rombout YB, Bosch S, van Der Giessen JW (2001) Detection and identification of eight *Trichinella* genotypes by reverse line blot hybridization. J Clin Microbiol 39:642–646

Rosca EC, Simu M (2018) Border zone brain lesions due to neurotrichinosis. Int J Infect Dis 67:43–45

Rostami A, Gamble HR, Dupouy-Camet J et al (2017) Meat sources of infection for outbreaks of human trichinellosis. Food Microbiol 64:65–71

Ryczak M, Sorber WA, Kandora TF, Camp CJ, Rose FB (1987) Difficulties in diagnosing trichinella encephalitis. Am J Trop Med Hyg 36:573–575

Sadaow L, Tantrawatpan C, Intapan PM et al (2013) Molecular differentiation of *Trichinella spiralis*, *T. pseudospiralis*, *T. papuae* and *T. zimbabwensis* by pyrosequencing. J Helminthol 13:1–6

Schellenberg RS, Tan BJ, Irvine JD et al (2003) An outbreak of trichinellosis due to consumption of bear meat infected with *Trichinella nativa*, in 2 northern Saskatchewan communities. J Infect Dis 188:835–843

Scudamore CL, Pennington AM, Thornton EM et al (1995) Release of the mucosal mast cell granule chymase, rat mast cell protease-II, during anaphylaxis is associated with the rapid development of paracellular permeability to macromolecules in rat jejunum. J Exp Med 182: 1871–1881

Scudamore CL, Jepson MA, Hirst BH et al (1998) The rat mucosal mast cell chymase, RMCP-II, alters epithelial cell monolayer permeability in association with altered distribution of the tight junction proteins ZO-1 and occludin. Eur J Cell Biol 75:321–330

Serhir B, MacLean JD, Healey S et al (2001) Outbreak of trichinellosis associated with arctic walruses in northern Canada, 1999. Can Commun Dis Rep 27:31–36

Shin K, Watts GF, Oettgen HC et al (2008) Mouse mast cell tryptase mMCP-6 is a critical link between adaptive and innate immunity in the chronic phase of *Trichinella spiralis* infection. J Immunol 180:4885–4891

Shupe K, Stewart GL (1991) Stimulated chemotactic response in neutrophils from *Trichinella pseudospiralis*-infected mice and the neutrophilotactic potential of *Trichinella* extracts. Int J Parasitol 21:625–630

de Silva NR, Sirisena JL, Gunasekera DP et al (1999) Effect of mebendazole therapy during pregnancy on birth outcome. Lancet 353(9159):1145–1149

Shimoni Z, Klein Z, Weiner P, Assous MV, Froom P (2007) The use of prednisone in the treatment of trichinellosis. IMAJ 9:537–539

Sohn WM, Kim HM, Chung DI et al (2000) The first human case of *Trichinella spiralis* infection in Korea. Korean J Parasitol 38:111–115

Somboonpatarakun C, Rodpai R, Intapan PM et al (2018) Immuno-proteomic analysis of *Trichinella spiralis*, *T. pseudospiralis*, and *T. papuae* extracts recognized by human *T. spiralis*-infected sera. Parasitol Res 117:201–212
Steel N, Faniyi AA, Rahman S et al (2019) TGFβ-activation by dendritic cells drives Th17 induction and intestinal contractility and augments the expulsion of the parasite *Trichinella spiralis* in mice. PLoS Pathog 15(4):e1007657
Stewart GL, Wood B, Boley RB (1985) Modulation of host response by *Trichinella pseudospiralis*. Parasite Immunol 7:223–233
Stewart GL (1995) Myopathogenesis and myoredifferentiation in trichinosis. Bas Appl Myol 5:213–222
Stoll NR (1947) This wormy world. J Parasitol 33:1–18
Sun S, Xu W, He N et al (1994) An antigenic recombinant fusion protein from *Trichinella spiralis* induces a protective response in BALB/c mice. J Helminthol 68:89–91
Suzdaltsev AA, Verkhovtsev VN, Spiridonov AM et al (1999) Trichinosis outbreak after ingestion of barbecued badger. Int J Infect Dis 3:216
Suzuki T, Sasaki T, Takagi H et al (2008) The effectors responsible for gastrointestinal nematode parasites, *Trichinella spiralis*, expulsion in rats. Parasitol Res 103:1289–1295
Taybouavone T, Hai TN, Odermatt P et al (2009) Trichinellosis during pregnancy: a case control study in the Lao Peoples' Democratic Republic. Vet Parasitol 159:332–336
Teunis PF, Koningstein M, Takumi K et al (2012) Human beings are highly susceptible to low doses of *Trichinella* spp. Epidemiol Infect 140:210–218
Tuohy M, Lammas DA, Wakelin D et al (1990) Functional correlations between mucosal mast cell activity and immunity to *Trichinella spiralis* in high and low responder mice. Parasite Immunol 12:675–685
Turner JE, Stockinger B, Helmby H (2013) IL-22 mediates goblet cell hyperplasia and worm expulsion in intestinal helminth infection. PLoS Pathog 9:e1003698
Urban JF Jr, Schopf L, Morris SC et al. (2000) Stat6 signaling promotes protective immunity against *Trichinella spiralis* through a mast cell- and T cell-dependent mechanism. J Immunol 164:2046–2052
Urban JF Jr, Noben-Trauth N, Schopf L et al (2001) Cutting edge: IL-4 receptor expression by non-bone marrow-derived cells is required to expel gastrointestinal nematode parasites. J Immunol 167:6078–6081
Vallance BA, Blennerhassett PA, Deng Y et al (1999) IL-5 contributes to worm expulsion and muscle hypercontractility in a primary *T. spiralis* infection. Am J Phys 277:G400–G408
Vallance BA, Matthaei KI, Sanovic S et al (2000) Interleukin-5 deficient mice exhibit impaired host defence against challenge *Trichinella spiralis* infections. Parasite Immunol 22:487–492
Viallet J, Maclean JD, Goresky LA et al (1986) Arctic trichinosis presenting as prolonged diarrhea. Gastroenterology 91:938–946
Wakelin D (1993) *Trichinella spiralis*: immunity, ecology, and evolution. J Parasitol 79:488–494
Wang ZQ, Cui J, Wu F, Mao FR, Jin XX (1998) Epidemiological, clinical and serological studies on trichinellosis in Henan Province, China. Acta Trop 71:255–268
Wang ZQ, Cui J, Wei HY et al (2006) Vaccination of mice with DNA vaccine induces the immune response and partial protection against *T. spiralis* infection. Vaccine 24:1205–1212
Wang ZQ, Cui J, Shen LJ (2007) The epidemiology of animal trichinellosis in China. Vet J173: 391–398
Wang S, Zhu X, Yang Y et al (2009) Molecular cloning and characterization of heat shock protein 70 from *Trichinella spiralis*. Acta Trop 110:46–51
Watanabe N, Bruschi F, Korenaga M (2005) IgE: a question of protective immunity in *Trichinella spiralis* infection. Trends Parasitol 21:175–178
Watt G, Silachamroon U (2004) Areas of uncertainty in the management of human trichinellosis: a clinical perspective. Expert Rev AntiInfect Ther 2:649–652
Watt G, Saisorn S, Jongsakul K et al (2000) Blinded Placebo-controlled trial of antiparasitic drugs for trichinosis myositis. J Infect Dis 182:371–374

Weatherly NF (1983) Anatomical pathology. In: Campbell WC (ed) *Trichinella* and trichinosis. Plenum Press, New York, pp 173–178

Woodbury RG, Miller HRP, Huntley JF et al (1984) Mucosal mast cells are functionally active during spontaneous expulsion of intestinal nematode infections in rat. Nature 312:450–452

Wranicz MJ, Gustowska L, Gabryel P et al (1998) *Trichinella spiralis*: induction of the basophilic transformation of muscle cells by synchronous newborn larvae. Parasitol Res 84(5):403–407

Yang J, Gu Y, Yang Y et al (2010a) *Trichinella spiralis:* Immune response and protective immunity elicited by recombinant paramyosin formulated with different adjuvants. Exp Parasitol 124: 403–408

Yang Y, Zhang Z, Yang J et al (2010b) Oral vaccination with Ts87 DNA vaccine delivered by attenuated *Salmonella typhimurium* elicits a protective immune response against *Trichinella spiralis* larval challenge. Vaccine 28:2735–2742

Yépez-Mulia L, Montaño-Escalona C, Fonseca-Liñán R et al (2009) Differential activation of mast cells by antigens from *Trichinella spiralis* muscle larvae, adults, and newborn larvae. Vet Parasitol 159:253–257

Yera H, Andiva S, Perret C et al (2003) Development and evaluation of a Western blot kit for diagnosis of human trichinellosis. Clin Diagn Lab Immunol 10:793–796

Zarlenga DS, Chute MB, Martin A et al (1999) A multiplex PCR for unequivocal differentiation of all encapsulated and nonencapsulated genotypes of *Trichinella*. Int J Parasitol 29:1859–1867

Zarlenga D, Thompson P, Pozio E (2020) *Trichinella* species and genotypes. Res Vet Sci 133:289–296

Zhang N, Li W, Fu B (2018) Vaccines against *Trichinella spiralis*: progress, challenges and future prospects. Transbound Emerg Dis 65:1447–1458

Zimmermann WJ (1983) Surveillance in swine and other animals by muscle examination. In: Campbell WC (ed) *Trichinella* and trichinosis. Plenum Press, New York, pp 515–528

Chapter 11
Soil-Transmitted Helminthiasis

Antonio Montresor and Albis Francesco Gabrielli

Abstract Soil-transmitted helminthiasis (STH) is estimated to infect more than 1 billion people worldwide, especially in tropical and subtropical countries. Associated morbidity adversely affects nutritional status and cognitive processes during childhood. Children and women of reproductive age are particularly vulnerable as both population groups are in a period of high demand for micronutrients and many suffer from an overall poor iron status.

Preventive chemotherapy interventions (large scale, periodical distribution of anthelminthics) is reaching over 600 million children every year and offers an opportunity to efficiently control STH at an affordable cost in endemic countries; in addition, the integration of STH control with public health interventions directed against several other NTDs allows the further expansion of health benefits at marginal cost.

11.1 Introduction

Soil-transmitted helminthiasis (STH) owes its name to the fact that the eggs of its causal agents, once expelled in the faeces of infected individuals, require a period of maturation in the soil before becoming infective. Morbidity associated with STH represents a serious public health problem in all those countries where sanitation and hygienic conditions are poor and where effective medicines for their treatment and public health control are neither widely available nor affordable by those in need.

STH is estimated to infect more than 1 billion people worldwide. Associated morbidity adversely affects nutritional status and cognitive processes during childhood (Hall et al. 2008; Nokes et al. 1992). Children, together with women of

A. Montresor · A. F. Gabrielli (✉)
Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland
e-mail: gabriellia@who.int

F. Bruschi (ed.), *Helminth Infections and their Impact on Global Public Health*,
https://doi.org/10.1007/978-3-031-00303-5_11

reproductive age, are particularly vulnerable as both population groups are in a period of high demand for micronutrients and many suffer from an overall poor iron status (Crompton and Nesheim 2002). Continuous contamination of the environment with human faeces carrying worm eggs perpetuates STH transmission, thus leading to infection and reinfection episodes. Morbidity in humans is proportionate to the number of worms hosted by an individual and can be controlled by regular treatment with anthelminthic medicines [preventive chemotherapy (PC)]. Interruption of STH transmission, however, is unlikely to be achieved unless access to effective sanitation, sewage treatment and safe disposal of human faeces becomes widely available.

As in the case of many neglected tropical diseases (NTDs), epidemiology of STH and socio-economic status of affected communities are intimately linked, with similar patterns across countries (de Silva et al. 2003). Where an improvement in sanitation levels has taken place as a natural component of a country's economic progress, a parallel progressive decline in the magnitude of the STH burden has almost invariably been observed.

11.2 The Agents

Ascaris lumbricoides (roundworm), *Trichuris trichiura* (whipworm) and *Necator americanus* and *Ancylostoma duodenale* (the latter two being known as hookworms) are the species of nematodes that cause STH. For all of them, humans represent the final host, and adult worms can be found in the human intestinal tract.

A. lumbricoides is the largest among the four nematodes causing STH. Its cylindrical, elongated body is creamy-white to pink in colour. Adult females can measure 20–35 cm, while males are slightly shorter (15–30 cm). Females can produce up to 200,000 eggs per day. Adult worms have a lifespan of 6 months to 2 years (1 year on average).

T. trichiura is white to pink in colour. The length of the male can reach 30–45 mm, while the female is larger (35–50 mm). The latter can produce between 2000 and 10,000 eggs per day. The average lifespan of the adult worms is about 1 year (longer lifespans are reported in literature).

The colour of *A. duodenale* resembles that of ivory; the adult measures 8–10 mm (male) or 10–14 mm (female); the female produces 10,000–25,000 eggs per day; *N. americanus* is grey-pink in colour and slightly smaller in size; its female can produce 5000–10,000 eggs per day. Both hookworms are characterized by well-developed buccal capsules with two pairs of teeth (*A. duodenale*) and two cutting plates (*N. americanus*). The average lifespan of an adult worm is estimated at 6 months to 1 year (Gunn and Pitt 2012), even though scientific literature reports that adult *A. duodenale* can live up to 7 years and *N. americanus* even longer (up to 20 years).

Ascariasis is the disease estimated to affect the largest number of individuals worldwide (819 million), followed by trichuriasis (465 million) and hookworm

infections (439 million) (Pullan et al. 2014). Ascariasis is particularly prevalent in eastern and southeastern Asia and in western and central Africa, while trichuriasis is mainly occurring in central Africa and in southern and southeastern Asia. Hookworm infections can be found especially in sub-Saharan Africa and in eastern and southeastern Asia. Ancylostomiasis probably originated in eastern Africa and later spread throughout the middle and the Far East, while necatoriasis is thought to have originated in western Africa, from where it was brought to the Americas through the slave trade. It is estimated that approximately 85% of the hookworm infections occurring worldwide are due to *N. americanus* and the remaining 15% to *A. duodenale* (Hotez et al. 2016). Although considered a neglected tropical disease (NTD), STH is not limited to tropical and subtropical regions. Its transmission has in fact been documented in many areas enjoying a temperate climate, even though the widespread improvements in sanitation since the mid-twentieth century have contributed to reduce its area of endemicity. Today, the largest part of the burden is found in developing countries in the warmest regions of the planet.

The risk of acquiring STH is significantly influenced by climatic and socioeconomic factors. Examples of the former include warmth and moisture, as warmer and wetter soils facilitate transmission. Occupation (agriculture-related work), household income (a fact that impacts on availability of safe water and sanitation facilities) and level of education (and consequently the adoption of hygienic practices) also influence transmission patterns. Although STH is generally regarded as a group of conditions predominantly occurring in rural areas, its prevalence can be extremely high in urban and peri-urban slums and informal settlements; this is especially true for ascariasis (Crompton and Savioli 1993; Gabrielli et al. 2005).

The causal agents of STH are transmitted through the parasites' eggs excreted in faeces of infected humans. It is estimated that severely infected individuals can discharge between 2 and 5 million eggs per day. In areas lacking adequate sanitation or where faeces are not properly disposed of, contamination of the external environment, and specifically the soil, can be significant. While in the soil if certain conditions relating to moisture, temperature and exposure to sunlight are met, eggs can survive from weeks to several years (Brudastov et al. 1970; Burden et al. 1976) and develop into fully infective stages.

Humans become infected when such infective stages of the worm enter the human body, a fact that can happen through:

1. Ingestion of infective eggs (*A. lumbricoides* and *T. trichiura*) or larvae (*A. duodenale*) contaminating food, hands or utensils
2. Penetration of the skin by infective larvae contaminating the soil (*N. americanus* and *A. duodenale*)

Following infection, *A. lumbricoides*, *A. duodenale* and *N. americanus* undergo a migration through different organs until they reach sexual maturity in the human gastrointestinal tract, while the cycle of *T. trichiura* is entirely intestinal.

After ingestion, *A. lumbricoides* eggs hatch in the intestinal lumen releasing infective larvae that migrate through the bloodstream to the lungs in 7–10 days. Larvae invade the alveoli and move up the respiratory tree until they reach the

pharynx and are swallowed; when they reach the small intestine, they mature to adult worms. Patent (egg-positive) infections usually develop in 8–10 weeks and might persist for 1–2 years.

Following ingestion of the eggs, *T. trichiura* larvae emerge and invade the intestinal mucosa where they develop into adults. Patent infections usually develop in 8–12 weeks and might persist for up to 4 years.

In the soil, hookworm larvae are released by discharged eggs within 1–2 days and develop into fully infective stages within a week. Such infective larvae penetrate the skin by secreting lytic enzymes such as collagenase and enter the bloodstream to reach the lungs within 1 week; here, they invade the alveoli and move up the respiratory tree until they attain the pharynx and are swallowed; when they reach the small intestine, they develop into adult worms. Patent infections develop in 4–7 weeks; *A. duodenale* infections might persist for up to 5 years while those due to *N. americanus* up to 15–20 years.

Because of the fact that none of the four species of parasites responsible for STH can multiply in the human host, the number of worms within a human host can only increase through subsequent reinfection episodes occurring as a result of human contact with egg or larval infective stages in the environment.

11.3 Epidemiology of Infection

The causal agents of STH are globally distributed, and more than a billion people living in tropical and subtropical countries are currently infected, with over 300 million of them suffering from morbidity. Figure 11.1 depicts each country's share of the global burden of STH, in terms of proportion of the total population requiring PC against STH in 2019. Country reports to the WHO indicate that in 2019 over 1 billion children (299 million preschool-age and 746 million school-age children) required PC (WHO 2020a). Overall, the largest proportion of the infections occurs in India where 27.3% of the world's children in need of treatment live. Indonesia, Nigeria and Bangladesh are also prominent given the size of their resident populations. Globally, 92 countries are in need of intervention (WHO 2020a).

The latest calculations made by the WHO's Global Burden of Disease project (WHO 2016) indicate that in the period 2010–2015, STH was overall responsible for over 3.4 million disability-adjusted life years (DALYs) lost. Data indicate that most of the burden laid in low-income countries (two-thirds of the DALYs lost) and in medium-income countries (one-third of the DALYs lost), while only a small fraction (less than 1% of the DALYs lost) was found in high-income countries.

Epidemiology of STH is characterized by a few distinctive features:

1. STH does not uniformly affect a population, but is characterized by an aggregated (or overdispersed) distribution within such population. Most individuals living in endemic communities harbour few worms, while few hosts harbour large worm burdens (Anderson and May 1991). It is estimated that in such communities approximately 70% of the adult worms live in 15–30% of the individuals.

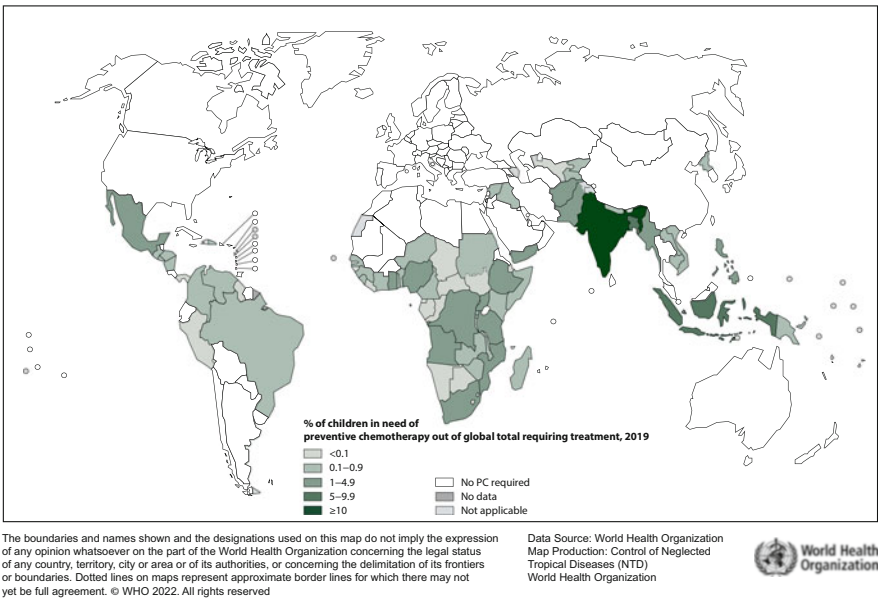

Fig. 11.1 Proportion of global population requiring preventive chemotherapy against STH, 2019 (WHO 2020d)

2. Infections of heaviest intensity usually occur in children and women (Bundy et al. 1992); in addition, these population groups are characterized by intense metabolism due to rapid physical growth, resulting in increased nutritional needs. Micronutrient deprivation associated with peak worm burdens and increased nutritional requirements explains why preschool-age children (1–4 years), school-age children (5–14 years) and women of reproductive age (15–45 years) are particularly vulnerable and are considered the population groups at greater risk of morbidity due to STH.
3. Heavy-intensity infections are the major source of morbidity, and morbidity is directly related to worm burden (Bundy et al. 1992). When the worm burden is low, the associated health damage might be mitigated by the human host, but when a certain threshold of intensity of infection is exceeded, overt morbidity invariably occurs (Gabrielli et al. 2011). In short, the greater the number of worms in the infected person, the greater will be the morbidity caused by these worms. A good example is offered by the case of hookworm infections: the amount of blood lost in the faeces (as an indicator of morbidity) is directly associated with hookworm egg count (as a measure of worm burden) (Stoltzfus et al. 1996).
4. Treatment administered at regular intervals will periodically decrease the worm burden of infected individuals, in spite of continuing reinfection episodes. Harbouring fewer worms will significantly reduce the health damage caused by these parasites (Guyatt et al. 1993), thus controlling morbidity. It will also reduce egg contamination of the environment, even though it is unlikely that this will be

reflected by a significant reduction in transmission and risk of reinfection. From a population perspective, until environmental and/or behavioural conditions have changed, the prevalence of infection that has been reduced by treatment interventions will therefore tend to return to original pretreatment levels if treatment interventions are discontinued. This will happen following reinfection episodes occurring as a consequence of the fact that the worm's infective stages will continue to contaminate the environment.

11.4 The Host Response to the Parasite

Humans acquire only partial immunity to STH reinfection. Antibodies are mainly elicited against the migrating larval stages, especially when they reach the intestinal lumen. Adult worms cause only limited immune reactions when they are located in the bowel; in ectopic locations (especially in the case of *A. lumbricoides*), they might provoke a cell-mediated reaction resulting in the development of a granuloma in the relevant tissue. Overall, the impact of human immune response on STH is limited. Significant research efforts have focused on the development of a human hookworm vaccine targeting both larval and adult antigens; in spite of this, no effective vaccine is currently available.

11.5 Immunopathological Processes

Soil-transmitted helminths can affect the nutritional status of their host through different mechanisms, both local and systemic (Hall et al. 2008). The main outcome is the resulting impairment of the overall nutritional status, which adversely affects physical growth and cognitive development of children, thus leading to stunting and difficulties in learning.

The pathophysiological mechanisms associated with STH include:

- Feeding on the content of the host's intestine
- Feeding on host tissues, such as blood, serum and tissue secretions, thus leading to loss of iron and protein and contributing to protein-energy malnutrition
- Impairment of digestion or absorption of nutrients as a result of physical damage to the gut surface
- Causing an inflammatory response that leads to the production of substances affecting appetite, intake, metabolism and storage of micronutrients
- Eliciting fever and immune response, thus increasing consumption of energy

Each species has peculiar pathogenic features:

Adult *A. lumbricoides* feed on intestinal content only, thus competing with the host for micronutrients and fluids and impairing absorption of carbohydrates and other organic compounds.

Adult *T. trichiura* embed their anterior ends in the mucosa and lyse cells, feeding on fluids, digested tissues and blood. They cause significant inflammation of the mucosa, a fact that is responsible for chronic haemorrhage and dysentery.

Adult hookworms use their buccal capsules to attach to the villi of the jejunum and the distal portion of the duodenum; by using their teeth (*A. duodenale*) or plates (*N. americanus*), they incise the mucosa and feed on blood. Worms move to new feeding sites frequently, leaving behind microscopic ulcers that contribute to blood loss.

11.6 Clinical Manifestations in Immunocompetent and Immunocompromised Patients

The burden of disease associated with STH is mainly attributable to their chronic and insidious impact on health, development and quality of life of those infected rather than to the overt morbidity or mortality they cause. The severity of the clinical manifestations mainly depends on the intensity of infection and on the underlying nutritional condition of the human host. Infections of heavy intensity are particularly severe in children, as they impair physical growth, can result in stunting and are a cause of micronutrient disorders including iron deficiency anaemia leading to impaired cognitive development, poor school performance and school absenteeism. Light infections may also be a contributory cause of growth deficits if the underlying nutritional status of infected individuals is poor. Infections occurring in preschool-age children can contribute to delaying primary school enrolment, thus triggering a cascade of prospective negative consequences in terms of educational and labour market outcomes. In adults, STH can be a cause of decreased physical fitness, reduced work productivity and adverse pregnancy outcomes.

The different causal agents of STH are responsible for specific clinical pictures in infected individuals:

Ascariasis can be associated with a pneumonitis, tracheitis and laryngitis occurring during the migratory stages of the larvae through the lungs and the respiratory tract; eosinophilia is common at this stage, thus giving rise to the so-called Loeffler's syndrome (eosinophilic pneumonia), with cough, chest pain and breathing difficulties. When worms reach their adult stage in the intestinal lumen, symptoms and signs might be light and unspecific and include abdominal discomfort, pain and weight loss; ectopic locations of the worms such as the pancreas (following migration though the ampulla of Vater), bile ducts, gallbladder, liver or oesophagus might be responsible for organ-specific symptoms. The most severe clinical manifestation caused by adult worms is represented by the obstruction of the small intestine by a bolus (mass) of worms, most commonly occurring in children and representing a surgical emergency. This form of acute intestinal obstruction can be a cause of death, as the wall of the strangled intestinal portion can quickly deteriorate. Generalized malaise with fever, gastrointestinal discomfort, colic pain and vomiting are the most

common associated findings. It is calculated that because of their significant size, just a dozen worms can occupy a volume of 100 ml. Worms might also occasionally be passed in faeces.

In trichuriasis, while low-intensity infections might not be associated with any patent symptoms, epigastric pain, vomiting, distension, flatulence, anorexia and weight loss may occur as intensity of infection increases. Eosinophilia is another common finding. In heavy-intensity infections, painful dysentery with blood and mucus in the stool has been observed. The caecum and colon are the sites most commonly colonized by the worms, but in heavy-intensity infections, worms can be found in the more distal segments of the intestine; the presence of high numbers of worms in the rectal mucosa induces tenesmus and can lead to rectal prolapse, especially in very young children. Anaemia might also occur, even though not as severe as in the case of hookworm infections: this is due to the fact the blood loss caused is only 0.005 ml/worm/day (approximately 30 times less than *A. duodenale* and 4 times less than *N. americanus*).

Hookworm infections can be associated with a dermatitis occurring at the site of penetration of the larvae; rash and pruritus are invariably present, and papulae or vesicles might also be present. When larvae undergo pulmonary migration, they might cause focal haemorrhages and allergic pneumonia. Eosinophilia is usually also present. Once in the intestinal lumen, the adult worms cause persistent loss of capillary blood (up to 0.15 ml/worm/day in the case of *A. lumbricoides* and 0.02 ml/worm/day in the case of *N. americanus*), thus causing iron deficiency anaemia. Anaemia thus results from a combination of chronic blood loss, depletion of iron stores and deficiency of iron intake and is associated with a delayed maturation of the erythroblasts (the precursors of the erythrocytes). Iron deficiency anaemia in pregnancy is associated with adverse maternal and foetal consequences, including neonatal prematurity, low birth weight and impaired lactation. Another common finding in hookworm infections is hypoproteinaemia (mainly hypoalbuminaemia), causing a form of oedema that is nonresponsive to diuretics.

11.7 Diagnosis (Inclusive of Histopathology)

Identification of eggs in stool samples remains the most commonly used means of diagnosis, especially in developing countries.

The Kato-Katz technique is recommended for mapping geographical distribution of STH, assessing its public health relevance and deciding on frequency of preventive chemotherapy interventions. As this technique does not require fixation or centrifugation, specimens are not subject to any dilution or concentration. As such, quantification of worm burden is possible, and infections can thus be arranged in classes of intensity, based on number of eggs per gram of faeces examined, as shown in Table 11.1. At community level, monitoring the evolution of classes of intensity over time (i.e. trends of the proportion of individuals in each class) is essential to assess the impact of a PC intervention (Montresor et al. 2013).

Table 11.1 Classes of intensity for STH (WHO 2002)

Organism	Light-intensity infections	Moderate-intensity infections	Heavy-intensity infections
A. lumbricoides	1–4999 epg	5000–49,999 epg	≥50,000 epg
T. trichiura	1–999 epg	1000–9999 epg	≥10,000 epg
Hookworms (A. duodenale and *N. americanus)*	1–1999 epg	2000–3999 epg	≥4000 epg

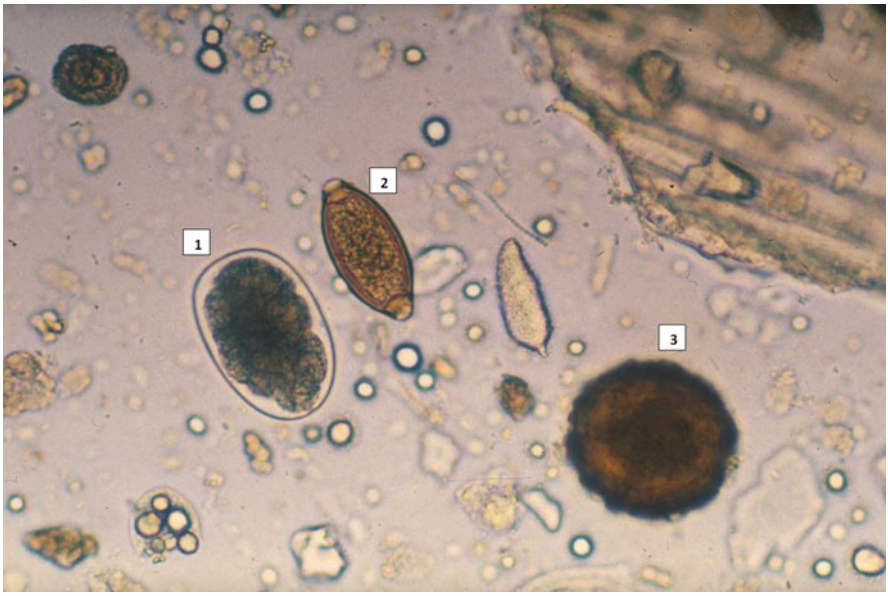

Fig. 11.2 Eggs of Hookworm (1), *Trichuris trichiura* (2) and *Ascaris lumbricoides* (3) in the same microscopic field, illustrating their relative sizes (WHO 1994)

More sensitive techniques relying on concentration are indicated for use in clinical practice, whose aim is both to detect any infection, even of low intensity, and to cure the infected individual by eliminating any harboured worm. In this case, tests can be repeated more than once, and on different stool samples, at diagnosis and at follow-up examinations, in order to increase sensitivity as much as possible at each step, thus minimizing the risk of falsely negative results.

Eggs of intestinal helminths are generally easy to identify because of their large size and distinctive morphology in faecal samples (Fig. 11.2; WHO 1994):

A. lumbricoides: Fertile eggs measure 55–75 μm by 35–50 μm, are golden yellow to brown in colour, have conspicuous mammillations on their surface and are usually unsegmented when passed (single-cell stage). Infertile eggs are elongated and much larger in size (85–95 μm by 43–47 μm) and have thin shells and a grossly irregular mammillated layer. The content of the egg is usually granular and lacks any organization.

T. trichiura: Eggs measure 50–55 μm by 22–24 μm; have a brown, smooth shell; and are lemon- or barrel-shaped, with typical bipolar prominences (plugs); they contain a single-cell ovum.

A. duodenale and *N. americanus*: Eggs measure 60–75 μm by 36–40 μm in size, are oval in shape and have a thin, colourless, hyaline shell. Eggs may be unsegmented but are usually found in the four- or eight-cell stage in fresh faeces. Faeces that have been kept at room temperature for even a few hours might show a more advanced stage of embryonic cleavage or reveal a developing larva.

Colon endoscopy or imaging techniques can be of help in case of suspected ectopic location of the worms (e.g. biliary ascariasis), intestinal obstruction and other complications.

11.8 Treatment

11.8.1 Clinical Management

In clinical management of STH, the available, WHO-recommended treatment options include albendazole, mebendazole, levamisole and pyrantel. All the mentioned medicines are to be administered orally and are available in solid (tablet) or liquid (suspension) formulations. Indications and dosages differ for each disease targeted (WHO 2009). As a precautionary measure, it should be noted that:

- Both albendazole and mebendazole are contraindicated in the first trimester of pregnancy
- Levamisole is contraindicated in the third trimester of pregnancy and during breastfeeding

11.8.1.1 Ascariasis

Albendazole

Adults and children over 2 years: albendazole 400 mg, single administration
Children 12 months to 2 years: albendazole 200 mg, single administration

Mebendazole

Adults and children over 1 year: mebendazole 500 mg, single administration or 100 mg twice daily for 3 days

Levamisole

Adults and children: 2.5 mg/kg, single administration

Pyrantel

Adults and children: 10 mg/kg, single administration

11.8.1.2 Trichuriasis

Albendazole

Adult and children over 2 years: albendazole 400 mg, single administration (moderate infection) or 400 mg daily for 3 days (severe infections)
Children 12 months to 2 years: albendazole 200 mg, single administration (moderate infections) or 200 mg initially and then 100 mg twice daily for 3 days (severe infections)

Mebendazole

Adults and children over 1 year: 100 mg twice daily for 3 days; if eggs persist in the faeces, second course after 3–4 weeks; alternatively, 500 mg, single administration

Ivermectin

In areas where albendazole or mebendazole have been administered on a large scale for several years, the therapeutic efficacy of these drugs especially against *T. trichiura* may have declined (Walker et al. 2021). In this case, it is advisable to supplement ivermectin (200 μg/kg) to maintain a good impact of the intervention

11.8.1.3 Hookworm Infections

Albendazole

Adults and children over 2 years: albendazole 400 mg, single administration
Children 12 months to 2 years: albendazole 200 mg, single administration

Mebendazole

Adults and children over 1 year: 100 mg twice daily for 3 days; if eggs persist in the faeces, second course after 3–4 weeks; alternatively, 500 mg, single administration

Levamisole

Adult and children: 2.5 mg/kg, single administration

Pyrantel

Adults and children: 10 mg/kg, single administration; in severe infections, 10 mg/kg daily for 4 days

11.8.2 Preventive Chemotherapy

In PC interventions, albendazole 400 mg, single administration, or mebendazole 500 mg, single administration, can be alternatively used against all species responsible for STH and in any child aged 2 years or more; mebendazole 500 mg or a reduced dose of albendazole 200 mg should be used in children aged 12–23 months (WHO 2006). Tablets are the recommended formulation under all circumstances. Children in the first year of life and pregnant women in the first trimester of pregnancy should be excluded from PC interventions (WHO 2006).

11.8.3 Drug Efficacy and Its Assessment

Two parasitological indicators are used to assess the efficacy of an anthelminthic drug in treating STH: the cure rate (CR) and the egg reduction rate (ERR). The cure rate can be defined as the percentage of egg-positive individuals who become egg-negative after treatment, while the egg reduction rate is the percentage reduction in the quantity of eggs per gram of faeces discharged by an infected individual before and after treatment.

While clinical management aims at curing infected individuals, a public health intervention such as PC aims at decreasing intensity of infection and keeping it at low levels, with the aim of controlling associated morbidity. Cure rate is therefore suitable to measure the success of a treatment protocol in clinical practice but is a non-appropriate indicator to assess efficacy of a single administration of anthelminthic medicines in the context of PC. The reason is that cure rate is dependent on intensity of infections: the same drug at the same dosage will produce a high cure rate if intensity is low and a low cure rate if intensity is high. Egg reduction rate, to be assessed after a single treatment, is a more appropriate indicator to show reduction of intensity of infection, monitor STH-associated morbidity and detect any possible surge of resistance to anthelminthic medicines (Montresor 2011).

The development of resistance to anthelminthic drugs in targeted parasites may be favoured by the fact that increasingly larger numbers of individuals are administered anthelminthic medicines, especially through PC interventions. As of today, however, scientific investigations have not demonstrated any presence of resistance among helminths that infect humans.

In order to tackle any possible threat of resistance, in 2013, the WHO released a publication aimed at providing national health authorities with up-to-date guidelines on how to assess drug efficacy in the context of PC interventions against STH (WHO 2013).

Between 2013 and 2019, STH drug efficacy trials were conducted in over ten countries. While in the large majority of cases the drug efficacy was reported as normal, data showed doubtful results for hookworm in Cambodia (WHO 2020b) and for *T. trichiura* in Tanzania (Walker et al. 2021).

11.9 Prognosis

Treatment at regular intervals, when started early in life, protects children from the worst consequences of STH and prevents the development of complications associated with heavy-intensity infections. Probabilities of controlling morbidity are higher when infections are recent and of light intensity. Once late-stage complications are established, anthelminthic treatment alone might not be sufficient for a full recovery; this is the case for long-standing malnutrition and its associated morbidities such as stunting. Acute complications, such as intestinal obstruction or rectal prolapse, might require surgery.

11.10 Prevention and Control

The WHO envisages a world free of childhood morbidity due to STH. The goal is to eliminate morbidity from STH among preschool-age children (aged 1–4 years) and school-age children (5–14 years). STH is no longer considered a public health problem when the prevalence of infections of moderate and high intensity is $<2\%$ in the sampled population (WHO 2020c–e).

The existence of two donation programmes to the WHO has certainly contributed to increase the global coverage of this intervention by enabling endemic countries to access free medicines for their PC programmes targeting both preschool-aged children and school-aged children. The two anthelmintics donated to the WHO for the large-scale treatment of STH are albendazole from GSK (since 2010) and mebendazole from Johnson & Johnson (since 2012). In 2020, donation of mebendazole was extended to a dissolvable formulation of the medicine, which is particularly indicated for the treatment of preschool-aged children (Johnson and Johnson 2019).

As a result of this global effort, the number of children receiving PC for STH has progressively increased to exceed 613 million in 2019 (WHO 2020a). Global coverage scaled up significantly over the last decade, from an average of 30% for children of all age in 2010 to 60% for school-aged children and 55% for preschool-aged children in 2019 (Montresor et al. 2020).

Nevertheless, while deworming coverage has progressively increased in children over the last decade, it has remained stable at around 20% for women of reproductive age on a global scale (Bangert et al. 2019). For this reason, additional efforts to sustain deworming interventions in this population group have been recently devised by the WHO (WHO 2018; Gyorkos et al. 2018) and have been implemented since 2021, in line with the recommendations included in the new global NTD road map 2021–2030 (WHO 2020e).

PC with albendazole or mebendazole has proven an effective intervention to reduce the burden of STH morbidity in affected populations. It has been estimated that in a single year (2015), it prevented the loss of 44% of the DALYs that would

have been caused by STH in children if the intervention had not been implemented (Montresor et al. 2017).

Since 2021, the WHO has been assessing the epidemiological situation of STH in countries that have conducted PC for over 6 years and will assess whether elimination of STH as a public health problem has been achieved in any countries.

11.10.1 Preventive Chemotherapy

The mainstay of the WHO strategy to reduce morbidity due to STH is PC. Preventive chemotherapy is "the use of anthelminthic drugs, either alone or in combination, as a public health tool against helminth infections" (WHO 2006) and is the key public health strategy recommended by the WHO to reduce morbidity and transmission of STH.

Operationally, PC is characterized by population-level diagnosis, population-level treatment and implementation at regular intervals (Gabrielli et al. 2011).

- *Population-based diagnosis*
  Population-based diagnosis consists of assessing the significance of STH in a population through surveys applied to a sample of its individuals. Population-based diagnosis can also be carried out retrospectively by analysing existing epidemiological data. Based on its results, the most appropriate frequency of treatment is selected. Population-based diagnosis distinguishes PC from the clinical approach in which diagnosis is performed at the individual level prior to treatment.
- *Population-based treatment*
  In PC, administration of anthelminthic drugs is not the outcome of a personalized, case management treatment approach performed by medical personnel on individuals reporting to health facilities. It rather entails actively targeting population groups at risk (preschool and school-age children, as well as women of reproductive age) with delivery of single administration medicines by both medical and nonmedical personnel (teachers, volunteers or community drug distributors).
- *Implementation at regular intervals*
  PC is implemented at regular intervals of time (once a year or twice a year); the most appropriate retreatment interval is based on the epidemiological characteristics of the disease as measured by the population-based diagnosis; the intervention is repeated without the need for further diagnostic interventions (WHO 2006), although implementation of a monitoring system is recommended.

The WHO recommends implementing PC interventions based on the administration of a single tablet of albendazole 400 mg (200 mg in children aged 12–23 months) or mebendazole 500 mg to the following population groups at risk of morbidity:

Table 11.2 Recommended treatment schedule by category of risk (WHO 2006, 2012a)

Category of risk	Prevalence of any soil-transmitted helminth infection (%)	Treatment schedule
High-risk areas	≥50	Twice a year
Moderate-risk areas	≥20 and ≥50	Once a year
Low-risk areas	<20	None (case-by-case treatment)

- Preschool-age children (aged 1–4 years)
- School-age children (aged 5–14 years)
- Women of reproductive age (aged 15–45 years), including pregnant women in the second and third trimesters and lactating mothers
- Adults professionally exposed to the risk of STH (e.g. tea pickers and miners)

The recommended treatment schedule is determined on the basis on the pre-intervention levels of prevalence of infection with any soil-transmitted helminth species, i.e. *A. lumbricoides*, *T. trichiura* or hookworms (*A. duodenale* and *N. americanus*), in a sample of the school-age population living in the target area (Table 11.2).

Implementing PC interventions to combat STH requires limited expenses; when implemented through schools, deworming 1,000,000 children has been estimated to cost US$72,000 (US$0.072 per child). This estimate includes procurement of albendazole or mebendazole (US$19,000), shipment of the medicines (US $20,000) and all the operational costs, such as distribution of medicines, training of teachers, supervision and monitoring (Montresor 2010; WHO 2012a). When deworming is provided in the context of vaccination and micronutrient campaigns or through maternal and child health services, the additional cost required is usually also limited because the infrastructure and the personnel are already made available by the programme that is piggybacked (Boselli et al. 2011).

11.10.2 Global Goals and Targets

In 2001, the World Health Assembly adopted Resolution WHA54.19, thus committing all endemic WHO Member States to provide regular deworming treatment to all population groups in need, with a minimum goal to reach at least 75% of school-age children at risk of morbidity due to STH by 2010 (WHO 2001).

However, despite the efforts in several endemic countries, the set global goal endorsed was not reached. By 2010, only a third of all children in need of deworming had received appropriate treatment (WHO 2011).

Anticipating this poor performance and the many other challenges faced by NTD programmes globally, in 2007, the WHO convened the first Global Partners' Meeting on NTDs. Some 200 participants attended the event, including representatives of WHO Member States, United Nations agencies, philanthropic foundations,

universities, pharmaceutical companies, international nongovernmental organizations and other institutions dedicated to contributing with their time, efforts and resources to tackle NTDs (WHO 2007). Since then, donors have made significant commitments, drug donation programmes have been set up, and national governments in endemic countries have shown their engagement in implementing and scaling up activities to control and eliminate NTDs in general and STH in particular. The development and the publication of the first WHO roadmap 2012–2020 (WHO 2012b) gave further strength to the partners efforts and provided clear directions for a decade.

More recently, the WHO has identified six global targets for 2030: (1) achieve and maintain elimination of STH morbidity in preschool- and school-age children, (2) reduce the number of tablets needed in PC for STH; (3) increase domestic financial support to PC for STH; (4) establish an efficient STH control programme in adolescent, pregnant and lactating women of reproductive age; (5) establish an efficient strongyloidiasis control programme in school-age children; and (6) ensure universal access to at least basic sanitation and hygiene in STH endemic areas (WHO 2020c).

A significant boost to the public health control of STH is expected to be generated by the successful adoption by the World Health Assembly, through Decision WHA73(33), of the new road map for neglected tropical diseases 2021–2030 on 13 November 2020 (WHO 2020d) and by its launch on 28 January 2021. The road map lists STH among the conditions targeted for elimination as a public health problem. The aim is for 96% of all countries endemic for STH (96/101) to be validated for elimination as a public health problem by 2030. The validation process can be triggered when STH infections of moderate and heavy intensity reach a prevalence of less than 2% of all STH infections detected through the country's monitoring and evaluation system. The WHO is in the process of establishing such process, in line with similar initiatives for other NTDs (WHO 2020e).

11.10.3 Integrated Approach

In all endemic countries and particularly where only limited resources are available, strategies for the control of parasitic infections, including STH, are being reconsidered in order to optimize the use of human and financial resources. Coordination and integration are therefore promoted among programmes dealing with different NTDs and among the health sector and other sectors. Examples include strengthening synergies in the work of health personnel involved in surveillance, data collection, monitoring and evaluation activities or fostering coordination and integration with regard to the use of health infrastructures (e.g. utilizing vaccination campaigns for the distribution of deworming medicines) as well as data reporting systems. This approach has enabled a broader range of health conditions to be tackled jointly and thus more effectively and at affordable and sustainable costs. In

short, integrated disease control entails merging resources, services and interventions at different levels and between sectors to improve the overall health outcome.

Since 1997, the WHO supports and endorses the process of development of integrated plans for the control of NTDs, which also entails approval and adoption by the relevant governments (WHO 1998). This process has experienced a significant acceleration since the release of the guidelines on PC, which promote coordination and integration among activities against four neglected tropical diseases (lymphatic filariasis, onchocerciasis, schistosomiasis and soil-transmitted helminthiasis; WHO 2006) and the availability of large anthelminthic drug donations since 2011.

STH is particularly suitable to integration as the approach to its control is sufficiently flexible to be adapted and adopted to combat other parasitic diseases such as schistosomiasis and lymphatic filariasis or to fit into other ongoing health interventions, such as those aimed at improving maternal and child health, or immunization campaigns. The Global Programme to Eliminate Lymphatic Filariasis (GPELF), based on regular treatment of communities with single administration drugs such as ivermectin and albendazole, also effective against STH, has historically represented an excellent opportunity for integration.

Indeed, control of STH can be a portal of entry for the control of other endemic communicable and non-communicable diseases (WHO 1996). This is the approach that was adopted with success by the Japanese Organization for International Cooperation in Family Planning (JOICFP) which utilized mass treatment for STH to reinforce confidence in the health system and stimulate the interest in family planning and in environmental hygiene (Yokogawa 1985).

Integration of disease control activities can be strengthened by the adoption of a multilayered approach. Integration of parasitic and communicable diseases should be implemented at all levels: inter sectoral (health, interior, agriculture, education), regional, district and primary health-care level. Special efforts should be made to strengthen the intersectoral collaboration and coordination between ministries at central level (e.g. Ministry of Health, Ministry of Education and Ministry of Infrastructure) and the intrasectoral coordination within departments of the Ministry of Health (e.g. between the department responsible for the control of infectious diseases and the one responsible for maternal and child health).

Practical examples of integration of STH control activities within other public health interventions include (WHO 2012a):

- *Deworming school-age children in schools*: The school system offers a well-established logistic framework for the following reasons—(1) in recent years, school enrolment has increased in most developing countries, (2) teachers are educated individuals who can administer anthelminthic medicines even without strict medical supervision and (3) children and their families trust the school and accept health interventions provided through the educational system.
- *Deworming preschool-age children during vaccination and micronutrient campaigns*: Such interventions represent a good opportunity to deworm preschool-age children for the following reasons: (1) adding anthelminthic tablets to the

health package usually increases the overall coverage of the campaign, and (2) health personnel are skilled in providing medicines or medical products to very young children.

- *Deworming women of reproductive age through maternal and child health services*: Maternal and child health services are regularly consulted by pregnant and lactating mothers, and focused health packages, such as iron supplementation, are usually offered to them. The inclusion of anthelminthic medicines into such packages makes sense as STH is known to adversely affect pregnancy and its outcomes.

11.10.4 Complementary Public Health Interventions

Health education and improved sanitation represent two public measures that can be implemented to complement and enhance the impact of PC.

Sanitation aims at reducing the contamination of soil and water by parasite eggs and consequently at decreasing transmission of STH. Even though its impact can take several years to be demonstrated, especially when implemented alone, sanitation is the only intervention that has proven successful in interrupting transmission of STH in those countries that have implemented it widely and managed to cover high proportion of the population living in endemic areas. Because of its high costs and demanding logistics, sanitation is usually not specifically recommended as a tool to control STH, but rather as a by-product of social and economic development that is able to produce a significant impact on a number of diseases, including STH.

Health education aims at reducing the risk of infection through the promotion of a healthy behaviour and lifestyle. When applied to STH, it specifically discourages open-air defecation and promotes the use of latrines and the adoption of hygienic practices, such as washing hands regularly and carefully. Its main target population is represented by school-age children, and dedicated health education packages are included in school health programmes in many developing countries.

A good synthesis of the two above-mentioned complementary public health interventions is offered by community-led total sanitation (CLTS); CLTS is a methodology that aims at mobilizing communities to completely abandon the practice of open-air defecation through education, increased awareness and behavioural change (Harvey 2011); CLTS was first pioneered in Bangladesh and later adopted in a number of developing countries in Asia, Africa and the Americas.

11.10.5 Vaccinology

Historically, efforts to develop a vaccine for STH have focused on hookworms, notably *N. americanus*, as in the case of the US-based Sabin Vaccine Institute

Product Development Partnership (Hotez et al. 2016). So far, however, such efforts have failed.

In principle, there is a strong rationale for developing anthelminthic vaccines: they could be incorporated into the regular child vaccination schedule (that usually achieves good coverage), would avoid the risk of selection of resistant parasites and provide long-term protection, thus avoiding the cost of annual treatment.

However, the development of helminth vaccines has been challenging: the identification of a target antigen has proven a difficult endeavour because of the multiple stage-specific antigens expressed at different phases of the parasite life cycle; in addition, helminths have an exceptional capacity to modulate and reduce host immunity (Zawawi and Else 2020).

For these reasons, a vaccine for STH is not expected to be available before the next decade. Further research would also be required to establish the role of a vaccine as a disease control tool complementary to other recommended interventions such as PC.

11.10.6 Conclusions

Preventive chemotherapy offers an opportunity to efficiently control STH at an affordable cost in endemic countries; in addition, the integration of STH control with disease control and elimination interventions directed against several other NTDs allows the further expansion of health benefits at marginal cost.

Interventions to address STH's burden can be adapted to the ecological and epidemiological characteristics of each endemic area, such as pattern of transmission and rate of reinfection, prevalence and intensity of infection and prevalent parasite species. They can also be adjusted during implementation: this is made possible by the fact that monitoring and evaluation are essential components of the STH control strategy. Through constant operational feedback, managers of helminth control programmes and health planners can therefore quantify the benefits and the costs of the intervention and possibly redirect it on the basis of the outcomes and the health impact produced (WHO 2012a).

STH is a disease of neglected populations, and its public health significance is inversely related to the social status of those affected. For example, widespread adoption of sanitation in endemic areas is likely to result in quick interruption of STH transmission, as it has been the case in Japan, Korea, Italy and other previously endemic countries.

Those countries that are experiencing rapid and significant socio-economic development are in a position to replicate the experience of the formerly endemic countries mentioned above, leading to long-term elimination of the problem, with no need of further interventions. In such contexts, implementation of PC can be gradually scaled down, as sanitation becomes widespread and hygiene practices improve.

In contrast, countries undergoing a slower development or affected by social crises delaying the wider development of infrastructures should ensure that PC is regularly implemented so as to protect children and other priority target groups from the worst consequences of infection. Evidence has shown that a number of countries that started control programmes in the recent past and successfully sustained interventions for a few years managed to obtain significant results in terms of reduction of prevalence of infection and elimination of infections of high intensity, thus controlling STH-associated morbidity (Tun et al. 2013; Casey et al. 2013).

The World Health Organization is currently focusing its efforts on the scale-up of global coverage of PC interventions in all countries affected by STH. This is considered an essential step to guarantee the protection of health among children and women.

References

Anderson RM, May RM (1991) Infectious diseases of humans. Oxford University Press, London

Bangert M, Bancalari P, Mupfasoni D, Mikhailov A, Gabrielli AF, Montresor A (2019) Provision of deworming intervention to pregnant women by antenatal services in countries endemic for soil-transmitted helminthiasis. PLoS Negl Trop Dis 13(5):e0007406

Boselli G, Yajima A, Eswara PA, Feldon KE, Xeuatvongsa A, Chitsavang C, Phengkeo S, Gabrielli AF, Politi C, Montresor A (2011) Integration of deworming into an existing immunization and vitamin A supplementation campaign is a highly cost-effective approach to maximize health benefits in Lao PDR. Int Health 3:240–245

Brudastov AN, Krasnonos LN, Lemelev VR, Kholmukhamedov SK (1970) Invasiveness of Ascaris lumbricoides eggs in guinea pigs and humans after 10 years in soil (Russian). Med Parazitol (Mosk) 39(4):447–451

Bundy DAP, Hall A, Medley GF, Savioli L (1992) Evaluating measures to control intestinal parasitic infections. World Health Stat Q 45(2–3):168–179

Burden DJ, Whitehead A, Green EA, Mcfadzean JA, Beer RJ (1976) The treatment of soil infested with the human whipworm, *Trichuris trichiura*. J Hyg (Lond) 77(3):377–382

Casey GJ, Montresor A, Cavalli-Sforza LT, Thu H, Tinh TT, Tien NT, Phuc TQ, Biggs BA (2013) Elimination of iron deficiency anemia and soil transmitted helminth infection: evidence from a fifty-four month iron-folic acid and de-worming program. PLoS Negl Trop Dis 11(7):e2146

Crompton DW, Nesheim MC (2002) Nutritional impact of intestinal helminthiasis during the human life cycle. Annu Rev Nutr 22:35–59. https://doi.org/10.1146/annurev.nutr.22.120501.134539. Epub 2002 Jan 4

Crompton DW, Savioli L (1993) Intestinal parasitic infections and urbanization. Bull World Health Organ 71(1):1–7

de Silva NR, Brooker S, Hotez PJ, Montresor A, Engels D, Savioli L (2003) Soil-transmitted helminth infections: updating the global picture. Trends Parasitol 19(12):547–551

Gabrielli AF, Ramsan M, Naumann C, Tsogzolmaa D, Bojang B, Khoshal MH, Connolly M, Stothard JR, Montresor A, Savioli L (2005) Soil-transmitted helminths and haemoglobin status among Afghan children in World Food Programme assisted schools. J Helminthol 79(4):381–384

Gabrielli AF, Montresor A, Chitsulo L, Engels D, Savioli L (2011) Preventive chemotherapy in human helminthiasis: theoretical and operational aspects. Trans R Soc Trop Med Hyg 105(12):683–693

Gunn A, Pitt SJ (2012) Parasitology. An integrated approach. Wiley-Blackwell, Chichester

Guyatt HL, Bundy DA, Evans D (1993) A population dynamic approach to the cost-effectiveness analysis of mass anthelminthic treatment: effects of treatment frequency on Ascaris infection. Trans R Soc Trop Med Hyg 87:570–575

Gyorkos TW, Montresor A, Belizario V, Biggs B-A, Bradley M, Brooker SJ et al (2018) The right to deworming: the case for girls and women of reproductive age. PLoS Negl Trop Dis 12(11): e000674

Hall A, Hewitt G, Tuffrey V, de Silva N (2008) A review and meta-analysis of the impact of intestinal worms on child growth and nutrition. Matern Child Nutr 4(Suppl 1):118–236

Harvey PA (2011) Zero subsidy strategies for accelerating access to rural water and sanitation services. Water Sci Technol 63(5):1037–1043

Hotez PJ, Beaumier CM, Gillespie PM, Strych U, Hayward T, Bottazzi ME (2016) Advancing a vaccine to prevent hookworm disease and anemia. Vaccine 34(26):3001–3005

Johnson & Johnson (2019) Johnson & Johnson extends long-standing donation program to support global effort to tackle intestinal worms. https://www.jnj.com/johnson-johnson-extends-long-standing-donation-program-to-support-global-effort-to-tackle-intestinal-worms

Montresor A (2010) Estimation of the cost of large-scale school deworming programmes with benzimidazoles. Trans R Soc Trop Med Hyg 104(2):129–132

Montresor A (2011) Cure rate is not a valid indicator for assessing drug efficacy and impact of preventive chemotherapy interventions against schistosomiasis and soil-transmitted helminthiasis. Trans R Soc Trop Med Hyg 105(7):360–363

Montresor A, Gabrielli AF, Yajima A, Lethanh N, Biggs BA, Casey GJ, Tinh TT, Engels D, Savioli L (2013) Markov Model to forecast the change in prevalence of soil-transmitted helminths during a control programme: a case study in Vietnam. Trans R Soc Trop Med Hyg 107(5): 313–318

Montresor A, Trouleau W, Mupfasoni D, Bangert M, Joseph SA, Mikhailov A, Fitzpatrick C (2017) Preventive chemotherapy to control of soil transmitted helminthiasis averted more than 500 thousand DALYs in 2015. Trans R Soc Trop Med Hyg 111(10):457–463

Montresor A, Mupfasoni D, Mikhailov A, Mwinzi P, Lucianez A, Jamsheed M, Gasimov E, Warusavithana S, Yajima A, Bisoffi Z, Buonfrate D, Steinmann P, Utzinger J, Levecke B, Vlaminck J, Cools P, Vercruysse J, Cringoli G, Rinaldi L, Blouin B, Gyorkos TW (2020) The global progress of soil-transmitted helminthiases control in 2020 and World Health Organization targets for 2030. PLoS Negl Trop Dis 14(8):e0008505

Nokes C, Grantham-McGregor SM, Sawyer AW, Cooper ES, Robinson BA, Bundy DA (1992) Moderate to heavy infections of Trichuris trichiura affect cognitive function in Jamaican school children. Parasitology 104(Pt 3):539–547

Pullan RL, Smith JL, Jasrasaria R, Brooker SJ (2014) Global number of infection and disease burden of soil transmitted helminth infections in 2010. Parasit Vectors 7:37

Stoltzfus RJ, Albonico M, Chwaya HM, Savioli L, Tielsch J, Schulze K, Yip R (1996) Hemoquant determination of hookworm-related blood loss and its role in iron deficiency in African children. Am J Trop Med Hyg 55(4):399–404

Tun A, Myat SM, Gabrielli AF, Montresor A (2013) Control of Soil transmitted helminthiasis in Myanmar. Result of 7 years of deworming. Tropical Med Int Health 18(8):1017–1020

Walker M, Cools P, Albonico M, Ame S, Ayana M, Dana D, Keiser J, Matoso LF, Montresor A, Mekonnen Z, Corrêa-Oliveira R, Pinto SA, Sayasone S, Vercruysse J, Vlaminck J, Levecke B (2021) Individual responses to a single oral dose of 400 mg albendazole against soil-transmitted helminth infections in schoolchildren suggests reduced therapeutic efficacy in areas with high drug pressure. PLoS Negl Trop Dis. (in press)

WHO (2011) Soiltransmitted helminthiases: estimates of the number of children needing preventive chemotherapy and number treated, 2009. Weekly epidemiological record No 25, vol 86, pp 257–266

World Health Organization (1994) Bench aids for the diagnosis of intestinal parasites. 2012 reprint. World Health Organization, Geneva

World Health Organization (1996) Report of the WHO informal consultation on the use of chemotherapy for the control of morbidity due to soil-transmitted nematodes in humans. Geneva 29 April to 1 May 1996. Division of control of tropical diseases. WHO/CTD/SIP.96.2. World Health Organization, Geneva
World Health Organization (1998) Integrating disease control: the challenge. Division of control of tropical diseases. WHO/CTD/98.7. World Health Organization, Geneva
World Health Organization (2001) Resolution WHA54.19. Schistosomiasis and soil-transmitted helminth infections. World Health Organization, Geneva
World Health Organization (2002) Prevention and control of schistosomiasis and soil-transmitted helminthiasis. Report of a WHO expert committee. WHO technical report series 912. World Health Organization, Geneva
World Health Organization (2006) Preventive chemotherapy in human helminthiasis. Coordinated use of anthelminthic drugs in control interventions: a manual for health professionals and programme managers. World Health Organization, Geneva
World Health Organization (2007) A turning point. Report of the global partners' meeting on neglected tropical diseases. World Health Organization, Geneva
World Health Organization (2009) WHO model formulary 2008. Based on the 15th model list of essential medicines 2007. World Health Organization, Geneva
World Health Organization (2012a) Soil-transmitted helminthiasis. Eliminating soil-transmitted helminthiasis as a public health problem in children. Progress report 2001–2010 and strategic plan 2011–2020. World Health Organization, Geneva
World Health Organization (2012b) Accelerating work to overcome the global impact of neglected tropical diseases. A roadmap for implementation. World Health Organization, Geneva
World Health Organization (2013) Assessing the efficacy of anthelminthic drugs against schistosomiasis and soil-transmitted helminthiases. World Health Organization, Geneva
World Health Organization (2016) Global health estimates 2000–2015: disease burden by cause, age, sex, by country and by region, 2000–2015. World Health Organization, Geneva
World Health Organization (2018) Reaching girls and women of reproductive age with deworming. World Health Organization, Geneva
World Health Organization (2020a) Soil-transmitted helminthiases: number of children treated in 2019. Wkly Epidemiol Rec 95(40):629–640
World Health Organization (2020b) Ninth meeting of the working group on monitoring of neglected tropical diseases drug efficacy. Lymphatic filariasis, onchocerciasis, schistosomiasis and soil-transmitted helminthiases. World Health Organization, Geneva
World Health Organization (2020c) 2030 targets for soil-transmitted helminthiases control programmes. World Health Organization, Geneva
World Health Organization (2020d) Road map for neglected tropical diseases 2021–2030. Decision WHA73(33). 13 November 2020. World Health Organization, Geneva
World Health Organization (2020e) Ending the neglect to attain the sustainable development goals: a road map for neglected tropical diseases 2021–2030. World Health Organization, Geneva
Yokogawa M (1985) JOICFP'S experience in the control of ascariasis within an integrated programme. In: Crompton DWT, Nesheim MC, Pawlowski ZS (eds) Ascariasis and its public health significance. Taylor and Francis, London, pp 265–277
Zawawi A, Else KJ (2020) Soil-transmitted helminth vaccines: are we getting closer? Front Immunol 11:576748

Chapter 12
Strongyloides stercoralis and Strongyloidosis

Masataka Korenaga and Fabrizio Bruschi

Abstract Strongyloidosis is a chronic, soil-transmitted, intestinal parasitic disease. *Strongyloides stercoralis* is a roundworm and main causative agent of this disease. *S. stercoralis* has a unique life cycle, which consists of direct (homogonic) development and indirect (heterogonic) development. Parasitic adult females produce both sexes of the next generation parthenogenetically. Female larvae can choose the direct or indirect development depending on various environmental conditions. Autoinfection is one of the characteristic features of this parasite, which causes hyperinfection and disseminated infection. Strongyloidosis occurs mostly in humid tropics and subtropics of more than 70 countries, affecting people between 30 million and 100 million or higher. However, the precise number is not known up to the present, because of difficulties in diagnosis. Even in highly developed countries, like the USA, serious problems have been caused by transmission of *S. stercoralis* through organ transplantation. We describe current status of strongyloidosis with special reference to biology, epidemiology, immunology, and vaccine development.

12.1 Introduction

Strongyloidosis is one of the chronic, soil-transmitted, intestinal helminth infections which affect the health of over one-third of the world population Based on the linear model, analyzing a total of 119 articles with systematic reviews and meta-analyses, the global burden of *S. stercoralis* infections has estimated at 386 million people, including 22 million school-age children (Fleitas et al. 2020).

Despite the great impact on public health at global level, the volume and growth of literature on strongyloidosis are still relatively poor (Sweileh 2019).

M. Korenaga (✉)
Department of Parasitology, Kochi Medical School, Kochi University, Kochi, Japan

Faculty of Health Sciences, Kochi Gakuen University, Kochi, Japan
e-mail: korenaga@kochi-u.ac.jp

F. Bruschi
Department of Translational Research, N.T.M.S., Università di Pisa, Medical School, Pisa, Italy

F. Bruschi (ed.), *Helminth Infections and their Impact on Global Public Health*,
https://doi.org/10.1007/978-3-031-00303-5_12

Strongyloides stercoralis is widespread, mainly in the tropics and subtropics which also contain species naturally infecting humans. Besides this species, *S. fuelleborni fuelleborni* infection in humans has been reported but restricted in Africa and the Southeast Asian country (Pampiglione and Ricciardi 1971; Hasegawa et al. 2010; Thanchomnang et al. 2017) and *S. f. kellyi* in Papua New Guinea (Ashford et al. 1992). The burden of strongyloidosis to humans has been underestimated in an aspect of global health. Strongyloidosis is one of the neglected tropical diseases and perhaps the most neglected disease (Olsen et al. 2009).

In this chapter, we focused mainly on human strongyloidosis together with recent advances of phylogenetic analysis and experimental models relating to human strongyloidosis. The comprehensive review articles regarding strongyloidosis and *Strongyloides* spp. have been published elsewhere (Grove 1989a, b; Sato 2003; Montes et al. 2010; Krolewiecki et al. 2013; Toledo et al. 2015; Viney and Lok 2015).

12.2 The Agent

12.2.1 Life Cycle and Morphology

The life cycle of *S. stercoralis* is unique. Infective third-stage larvae (L3i) penetrate the intact skin of hosts and migrate into the lungs via the bloodstream. The larvae pass the capillary walls and move to the alveoli, bronchus, and trachea and then go down the esophagus via the pharynx. Finally, the larvae molt twice and mature to parasitic females. Adult worms parasitize in the mucosa of the small intestine. The size of the adult worms is 2.1–2.7 (2.4 in average) mm in length and 30.0–40.0 (37.0) μm in width, whereas those of *S. f. fuelleborni* are 2.9–4.2 (3.5) mm in length and 43.0–55.0 (51.0) μm in width (Table 12.1). The ovaries of *S. f. fuelleborni* spiral around the intestine (Little 1966). Parasitic females lay eggs parthenogenetically. The early stages of *S. stercoralis* larvae pass through the gut of the host with feces and develop in the external environment (Little 1966). Female and male first-stage larvae may develop to free-living adults, mate, and reproduce offspring (which become L3i eventually). This type of development is known as heterogonic (indirect). Under certain conditions (temperature, nutrients, pH, etc.), female larvae can take either of two different life cycles: a heterogonic development as above or a homogonic (direct) development. In homogonic development, first-stage rhabditiform larvae molt twice to grow to L3i. L3i are threadlike in shape (filariform), 490–630 (563) μm in length and 15–16 (15.8) μm in width in *S. stercoralis* and 560–680 (616) μm in length and 14–17 (15.8) μm in width in *S. f. fuelleborni*. Filariform larvae are characterized in the notched tip of the tail. Four molts occur in the development of both the parasitic and free-living adults (Little 1966). When second-stage larvae transform within the intestine into L3i, they can penetrate the perianal skin or bowel mucosa to complete their life cycle, which is called an autoinfection. The life cycle of *S. stercoralis* is shown in Fig. 12.1.

Table 12.1 Morphology of *Strongyloides* spp.

Species	Stages	Length (μm)	Width (μm)	Remarks	References
S. stercooralis	Parasitic females	2100–2700 (av. 2420)	30–40 (av.37)	From canines experimentally infected with L3i cultured from patient's feces	Little (1966)
	Filariform L3	490–630 (av. 563)	15–16 (av. 15.8)		
S. stercooralis	Parasitic females	2100–2790 (av. 2465)	40–90 (av. 53)	Intestine recovered from a patient autopsied	Takagi et al. (1973)
	Filariform L3	380–476 (av. 434.5)	12–40 (av. 22.8)	Lungs	
S. fulleborni fulleborni	Parasitic females	2900–4200 (av. 3470)	43–55 (av. 51)		Little (1966)
	Filariform L3	560–680 (av. 616)	14–17 (av.15.8)		
	Filariform L3	568–662 (av. 620)	13–16 (av. 15.2)		Hasegawa et al. (2010)
	Eggs	42–58	32–34	Containing morula to tadpole stage	
S. fulleborni kelly	Parasitic females	3000–4200 (av. 3420)	39–58 (av. 45.3)	From Papua New Guinea	Kerry et al. (1976)
	Filariform L3	610	16		

12.2.2 Phylogenetic Analysis

Genetic diversity of genus *Strongyloides* has been analyzed by Hasegawa et al. (2010) and Barratt et al. (2019), sequencing the hypervariable (HVR)-I and HVR-IV regions of the *Strongyloides* 18S rRNA gene and a fragment of the mitochondrial cytochrome c oxidase subunit 1 (*Cox 1*) gene. Hasegawa et al. (2010) have suggested that isolates of *S. f. fuelleborni* in their study are divided into three groups corresponding to geographical localities but not to host species, while those of *S. stercoralis* are grouped into dog-parasitic and primate-parasitic clades, but not to geographical regions. They have also suggested that *S. stercoralis* has been dispersed by migration and the activities of modern humans. Jaleta et al. (2017) have shown that there exist two populations of *S. stercoralis*, based on the phylogenetic analysis of the nuclear 18S rDNA, the mitochondrial *Cox1* locus, and the whole-genome sequence of the parasites from humans and their canines in northern Cambodia. Interestingly, they have evidenced that one population is canine-specific, but the other one is shared with humans. In accordance with this, Nagayasu et al.

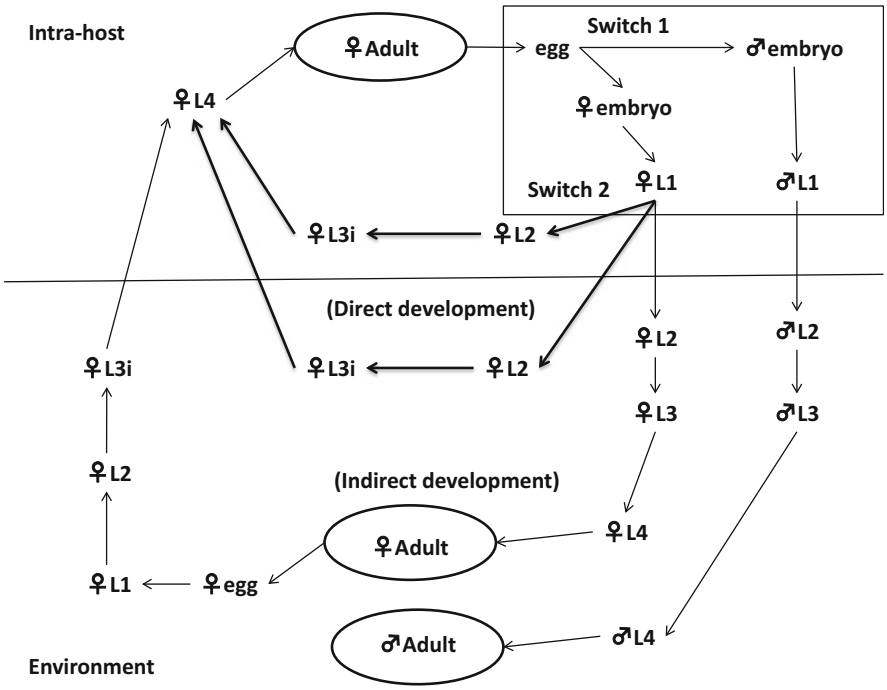

Fig. 12.1 The life cycle of *Strongyloides stercoralis*. The life cycle consists of direct (homogonic) and indirect (heterogonic) developments. Two developmental switches, a sex determination and a female-only developmental choice, have been demonstrated to control the development in *S. ratti* which is close phylogenetically to *S. stercoralis* (Nemetschke et al. 2010; Viney 2006). Such switches might be hypothesized in *S. stercoralis*

(2017) have shown the presence of two distinct lineages of *S. stercoralis* (types A and B), by analyzing mitochondrial *Cox1*, nuclear 18S rDNA, 28S rDNA, and a major sperm protein domain-containing protein genes. Type A parasites were recovered both from humans and canines, although type B parasites were collected only from canines. On the other hand, in northern and western Thailand, Aupalee et al. (2020) have studied on *Cox1*, *SSU* HVR-I, and HVR-IV haplotypes of *S. stercoralis* recovered from human patients, resulting that all worms tested were *S. stercoralis* and phylogenetically very close to other specimens from the Southeast Asian peninsula. Prior to these studies, phylogenetic analyses of SNP (Kikuchi et al. 2016) have clearly shown that unambiguous geographical separation and subpopulations correlate with the host geographical origin and the parasites were collected in Okinawa, Japan (non-prevalent area), and in Htanbin, Myanmar (prevalent area). Whole-genome amplification and next-generation sequencing techniques detected nearly 30,000 variant positions when compared with the reference genome (US strain). They have suggested that the relatively higher heterozygosity in the genomes of the Japanese samples could be explained by the independent evolution

of two haplotypes of diploid genomes through asexual reproduction during the autoinfection cycle.

12.2.3 Mechanisms of Development

The temperature-sensitive developmental switch was demonstrated clearly to be controlled by the neuron pair ALD (amphidial neuron/lamellar dendrite, cell body "D") (Nolan et al. 2004). Sensing the environment is the function of the amphidial neurons, serving as thermoreceptors similar to neuron pair AFD in *Caenorhabditis elegans* (Mori and Ohshima 1995). Nolan et al. (2004) showed that first-stage larvae enter the homogonic development at temperature of 34 °C and above, whereas larvae enter the heterogonic pathway and develop to free-living adult worms at temperature below 34 °C. These results coincide with former observation that some larvae developed to the infective third-stage larvae when passage along the gut was delayed in an experimental canine model (Nishigori 1928). Thus, internal development to infectivity makes autoinfection possible (Schad 1989). Autoinfection continues throughout a lifetime of the hosts. Persistent infections for 40 years long have been recorded, for example, as "war strongyloidosis" from various countries (Pelletier et al. 1988; Suzuki et al. 1989; Robson et al. 2009).

In the heterogonic development, eggs reproduced develop only into L3i (Yamada et al. 1991). It has been suggested that *S. stercoralis* free-living females reproduce by automictic thelytoky and pseudogamy (Hammond and Robinson 1994). In contrast, Jeleta et al. (2017), analyzing 18S rDNA [small subunit (*SSU*)] haplotypes, have suggested that reproduction in the free-living generation of both wild and laboratory isolates of *S. stercoralis* is sexual and not pseudogamic. Molecular biology and genomics of *Strongyloides* spp. are reviewed elsewhere (Charlesworth 2010; Nemetschke et al. 2010; Streit 2008; Viney 2006; Jaleta and Lok 2020).

S. stercoralis L3i was shown to be strongly attracted to an extract of mammalian skin. The active component in the skin extract was urocanic acid, which is abundant in mammalian skin and skin secretions. The attractant activity of urocanic acid was inhibited by divalent metal ions. This suggests possibility to develop an inexpensive, practical, topical preventive for use on exposed body surfaces in people at risk of infection with *S. stercoralis* (Safer et al. 2007).

Metalloproteinases play roles widely in parasitism, ranging from tissue penetration, digestion of host tissue for nutrition and evasion of host immune responses, and developmental molts of larvae (Tort et al. 1999). With several *Strongyloides* spp., a proteinase activity was implicated in skin penetration by the larvae (Lewert and Lee 1954; Gallego et al. 2005). Cysteine and metalloproteinases were active during the skin penetration process (Dresden et al. 1985; Rege and Dresden 1987). With *S. stercoralis*, the larvae rapidly penetrated the dermal extracellular matrix with the aid of a secreted, neural metalloproteinase (McKerrow et al. 1990). An astacin-like metalloproteinase transcript was reported from the infective larvae of *S. stercoralis* (Gallego et al. 2005). The *S. stercoralis* metalloproteinase has been

designated as strongylastacin depending on the results of phylogenetical and structural analysis (Gallego et al. 2005).

Hallem and her colleagues (Gang et al. 2017) have demonstrated the applicability of CRISPR/Cas9-mediated mutagenesis to *S. stercoralis* with the aim to study the gene function. Using this targeted mutagenesis, they have clearly shown that the *S. stercoralis* (Ss) *tax-4* gene (cGMP-gated cation channel subunit gene) highly conserved in sensory neurons of nematode is required for attraction to a human-emitted odorant and in-host development (Gang et al. 2020). The knockout of Ss-tax-4 gene was shown to affect the normal thermotaxis in *S. stercoralis* (Bryant et al. 2018).

The reader is referred to a comprehensive review where Lok et al. (2017) give us knowledge on genetics and transgenesis in *Strongyloides* spp.

12.3 Epidemiology of Infection

Strongyloidosis occurs mostly in humid tropics and subtropics of more than 70 countries (Olsen et al. 2009). The precise number of individuals infected with the parasite is not known up to the present, because the prevalence obtained in each research depends on sensitivity and specificity of the methodology applied. Several reports, however, give us current epidemiological status showing the worldwide spread of strongyloidosis (Table 12.2). These figures imply that the number of population suffering strongyloidosis is more than we imagined. Most of them live in conditions of poor hygiene. Wang et al. (2013) reviewed that most of the patients with strongyloidosis in China were peasants or field-workers and that evident clustering in families in rural areas (e.g., Guangdong and Guangxi Provinces, etc. in southern China) was seen when they examined cumulative cases and distribution of strongyloidosis during 1973–2011.

Some authors suggested a possible cross transmission of *Strongyloides* between humans and dogs (Thamsborg et al. 2017).

Strongyloidosis poses a serious problem even in industrialized countries, like the USA (Concha et al. 2005) and western Europe (Winnicki et al. 2018). Transmission of *S. stercoralis* has occurred through organ transplantation in the USA. The donor was from a Caribbean endemic area. The kidneys, pancreas, liver, and heart were transplanted. This fact emphasizes the importance of considering the possible occurrence of donor-derived infection with *S. stercoralis*, although the most relevant problem in organ transplant recipients is represented by reactivation of chronic infection after initiation of immunosuppressive treatment (Hasan et al. 2013). Two cases with strongyloidosis were recorded on 1046 kidney and 708 liver transplant recipients registered in four medical centers in Brazil from 2001 to 2006 (Batista et al. 2011). Fabiani et al. (2017, 2018) have reviewed this topic in both stem cell solid organ transplanted patients, and Fabiani and Bruschi (2014) have reviewed this issue in rheumatologic patients treated with biological drugs. Expanded infectious disease screening program was done in the USA for Hispanic transplant candidates

Table 12.2 Prevalences of strongyloidosis in various regions and/or countries in the world

Regions and/or countries	No. of subjects surveyed	No. of positives	%	CI(95%)	Year of survey	Methods of survey	Reference
Oran, Argentina	228 patients	67	29.4		2007	Agar plate, Baermann, sedimentation-conc Harada-Mori	Krolewiecki et al. (2010)
	262 patients	214	81.7		2007	NIE-LIPS	
Rome, Italy a large teaching hospital	4695 Italian	2	0.04		2006–2008	Agar plate	Masucci et al. (2011)
	656 Non-Italian	2	0.3				
Rural area, Brazil	ND	ND	4.8		1999–2009	Parasitological methods	Paula and Costa-Cruz (2011)
Urban area, Brazil	ND	ND	5				
Eastern Uganda	113 mothers	9	8	3.7–14.7	2009	Baermann	Sousa-Figueiredo et al. (2011)
	213 pre-school children	8	3.8	1.6–7.3			
	120 mothers	88	73.3	64.5–81.0		ELISA	
	225 pre-school children	61	27.1	21.4–33.4			
North-east, Poland	120, 5 months to 18 years old	7	5.83		2008–2009	Decantation	Zukiewicz et al. (2011)
Northern Laos	14 households × 6 villages household members >6 years old randomly selected	ND	8.9	7.4–10.4	2009	Formalin-ether-concentration	Conlan et al. (2012)

(continued)

Table 12.2 (continued)

Regions and/or countries	No. of subjects surveyed	No. of positives	%	CI(95%)	Year of survey	Methods of survey	Reference
41 GeoSentinel Clinics in 19 countries	854 children (<18 years old)	40	4.7		1997–2009	ND	McCarthy et al. (2013)
	6751 adult (>19 years old)	344	5.1				
	international migrants[a]						
Flores Island, Indonesia semi-urban area	675, 18–80 years old	5	0.7		2009	qPCR	Wiria et al. (2013)

CI confidence intervals, *ND* not described

[a]Diagnoses with strongyloidosis by region of migrant origin were of 7% in Southeast Asia ($n = 1200$), 3% in South Asia ($n = 844$), 6% in North Africa ($n = 503$), 4% in East Africa ($n = 1253$), 5% in West Africa, and 5% in South Africa ($n = 698$)

(recipients) between 2006 and 2008, minimizing the risk of posttransplant infectious complications. Of 83 patients screened, most were from Mexico (74.7%) and others from Ecuador, Puerto Rico, and Peru. The seropositive rate was 6.7% for *S. stercoralis* (Fitzpatrick et al. 2010).

Roxby et al. (2009) have warned that physicians in the USA often miss opportunities to identify patients with chronic strongyloidosis and stressed an importance of screening and treatment before transplantation. Repetto et al. (2010) also suggested the need to include strongyloidosis as a presumptive diagnosis in patients with past risk of infection and especially if they develop eosinophilia although not originating from endemic areas. Based on mortality data during 1991–2006 in the USA, a population-based case-control study showed that strongyloidosis caused 347 deaths (0.79 per 10 million deaths, 14–29 deaths per year) and that strongyloidosis deaths were related with chronic obstructive pulmonary disease (COPD) and infection with human immunodeficiency virus (HIV). However, in the second half of the study period (1999–2006), strongyloidosis deaths were associated only with HIV infection (Croker et al. 2010).

12.4 The Host Response to the Parasite and Immunopathological Processes

Human strongyloidosis varies from a chronic but limited infection in normal hosts to hyperinfection in patients treated with corticosteroids, with alcoholism or HTLV-1 coinfection. Khieu et al. (2013) have reported that one-third of schoolchildren, who were treated with ivermectin for *S. stercoralis* infection at baseline survey on 2009 in Cambodia, had been reinfected at a follow-up study on 2011. They showed that 68.5% of infected children remained free of the infection for at least 2 years after ivermectin treatment. How human strongyloidiasis is controlled and how various factors affect this control have not been resolved completely. Pathophysiological aspects in human strongyloidosis were reviewed extensively by Genta and Caymmi Gomes (1989) and Toledo et al. (2015). Patients with chronic strongyloidosis had parasite-specific IgE antibodies (Genta et al. 1983). Total IgE levels were above 200 IU/mL in 10 of the 15 patients examined (66.7%), and eosinophilia in peripheral blood was seen in 73.3% of the patients (Genta et al. 1983). Eotaxin and IL-5 serum levels were found significantly increased in patients with strongyloidosis (Mir et al. 2006). The antigen-specific Th2 responses are protective against helminth infections including *Strongyloides* spp. In relation to this, the role of basophils was reported: basophils derived from mice infected with *S. venezuelensis* produce spontaneously in vitro IL-4, IL-6, and IL-13, along with IL-3. They express MHC class II and induce the development of naïve $CD4^+$ cells into Th2 cells (Yoshimoto et al. 2009).

The number of basophils increases in blood and mesenteric lymph; these cells resulted able to control early intestinal parasite expulsion, during intestinal infection with *Strongyloides ratti* in mice (Reitz et al. 2017, 2018).

Larvae of *S. stercoralis* possess collagenase-like and other proteolytic activities (Rege and Dresden 1987; Mckerrow et al. 1990; Brinley et al. 1995). Penetration by *Strongyloides* larvae caused alteration of the extracellular glycoprotein-containing materials of the skin, especially in the basement membrane. The larvae were able to pass through the basement membrane easily and to reach within the dermis 3 min after they were placed on the skin in an experimental rodent model using *Strongyloides ratti* (Lewert and Lee 1954).

Immune responses caused by larval penetration/migration are an important study subject. Recently, tissue factors (TFs) have been considered important for initiating innate and adaptive responses. Thymic stromal lymphopoietin (TSLP) is one of the TFs, an interleukin 7 (IL-7)-like cytokine. TSLP is expressed mainly by epithelial cells at barrier surfaces (the skin, gut, and lungs) (Ziegler and Artis 2010). Myeloid dendritic cells (DCs) express TSLP receptor and IL-7 receptor-α (Reche et al. 2001). Since parasitic infections cause epithelial damage, it might be suggested that TSLP expression is induced through the protease-activated receptor pathway (Demehri et al. 2009). TSLP can drive a Th2 response, potentially through effects on DCs, granulocytes, natural killer (NK) cells, and CD4+ T cells (Ziegler and Artis 2010). TSLP was shown to promote protective immunity to *Trichuris muris, Nippostrongylus brasiliensis, Heligmosomoides polygyrus,* and *Schistosoma mansoni* in mice, but the role in protective immunity to *S. stercoralis* still remains uncertain (Ziegler and Artis 2010).

Trefoil factor 2 (TFF2) produced by epithelial cells has a critical role in their wound healing during larval migration through the lungs in mice infected with *N. brasiliensis*, a rodent nematode which is very similar to hookworm (Wills-Karp et al. 2012). This factor regulates interleukin-33 (IL-33) production by epithelial cells. This cytokine stimulates IL-5 production resulting in eosinophilia, contributing to protective immunity against *S. venezuelensis* in mice (Yasuda et al. 2012). IL-5 and/or eosinophils induced by IL-5 were shown to be involved in reducing susceptibility and/or fecundity in a primary infection with *S. ratti* (Ovington et al. 1998; Watanabe et al. 2003) and *S. venezuelensis* (Korenaga et al. 1994) in mice, while duration of the infection is similar in normal and IL-5-deficient mice. IL-5 was shown to be critical for the protective immunity to migrating larvae in a secondary infection with *S. ratti* (Watanabe et al. 2003) and *S. venezuelensis* (Korenaga et al. 1991) in mice, but not for adult worm expulsion from the gut.

Granulocytes are also crucial for the host's early defense against larval *S. stercoralis* (Galioto et al. 2006) and migrating larvae of *S. ratti* (Nawa et al. 1988; Watanabe et al. 2000). A histopathological study indicated that migrating larvae of *S. ratti* at the inoculation site are surrounded by neutrophils and eosinophils at 12–24 h after infection (Dawkins et al. 1981). Motile *S. ratti* larvae were shown to stimulate neutrophils' release of eosinophil chemotactic factor (ECF). Neutrophils were considered to be an important source of ECF, responsible for eosinophil accumulation around the larvae (Owhashi et al. 1986). Furthermore, eosinophil chemoattractants are produced by larval *S. stercoralis.* The chemoattractants are both protein and chitin that are major components of nematode cuticle, stimulating multiple receptors on the eosinophil surface (Stein et al. 2009).

Classical NK cells and T-bet+ cells (ILC1), Th2-type innate lymphocytes (ILC2), and retinoid-related orphan receptor γ^+ ($ROR\gamma^{+)}$ lymphoid tissue inducer-related cells (ILC3) have distinct roles in innate immune responses, producing Th1, Th2, and Th17 cytokines, respectively (Koyasu and Moro 2012). Th2-type innate lymphocytes include natural helper cell (NH cell) (Moro et al. 2010), nuocyte (Neill et al. 2010), innate helper 2 cell (Ih2) (Price et al. 2010), and multipotent progenitor type 2 cell population (MPP^{type2}) (Saenz et al. 2010). Recent evidences indicate an involvement of ILC2 in the early phase of following Th2-type responses in murine helminthiasis models (Maizels et al. 2012).

Toll-like receptors (TLRs) on dendritic cells and other various cells recognize invading pathogens through pathogen-associated molecular patterns (PAMPs) during both innate and adaptive responses (Akira et al. 2001). Among them, TLR4 is critical for protective adaptive immunity to migrating larvae of *S. stercoralis* in murine model. TLR4 is expressed on the surface of neutrophils. TLR4 has been shown to be required for activating neutrophils in mediating larval killing but not for T- and B-cell function (Kerepesi et al. 2007). Since the first report of Abraham (1995), his group has published excellent papers on protective immunological mechanisms against *S. stercoralis* using an innovative method consisting in a diffusion chamber containing L3i implanted subcutaneously in mice, to assess in vivo survival rates of larvae. This allowed to identify the different factors involved in protective immunity against *S. stercoralis* (Table 12.3). Refer to an excellent review of Bonne-Année et al. (2011).

A macrophage migration inhibitory factor (MIF) is one of the cytokines identified originally as an inhibitor of the random migration of macrophage. It regulates both innate and adaptive immune responses and inflammation (Nishihira 2012). L3i of *S. ratti* secretes MIF (*Sra*-MIF) which binds monocyte/macrophage lineage to induce IL-10 but not TNF-α production. Sequence analysis of the full-length cDNA of the parasite-derived cytokine indicated the highest homology to *S. stercoralis* (Younis et al. 2012). There is a possibility that MIF derived from *S. stercoralis* might regulate host immune responses.

It is hard to analyze immunological and inflammatory responses of the adult stage of *S. stercoralis*, due to the lack of adequate experimental systems except an immunosuppressed canine model (Schad et al. 1984). Although rodents are not definitive hosts for *S. stercoralis*, a Mongolian gerbil (jird) (*Meriones unguiculatus*) infection model in which the parasite can develop to the adult stage has been used to analyze hyperinfection of *S. stercoralis* (Nolan et al. 1993, 1995). Autoinfection occurs only when the intestinal population of the first-stage larvae was very large in the jird model (Nolan et al. 2002).

A significant improvement of this model was obtained by Charuchaibovorn et al. (2019) as regards the potential usefulness of gerbils infected with a human isolate of *S. stercoralis*.

Finally, in general, regions of developing countries with high parasitic infection rates have a reduced incidence of autoimmune diseases relating to Th1 immune responses and/or CD4+ regulatory T-cell function. Chronic liver diseases such as primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH) are thought to have

Table 12.3 Factors of protective immunity against *S. stercoralis*

Innate immunity	Adaptive immunity	References
	Granulocytes (Neutrophils, Eosinophils)	Brigandi et al. (1996)
	Compliment (C3)	
	IgM	
	Granulocytes, Eosinophils	Rotman et al. (1996)
	Eosinophils	Brigandi et al. (1997)
	IgM	
	CD4, IL-4, IL-5	Rotman et al. (1997)
	rIL-12 (suppress immunity)	
	Eosinophils	Brigandi et al. (1998)
IL-5, Eosinophils	IgM (induced by IL-5)	Herbert et al. (2000)
B cells (-)	B-1 cells (IgM)	Herbert et al. (2002a)
	IgM, IgG, Complement (C3)	Ligas et al. (2003)
	Granulocytes (Neutrophils)	
	IL-5 (-)	
	Human IgG, Complement (C3)	Kerepesi et al. (2004)
	Granulocytes	
	IgA + IgE + IgM (-)	
	IL-5 + Eosinophils (-)	
	Ab dependent cytotoxicity (-)	
Eosinophils (CCR3)	Neutrophils (CXCR2)	Galioto et al. (2006)
Neutrophils (CXCR2)		
Eosinophils (Ag presenting)	Eosinophils (Ag presenting)	Padigel et al. (2006)
C3	C3, C3a	Kerepesi et al. (2006)
C5 (-)	C5 (-)	
TLR4 (-)	TLR4	Kerepesi et al. (2007)
	PEC (Neutrophils?)	
Eosinophils (Ag presenting)	Eosinophils (Ag presenting)	Padigel et al. (2007a)
	Gαi2 protein signaling	Padigel et al. (2007b)
	(Neutrophil recruitment)	
	Immune serum	
MPO (Neutrphils)	MPO (Neutrophils)	O'Connell et al. (2011a)
MBP (Eosinophils)		
IL-17A (-), IL-17F (-)	IL17A (-), IL17F (-)	O'Connell et al. (2011b)
CXCR2 (Neutrophil recruitment)	CXCR2 (Neutrophil recruitment)	

(-) not essential

an autoimmune basis to their pathogenesis (Aoyama et al. 2007). A particular situation to study is represented by Okinawa prefecture in Japan which is endemic for strongyloidosis. Aoyama et al. (2007) examined the relationship between autoimmune liver diseases and *S. stercoralis* infection. They found that the frequency of *S. stercoralis* infection in the autoimmune liver disease group (1%) was significantly lower than that in the control group (7%). It might be postulated that the pathogenesis of autoimmune liver diseases is modulated by *S. stercoralis* infection through Th1–Th2 cross-inhibitory process and/or induction of CD4+ regulatory T cell which produce IL-10 and transforming growth factor-β (Aoyama et al. 2007).

12.5 Clinical Manifestations and Prognosis in Immunocompetent and Immunocompromised Patients

Morbidity caused by *S. stercoralis* infection ranges from asymptomatic light infections to severe and often fatal clinical manifestations. Symptoms are abdominal pain, anorexia, nausea with or without vomiting, diarrhea, constipation, pruritus ani, urticaria, larva currens, chest pain, dyspnea, weight loss, malaise, and nervousness (Grove 1989a). Severe infections produce various manifestations depending on the intensity of infection, the organs involved, and the presence or absence of secondary bacterial infection (Grove 1989a). Disseminated infection is related to the migrating larvae to the organs beyond the range of the normal migratory route and is often complicated by Gram-negative sepsis (Kishimoto et al. 2008).

Chronic strongyloidosis is sustained by a relatively low and stable number of adult worms by means of well-regulated autoinfection. When the stable interaction between the parasite and host is impaired, an increasing number of autoinfective larvae complete the life cycle, and the population of adult worms increase. This status is called hyperinfection (Siddiqui et al. 2006). Since Purtilo et al. (1974) described 32 cases hyperinfected by *S. stercoralis*, its association with host immunosuppression has become recognized (Grove 1989a). Those patients showed depression of cell-mediated immunity, protein-calorie malnutrition, malignant conditions (carcinoma, lymphoma, leukemia, etc.), and chronic illnesses (tuberculosis, syphilis, lepromatous leprosy, etc.). Hyperinfection has been described in various reports in patients receiving renal transplantation or affected by systemic lupus erythematosus, nephritic syndrome (Grove 1989a), rheumatoid and bronchial asthma (Altintop et al. 2010), hypogammaglobulinemia (Seet et al. 2005), and malignant lymphoma (Suzuki et al. 1989; Abdelrahman et al. 2012). These diseases/clinical conditions are treated with corticosteroids and other immunosuppressants or can cause immunosuppression by themselves (Grove 1989a). It has been hypothesized, but not proven, that hyperinfection might be mediated through steroid hormone receptors in *S. stercoralis* larvae (Siddiqui et al. 2000b).

IgG subclasses in the humoral response to *S. stercoralis* were examined in 20 patients with uncomplicated strongyloidosis and 21 immunocompromised patients with extraintestinal disease (hyperinfection). Specific IgG2 and IgG4 levels were significantly higher in immunocompetent than in immunocompromised patients. Especially IgG4 response was prominent. By immunoblotting, there was no difference in parasite antigens which were recognized by antibodies of sera from either immunocompetent or immunocompromised patients with strongyloidosis (Genta and Lillibridge 1989).

The first report indicating an association between *S. stercoralis* infection and human T-lymphotropic virus 1 (HTLV-1) infection was done by Nakada et al. (1984). HTLV-1 infection in certain individuals coinfected with *S. stercoralis* might cause an immunological unbalance which favors the parasite (Newton et al. 1992; Satoh et al. 2002a). In fact, the coinfection with HTLV-1 decreases IL-5 levels, peripheral eosinophil counts, and IgE responses consistent with a relative switch from Th2 to Th1 response (Hirata et al. 2006; Porto et al. 2001) while expanding the regulatory T-cell subset (Montes et al. 2009). Furthermore, *S. stercoralis* infection induces polyclonal expansion of HTLV-1-infected cells through activating the IL-2/IL-2R system (Satoh et al. 2002b). Thus, host's immune systems seem to be modulated by coinfection with *S. stercoralis* and HTLV-1. It has been suggested that regulatory T cells play an important role in susceptibility to *S. stercoralis* hyperinfection (Montes et al. 2009).

HTLV-1 infection promotes Th1 responses, like serum levels of IFN-γ and TNF-α. It has been observed that the levels of IFN-γ and TNF-α were higher in subjects with HTLV-1 without strongyloidosis than in subjects coinfected with HTLV-1 and *S. stercoralis*. Further, an increase of TNF-α in serum level was observed after strongyloidosis was treated. On the contrary, the serum levels of sIL-2R decreased after anthelminthic treatment, although subjects with HTLV-1 without *S. stercoralis* had higher serum levels of sIL-2R (Salles et al. 2013). In a case report of *S. stercoralis* hyperinfection in a subject with HTLV-1, an increase of about 19 times in IL-17 level has been observed following the parasitological cure, in addition to decrease in eosinophil counts, both total and specific IgE level and specific IgG1 level (De Souza et al. 2018). In accordance with these, Montes et al. (2009) have demonstrated that population of regulatory T cells (CD4+CD25+-FoxP3+ cells) are increased in subjects with HTLV-1 and *S. stercoralis* coinfection and correlate with both low circulating eosinophil counts and reduced antigen-driven IL-5 production. They have suggested that regulatory T cells play a significant role in susceptibility to the parasites, resulting in the hyperinfection syndrome. It has been shown that expression of IL-17 mRNA is induced in HTLV-1-infected T-cell lines (Duc Dodon et al. 2004). In addition, increased proportions of regulatory T cells in strongyloidosis and HTLV-1-coinfected patients have been observed when compared to those of regulatory T cells in HTLV-1-infected patients (Montes et al. 2009).

An immunohistochemical study by Malpica et al. (2019) has shown that the number of stained regulatory T cells (FoxP3+) increased in number in duodenal biopsies obtained from strongyloidiasis and HTLV-1-coinfected patients. They have

also observed that CD3+, CD8+, or IgE+ lymphocytes decreased in number in areas adjacent to parasites compared to non-adjacent areas and that eosinophils in the mucosa are infiltrated with the same tendency as those cells. They suggest that the role of regulatory T cells might be critically important to downregulate local effector responses against the parasites. These findings might support the hypothesis that regulatory responses in coinfection with HTLV-1 and *S. stercoralis* are evoked not only systemically but also locally in duodenal mucosa at the site of adult worms and production of larvae. As a result, regulatory responses generated by the coinfection might diminish host's protective immunity against the parasites (Montes et al. 2009; Malpica et al. 2019).

Coinfection with HIV and *S. stercoralis* is common in endemic areas. However, HIV infection is not always a cause for disseminated strongyloidosis and hyperinfection syndrome (Lucas 1990). HIV-associated immune reconstitution disease (IRD) is the clinical presentation or deterioration of ongoing opportunistic infections that results from enhancement of pathogen-specific immune responses among patients responding to antiretroviral treatment (ART) (Lawns and Wilkinson 2006). The number of reports of IRD associated with parasitic diseases (leishmaniasis, toxoplasmosis, schistosomiasis, and strongyloidosis) has been increasing (Kim and Lupatkin 2004; Lanzafame et al. 2005; Lawns and Wilkinson 2006). IRD develops when immune responses suppressed markedly by HIV are rapidly restored during ART. In cases of disseminated strongyloidosis and hyperinfection syndrome in HIV patients, a relation between $CD4^+$ T cell and parasite's developmental pathway seems to be most important. Interestingly, significant negative correlations were shown between $CD4^+$ cell counts and the proportions of free-living male and female worms. Homogonic development of *S. stercoralis* seems to be favored in individuals with preserved immune function (Viney et al. 2004).

In contrast to these, no cases of hyperinfection syndrome have occurred in an urban US AIDS cohort studied by Nabha et al. (2012), with the exception of a few patients with signs and symptoms referable to *Strongyloides*-associated IRD following ART. However, HIV-infected patients remain at risk of hyperinfection with *S. stercoralis*, when receiving corticosteroids to treat *Pneumocystis jirovecii* pneumonia, extrapulmonary tuberculosis, and so on. HIV-positive immigrants from endemic areas should be screened systemically for strongyloidosis (González et al. 2010; Llenas-García et al. 2012; Mascarello et al. 2011).

12.6 Diagnosis (Inclusive Histopathology)

12.6.1 Microscopic Examination and Histopathology

Detection of *S. stercoralis* larvae can be done by microscopic examination of feces, duodenal aspirates, or bronchoalveolar lavage. A filter paper method is useful to recover filariform larvae for identification of the parasites. Using an agar plate (Fig. 12.2), fecal cultures can increase the sensitivity even if larvae are low in

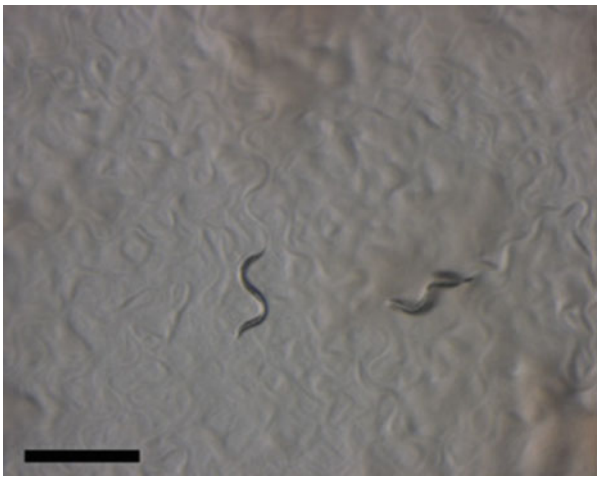

Fig. 12.2 Motile larvae of *Strongyloides venezuelensis* and furrows seen on agar plate. A bar indicates 0.5 mm in length

number in feces examined (Arakaki et al. 1990; Ines et al. 2011; Kaminsky 1993; Machicado et al. 2012; Salazar et al. 1995). When compared the efficacy of four different methods (direct fecal smear, formalin-ether concentration, Harada-Mori filter paper culture, and agar plate culture), the agar plate culture (using 3 g of feces) was highly effective (Sato et al. 1995). Results of a single stool examination by the use of conventional technique fail to detect larvae in up to 70% of cases (Siddiqui and Berk 2001). Even when the examinations were repeated daily for 3 days, the reconfirmation rate was 51.5% by the direct smear and 45.5% by the concentration method (Sato et al. 1995). These results indicate that it is difficult to detect *S. stercoralis* larvae in stool specimens because the majority of cases involve chronic low-level infection (Sato et al. 1995).

Khieu et al. (2013) conducted a cross-sectional study in 458 children from four primary schools of semirural villages in Cambodia, using agar plate culture (for a hazelnut-sized stool sample) and Baermann techniques (for a walnut-sized stool sample) on three stool samples. The sensitivity of agar plate culture and Baermann was 88.4% and 75.0%, respectively. The negative predictive values were 96.4% and 92.5%, respectively. The estimated prevalence according to a model of Marti and Koella (1993) was 24.8% of the study population. The cumulative prevalence increased from 18.6% with a single test to 24.4% after analyzing three stool samples. This figure was close to the Marti and Koella model's true prevalence. Khieu et al. (2013) suggested that the examination of multiple stool samples with different diagnostic methods is required to reach a reliable estimate of the prevalence in the absence of a gold standard.

Histological examination of duodenal or jejunal biopsy specimens might reveal adults and/or larvae embedded in the mucosa. Kishimoto et al. (2008) clearly showed that observation and biopsy from a total of 25 cases by an esophagogastroduodenoscopy (EGD) were effective tools for diagnosing strongyloidosis, besides gastroduodenal drainage and stool analyses. Abnormal endoscopic

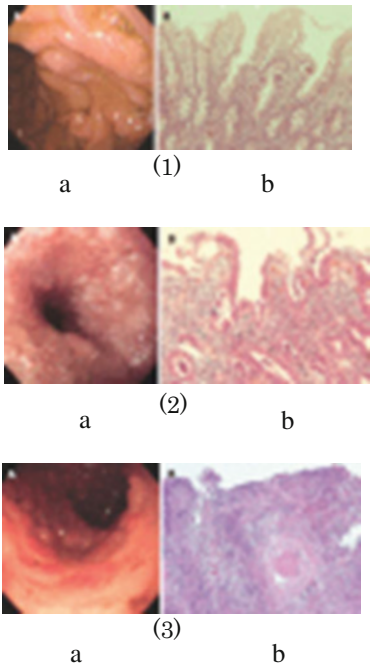

Fig. 12.3 Endoscopic and histopathological observations on the duodenum of *Strongyloides stercoralis* hyperinfection. (1) (a) Endoscopic image showing white villi and edematous mucosa in the second part of the duodenum, (b) Biopsy specimen from the mucosa showing numerous larvae with villous atrophy and mild inflammatory cell infiltration (HE staining, ×200); (2) (a) Endoscopic image showing white villi and stenosis in the second part of the duodenum, (b) Biopsy specimen from the mucosa showing numerous larvae with severe villous atrophy and moderate inflammatory cell infiltration (HE staining, ×200); (3) (a) Endoscopic image showing large ulcers and pseudopolyps in the second part of the duodenum, (b) Biopsy specimen from the margin of the ulcer showing formation of granulation tissue and complete destruction of the villi. Numerous larvae are observed within the granulation and lymph vessels (HE staining, ×100). By courtesy of Kishimoto et al.: World Journal of Gastroenterology 14(11): 1768–1773, 2008. The publisher and Hokama (correspondent author) gave us permission

findings in the duodenum were edema (69.5%), white villi (56.5%), erythema (39.1%), erosion (26.0%), stenosis (17.3%), fine granule (17.3%), hemorrhage (13.0%), dilatation (13.0%), and ulcer (8.6%) (Fig. 12.3, after Kishimoto et al. 2008). The histopathological changes in fatal cases were classified into three categories (De Paola et al. 1962). First, catarrhal enteritis is a minor form characterized by mild mucosal congestion with larvae restricted to the mucosal membrane. Second, edematous enteritis is a moderately serious form characterized by edematous thickening of the wall, swelling folds, and villous atrophy with larvae invading lymph vessels. Third, ulcerative enteritis is a serious form characterized by ulcers and fibrosis. Larvae were found in the entire wall.

S. stercoralis infection disturbs the mucosal integrity and compromises the intestinal barrier. Infection is associated with high apoptosis rates concomitant

with low cell proliferation in duodenal and jejunal biopsies. The proliferative index is significantly reduced in patients compared to controls in both duodenal and jejunal biopsies, using an immunostaining method with Ki-67 which identifies cells in different cell-cycle phases (Werneck-Silva et al. 2006).

12.6.2 Serological Diagnosis

Serological tests have been developed to detect antibodies against *S. stercoralis* crude (CrAg), purified or recombinant antigens.

Indirect immunofluorescence using larval *S. stercoralis* antigen showed a 92% positivity for IgG antibodies with no cross reactivity to *Schistosoma mansoni*, *Loa loa*, or hookworm or in patients with idiopathic hypereosinophilia. A weak positivity was found in Bancroftian filariasis patients (Genta and Weil 1982). Relatively low molecular weight proteins (41, 26, and 22 kDa or 41, 31, and 28 kDa) from larval *S. stercoralis* were shown to be reactive to IgG and to be applicable for immunodiagnostic tools such as enzyme-linked immunosorbent assay (ELISA) and immunoblotting (Sato et al. 1990; Conway et al. 1993). Highly immunodominant 41-kDa antigen (P5) exhibited immunoreactivity with 83% of patients with strongyloidosis. Sequential analysis showed that P5 antigen is γ-subunit of isocitrate dehydrogenase (NAD^+) (Siddiqui et al. 2000a).

Although ELISA using larval antigens is thought to be useful for immunodiagnosis, there is a problem with supplying antigenic materials sufficiently. Therefore, a recombinant 31 kDa antigen (NIE) derived from L3i of *S. stercoralis* was developed, which resulted in the specificity of 87.5% with 48 sera from the patients with strongyloidosis. The NIE antigen was reactive with both parasite-specific IgE and IgG from the pooled patients' sera. There was no cross reactivity to *Onchocerca volvulus*, *L. loa*, and *Mansonella perstans,* but in tropical pulmonary eosinophilia presumably caused by *Wuchereria bancrofti*, false-positive results were obtained (Ravi et al. 2002).

Furthermore, luciferase immunoprecipitation systems (LIPS) were applied to detect parasite-specific IgG using recombinant antigens, NIE and SsIR. LIPS assays using either NIE or SsIR as antigen exhibited the same or higher performance in sensitivity or specificity compared to ELISA using the same antigens. When the assay was applied to combine NIE with SsIR as antigens, LIPS was 100% sensitive and specific, with an optimal negative (NPV) and positive predictive values (PPV) (Ramanathan et al. 2008). An excellent community-wide study on strongyloidosis was reported using stool examination (agar plate, Baermann, sedimentation concentration, and Harada-Mori) and serodiagnosis (CrAg-ELISA, NIE-ELISA, NIE-LIPS, and NIE-SsIR-LIPS). The prevalence of *S. stercoralis* infection was 29.4% by stool examination using agar plate, Baermann, sedimentation concentration, or Harada-Mori methods. The optimal cutoff point for each immunoassay was determined by plotting the sensitivity and specificity for cutoff point values by means of the receiver operating characteristic (ROC) curves. NIE-LIPS revealed the highest

sensitivity (97.8%) and specificity (100%) for detecting specific IgG (Krolewiecki et al. 2010).

While serodiagnosis using CrAg and NIE are slightly cross reactive to Bancroftian filariasis as mentioned above, recombinant strongylastacin, a 40-kDa metalloproteinase, does not cross react with IgE antibodies either from patient with *W. bancrofti* or patients with tropical pulmonary eosinophilia and increased level of IgE antibodies (Varatharajalu et al. 2011). Interestingly, the immunoblots and ELISA revealed the presence of IgG antibodies to strongylastacin in all individuals, irrespective of *S. stercoralis* infection status. IgG antibodies to strongylastacin are ubiquitous, because they are thought to result from zinc metalloproteinases, including astacin-like enzymes in food and/or in the gut's normal biota (Varatharajalu et al. 2011).

12.6.3 PCR-Based Examination

Since the paper by Putland et al. (1993), 18S rDNA and mitochondrial DNA of *S. stercoralis* have been utilized for phylogenetic analysis and diagnostic purposes (Dorris et al. 2002; Hu et al. 2003). Hasegawa et al. (2009) critically showed that hypervariable regions in 18S rDNA are suitable for markers with species-specific diagnosis in strongyloidosis. Some isolates of *Strongyloides* spp. were analyzed with 18S rDNA, showing the genetic relationship among parasite populations is not related to the host species (human, chimpanzee, and canine) but to geographical distribution (Pakdee et al. 2012).

A *S. stercoralis* real-time PCR has been developed and achieved higher specificity and sensitivity comparing to Baermann sedimentation and coproculture (Verweij et al. 2009). The primer and probe set from the 18S rRNA gene sequence was 10-fold to 100-fold more sensitive than the PCR designed from the cytochrome c oxidase subunit I gene or the *S. stercoralis*-specific repeated sequence. In order to optimize DNA extraction and PCR assays, Repetto et al. (2013) have developed an in-house method for nematode DNA isolation. Their results have clearly shown that the in-house and combined methods of DNA isolation increase the sensitivity of the molecular diagnosis based on a conventional PCR. They have proposed to apply diagnostic algorithm for *S. stercoralis* in asymptomatic patients, which combined both real-time PCR and blood eosinophil counts together with stool examination (Repetto et al. 2016).

The real-time PCR applied in asymptomatic cases in Cambodia showed a lower sensitivity compared to studies undertaken with symptomatic patients (Schär et al. 2013). In contrast, Paula et al. (2018) have shown that real-time PCR obtained the best results for detection of *S. stercoralis* infection among transplant candidates, being applied in stool samples.

Fluorescence resonance energy transfer (FRET) real-time PCR techniques have been applied to detect 18S rRNA (Janwan et al. 2011) or 28S rRNA gene sequences (Kramme et al. 2011) in fecal samples. Kramme et al. (2011) suggested that FRET

real-time PCR reduced nonspecific binding in comparison with TaqMan minor groove binder probe for amplicon detection used by Verweij et al. (2009).

A nested PCR targeting the internal transcribed spacer I (ITS1) region of the ribosomal DNA gene has been used to amplify *S. stercoralis* DNA (Nilforoushan et al. 2007) and to apply to fecal samples for field survey (Ahmad et al. 2013). Sampling and isolation methods for genomic analysis have been described by Zhou et al. (2019).

In addition to fecal samples, cell-free DNA (18S rRNA and Cox1 genes) has been detected both in human serum (Gorgani-Firouzjaee et al. 2018) and urine samples (Lodh et al. 2016). However, it has been shown that the sensitivity of PCR on urine is lower than that of PCR on serum (Formenti et al. 2019).

Finally, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) would be introduced as a diagnostic technique in parasitology as the first-line tool to identify pathogens because of its diagnostic accuracy, robustness, reliability, and rapid turnaround time (Feucherolles et al. 2019).

12.7 Treatment

According to the Centers for Disease Control and Prevention (USA) (www.cdc.gov/parasites/strongyloides/health_professionals/index.html) and Segarra-Newnham (2007), a treatment for strongyloidosis is recommended as follows:

12.7.1 First-Line Therapy

Ivermectin (Merck Sharp & Dohme Research Laboratories, NJ, USA)

200 μg/kg/day, one dose; repeat same dose after 2 weeks.

In case of immunosuppressive patients or disseminated patients, repeat totally four doses or more every 1–2 weeks. Follow-up stool examination should be done to verify eradication of worms. In addition, eosinophilia is thought to be a good marker of parasite reactivation (Loutfy et al. 2002; Repetto et al. 2010). It has been recommended that the patients should be monitored posttreatment at least every 3 months during the first year and then twice a year (Repetto et al. 2018).

Contraindications are as follows: there is no safety data for pregnant or lactating women and child patients weighing <15 kg. Confirmed or suspected concomitant *Loa loa* infection may cause serious side effects.

Most of the patients treated with ivermectin had no side effects in Japan. But some complained of nausea, anorexia, dizziness or vertigo, blurred vision, and malaise after the first treatment and itching and borborygmus after the second treatment (Shikiya et al. 1992).

Based on a randomized controlled trial, single-dose ivermectin rather than a four-dose regimen is recommended for the treatment of chronic, non-severe

strongyloidiasis in immunocompetent patients (Buonfrate et al. 2018). A subcutaneous ivermectin use has been reported in two cases of severe strongyloidosis (Barrett et al. 2016).

Refer WHO recommendations:
http://whqlibdoc.who.int/publications/2006/9241547103_eng.pdf

12.7.2 Alternative

Albendazole, 400 mg orally twice a day for 7 days.

Some patients complained diarrhea and abdominal pain (Segarra-Newnham 2007).

Contraindications as follows: patients with hypersensitivity to benzimidazole. Its use should be avoided in the first trimester of pregnancy.

Refer to WHO recommendation: http://whqlibdoc.who.int/publications/2006/9241547103_eng.pdf

Basic pharmacology of various drugs for strongyloidosis was reviewed by Grove (1989b).

12.8 Prevention and Control

Personal hygiene is important to prevent strongyloidosis, wearing shoes and using lavatory not to contaminate soil of living places and working fields. For public health, unfortunately, no vaccine for *Strongyloides* has been put into practical use so far. Recent advances in molecular biology give us some clues to potential chemotherapeutic and/or vaccine targets for strongyloidosis.

DNA microarrays are powerful tools to advance the development of vaccine discovery and chemotherapeutics. The microarray-based analysis of differential gene expression between L3i and L1 revealed differences in the expression of genes encoding putatively as well as between *S. stercoralis* L3i and *C. elegans* dauer stage larvae (Ramanathan et al. 2011). Furthermore, transcriptome analysis of L3i has provided us targets for potential chemotherapeutics using 454 sequencing coupled with semiautomated bioinformatic analyses. More than 50% of *S. stercoralis* putative proteins examined have no homologues present in humans. Among them, several putative proteins have been searched for homologues to *C. elegans* proteins with lethal RNAi phenotype, which cause death of *C. elegans* when knocked down via RNA interference (Marcilla et al. 2012).

Deoxycholate (DOC)-soluble proteins extracted from *S. stercoralis* L3i were shown to induce protective immunity, using Abraham's implantation method. Then, larval antigens were purified by an IgG affinity chromatography. Eluted antigens, in combination with alum, generated significant protective immunity in mice (Herbert et al. 2002b). DNA vaccine induced protective immunity against

S. stercoralis L3i in mice. Three proteins recognized by patients' serum IgG were candidates for vaccine. Successful immunization was done with plasmid containing DNA encoding Na^+-K^+ ATPase and plasmid containing DNA encoding granulocyte-macrophage colony-stimulating factor (GM-CSF) (Kerepesi et al. 2005). Furthermore, a recombinant antigen Ss-IR that is highly immunogenic in humans generated protective immunity through an antibody-dependent manner, so that SsIR plus alum may have potential to be used for a prophylactic vaccine in humans (Table 12.3) (Abraham et al. 2011).

12.9 Concluding Remarks

The most important measure to prevent tropical infectious diseases such as strongyloidosis is the development of society and promotion of healthcare system in developing countries. According to the report of Khieu et al. (2013), almost two-thirds of the soil-transmitted helminth infections could be avoided by proper sanitation in Cambodia.

Educational program on strongyloidosis for medical students and residents has been suggested to let them recognize the risk of strongyloidosis as well as to improve basic parasitological knowledge (Bjorklund et al. 2011). Strongyloidosis is a silent disease in most cases so that physician and health professionals may misdiagnose and/or tend to underestimate its morbidity. Precise knowledge on strongyloidosis for people concerned is needed as well as the development of effective vaccine and diagnostic tools that have specificity, sensitivity, and simplicity.

Acknowledgments The authors acknowledge Professor Eisaku Kimura for his critical reading of the manuscript. One of the authors (MK) is grateful to Professors Isao Tada and Yoshihisa Hashiguchi for their encouragements through this study and Ms. Kyoko Imamura for her secretarial assistance.

References

Abdelrahman MZ, Zeehaida M, Rahmah N et al (2012) Fatal septicemic shock associated with *Strongyloides stercoralis* infection in a patient with angioimmunoblastic T-cell lymphoma: a case report and literature review. Parasitol Int 61:508–511

Abraham D, Rotman HL, Haberstroh HF et al (1995) *Strongyloides stercoralis*: protective immunity to third-stage larvae in BALB/cByJ mice. Exp Parasitol 80:297–307

Abraham D, Hess JA, Mejia R et al (2011) Immunization with the recombinant antigen Ss-IR induces protective immunity to infection with *Strongyloides stercoralis* in mice. Vaccine 29: 8134–8140

Ahmad AF, Hadip F, Ngui R et al (2013) Serological and molecular detection of *Strongyloides stercoralis* infection among an Orang Asli community in Malaysia. Parasitol Res. https://doi.org/10.1007/s00436-013-3450-z

Akira S, Takeda K, Kaisho T (2001) Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol 2:675–680

Altintop L, Cakar B, Hokelek M et al (2010) *Strongyloides stercoralis* hyperinfection in a patient with rheumatoid arthritis and bronchial asthma: a case report. Ann Clin Microbiol Antimicrob 9: 27. http://www.ann-clinmicrob.com/content/9/1/27

Aoyama H, Hirata T, Sakugawa H et al (2007) An inverse relationship between autoimmune liver diseases and *Strongyloides stercoralis* infection. Am J Trop Med Hyg 76:972–976

Arakaki T, Iwanaga M, Kinjo F et al (1990) Efficacy of agar plate culture in detection of *Strongyloides stercoralis* infection. J Parasitol 76:425–428

Ashford RW, Burnish G, Viney ME (1992) *Strongyloides fuelleborni kellyi*: infection and disease in Papua New Guinea. Parasitol Today 8:314–318

Aupalee K, Wijit A, Singphai K et al (2020) Genomic studies on *Strongyloides stercoralis* in northern and western Thailand. Parasit Vectors 13:250. https://doi.org/10.1186/s13071-020-041150-0

Barratt JLN, Lane M, Talundzic E et al (2019) A global genotyping survey of *Strongyloides stercoralis* and *Strongyloides fuelleborni* using deep amplicon sequencing. PLoS Neg Trop Dis. https://doi.org/10.1371/journal.pntd.0007609

Barrett J, Broderick C, Soulsby H et al (2016) Subcutaneous ivermectin use in the treatment of severe *Strongyloides stercoralis* infection: two case reports and a discussion of literature. J Antmicrob Chemother 71:220–225

Batista MV, Pierrotti LC, Abdala E et al (2011) Endemic and opportunistic infections in Brazilian solid organ transplant recipients. Tropical Med Int Health 16:1134–1142

Bjorklund AB, Cook BA, Hendel-Paterson BR et al (2011) Impact of global health residency training on medical knowledge of immigrant health. Am J Trop Med Hyg 85:405–408

Bonne-Année S, Hess JA, Abraham D (2011) Innate and adaptive immunity to the nematode *Strongyloides stercoralis* in a mouse model. Immunol Res 51:205–214

Brigandi RA, Rotman HL, Yutanawiboonchai W et al (1996) *Strongyloides stercoralis:* role of antibody and complement in immunity to the third stage larvae in BALB/cByJ mice. Exp Parasitol 82:279–289

Brigandi RA, Rotman HL, Nolan TJ et al (1997) Chronicity in *Strongyloides stercoralis* infections: dichotomy of the protective immune response to infective and autoinfective larvae in a mouse model. Am J Trop Med Hyg 56:640–646

Brigandi RA, Rotman HL, Leon O et al (1998) *Strongyloides stercoralis* host-adapted third-stage larvae are the target of eosinophil-associated immune-mediated killing in mice. J Parasitol 84: 440–445

Brinley PJ, Gam AA, McKerrow JH et al (1995) Ss40: the zinc endopeptidase secreted by infective larvae of *Strongyloides stercoralis*. Exp Parasitol 80:1–7

Bryant AS, Ruiz F, Gang SS, Castelletto ML, Lopez JB, Hallem EA (2018) A critical role for thermosensation in host seeking by skin-penetrating nematodes. Curr Biol 28:2338–2347. https://doi.org/10.1016/j.cub.2018.05.063

Buonfrate D, Requena-Mendez A, Angheben A et al (2018) Accuracy of molecular biology techniques for the diagnosis of *Strongyloides stercoralis* – a systematic review and meta-analysis. PLoS Negl Trop Dis. https://doi.org/10.1371/journal.pnd.0006229

Charlesworth B (2010) Sex determination: a worm does it by elimination. Curr Biol 20:R841–R843

Charuchaibovorn S, Sanprasert V, Nuchprayoon S (2019) The experimental infections of the human isolate of *Strongyloides stercoralis* in a rodent model (the Mongolian gerbil, *Meriones Unguiculatus*). Pathogens 21. https://doi.org/10.3390/pathogens8010021

Concha R, Harrington W Jr, Rogers AI (2005) Intestinal strongyloidiasis: recognition, management, and determinants of outcome. J Clin Gastroenterol 39:203–211

Conlan JV, Khamlome B, Vongxay K et al (2012) Soil-transmitted helminthiasis in Laos: a community-wide cross-sectional study of humans and dogs in a mass drug administration environment. Am J Trop Med Hyg 86:624–634

Conway DJ, Bailey JW, Lindo JF et al (1993) Serum IgG reactivity with 41-, 31-, and 28-kDa larval proteins of *Strongyloides stercoralis* in individuals with strongyloidosis. J Infect Dis 168:784–787

Croker C, Reporter R, Redelings M et al (2010) Strongyloidiasis-related deaths in the United States, 1991–2006. Am J Trop Med Hyg 83:422–426

Dawkins HJ, Muir GM, Grove DI (1981) Histopathological appearances in primary and secondary infections with *Strongyloides ratti* in mice. Int J Parasitol 11:97–103

De Paola D, Dias LB, Suva JR (1962) Enteritis due to *Strongyloides stercoralis* – a report of 5 fatal cases. Am J Dig Dis 7:1086–1098

De Souza JN, Soares BNRR, Goes LL et al (2018) Case report: *Strongyloides stercoralis* hyperinfection in a patient with HTLV-1: an infection with filariform and rhabditiform larvae, egg, and free-living adult females output. Am J Trop Med Hyg 99:1583–1586

Demehri S, Morimoto M, Holtzman MJ et al (2009) Skin-derived TSLP triggers progression from epidermal-barrier defects to asthma. PLoS Biol 7:e1000067

Dorris M, Viney ME, Blaxter ML (2002) Molecular phylogenetic analysis of the genus *Strongyloides* and related nematodes. Int J Parasitol 32:1507–1517

Dresden MH, Rege AA, Murrell KD (1985) *Strongyloides ransomi*: proteolytic enzymes from larvae. Exp Parasitol 59:257–263

Duc Dodon M, Li Z, Hamaia S et al (2004) Tax protein of human T-cell leukaemia virus type 1 induces interleukin 17 gene expression in T cells. J Gen Virol 85:1921–1932

Fabiani S, Bruschi F (2014) Rheumatological patients undergoing immunosuppressive treatments and parasitic diseases: a review of the literature of clinical cases and perspectives to screen and follow-up active and latent chronic infections. Clin Exp Rheumatol 32:587–596

Fabiani S, Fortunato S, Petrini M, Bruschi F (2017) Allogeneic hematopoietic stem cell transplant recipients and parasitic diseases: a review of the literature of clinical cases and perspectives to screen and follow-up active and latent chronic infections. Transpl Infect Dis 19:2. https://doi.org/10.1111/tid.12669

Fabiani S, Fortunato S, Bruschi F (2018) Solid organ transplant and parasitic diseases: a review of the clinical cases in the last two decades. Pathogens 7:65. https://doi.org/10.3390/pathogens7030065

Feucherolles M, Poppert S, Utzinger J, Becker SL (2019) MALDI-TOF mass spectrometry as a diagnostic tool in human and veterinary helminthology: a systematic review. Parasit Vectors 12:245–258. https://doi.org/10.1186/s13071-019-3493-9

Fitzpatrick MA, Caicedo JC, Stosor V et al (2010) Expanded infectious diseases screening program for Hispanic transplant candidates. Transpl Infect Dis 12:336–341

Fleitas PE, Travacio M, Martí-Soler H, Socías ME, Lopez WR, Krolewiecki AJ (2020) The *Strongyloides stercoralis*-hookworms association as a path to the estimation of the global burden of strongyloidiasis: a systematic review. PLoS NTD 14(4):e0008184

Formenti F, La Marca G, Perandin F et al (2019) A diagnostic study comparing conventional and real-time PCR for *Strongyloides stercoralis* on urine and on faecal samples. Acta Trop 190:284–287

Galioto AM, Hess JA, Nolan TJ et al (2006) Role of eosinophils and neutrophils in innate and adaptive protective immunity to larval *Strongyloides stercoralis* in mice. Infect Immun 74:5730–5738

Gallego SG, Loukas A, Slade RW et al (2005) Identification an astacin-like metallo-proteinase transcript from the infective larvae of *Strongyloides stercoralis*. Parasitol Int 54:123–133

Gang SS, Castelletto ML, Bryant AS et al (2017) Targeted mutagenesis in a human-parasitic nematode. PLoS Pathog. https://doi.org/10.1371/journal.ppat.1006675

Gang SS, Castelletto ML, Yang E et al (2020) Chemosensory mechanisms of host seeking and infectivity in skin-penetrating nematodes. PNAS 117:17913–17923

Genta RM, Caymmi Gomes M (1989) Pathology. In: Grove DI (ed) Strongyloidiasis – a major roundworm infection of man, 1st edn. Taylor & Francis, London, pp 105–132

Genta RM, Lillibridge JP (1989) Prominence of IgG4 antibodies in the human responses to *Strongyloides stercoralis* infection. J Infect Dis 160:692–699
Genta RM, Weil GJ (1982) Antibodies to *Strongyloides stercoralis* larval surface antigens in chronic strongyloidiasis. Lab Investig 47:87–90
Genta RM, Ottesen EA, Poindexter R et al (1983) Specific allergic sensitization to *Strongyloides* antigens in human strongyloidiasis. Lab Invst 48:633–638
González A, Gallo M, Valls ME et al (2010) Clinical and epidemiological features of 33 imported *Strongyloides stercoralis* infections. Trans R Soc Trop Med Hyg 104:613–616
Gorgani-Firouzjaee T, Kalantari N, Javanian M et al (2018) *Strongyloides stercoralis*: detection of parasite-derived DNA in serum samples obtained from immunosuppressed patients. Parasitol Res 117:2927–2932
Grove DI (1989a) Clinical manifestations. In: Grove DI (ed) Strongyloidiasis – a major roundworm infection of man, 1st edn. Taylor & Francis, London, pp 155–176
Grove DI (1989b) Treatment. In: Grove DI (ed) Strongyloidiasis – a major roundworm infection in man. Taylor & Francis, London, pp 199–231
Hammond MP, Robinson RD (1994) Chromosome complement, gametogenesis, and development of *Strongyloides stercoralis*. J Parasitol 80:689–695
Hasan A, Le M, Pasko J et al (2013) Transmission of *Strongyloides stercoralis* through transplantation of solid organs-Pennsylvania, 2012. CDC Morb Mort Wkly Rep 62:264–266
Hasegawa H, Hayashida S, Ikeda Y et al (2009) Hyper-variable regions in 18S rDNA of *Strongyloides* spp. as markers for species-specific diagnosis. Parasitol Res 104:869–874
Hasegawa H, Sato H, Fujita S et al (2010) Molecular identification of the causative agent of human strongyloidiasis acquired in Tanzania: dispersal and diversity of *Strongyloides* spp. and their host. Parasitol Int 59:407–413
Herbert D'BR, Lee JJ, Lee NA et al (2000) Role of IL-5 in innate and adaptive immunity to larval *Strongyloides stercoralis* in mice. J Immunol 165:4544–4551
Herbert D'BR, Nolan TJ, Schad GA et al (2002a) The role of B cells in immunity against larval *Strongyloides stercoralis* in mice. Parasite Immunol 24:95–101
Herbert D'BR, Nolan TJ, Schad GA et al (2002b) Immunoaffinity-isolated antigens induce protective immunity against larval *Strongyloides stercoralis* in mice. Exp Parasitol 100:112–120
Hirata T, Uchima N, Kishimoto K et al (2006) Impairment of host immune response against *Strongyloides stercoralis* by human T cell lymphotrophic virus type 1 infection. Am J Trop Med Hyg 74:246–249
Hu M, Chilton NB, Gasser RB (2003) The mitochondrial genome of *Strongyloides stercoralis* (Nematoda) – idiosyncratic gene order and evolutionary implications. Int J Parasitol 33:1393–1408
Ines EJ, Souza JN, Santos RC et al (2011) Efficacy of parasitological methods for the diagnosis of *Strongyloides stercoralis* and hookworm in faecal specimens. Acta Trop 120:206–210
Jaleta TG, Lok JB (2020) Advances in the molecular and cellular biology of *Strongyloides* spp. Curr Trop Med Rep 6:161–178
Jaleta TG, Zhou S, Bemm FM et al (2017) Different but overlapping populations of *Strongyloides stercoralis* in dogs and humans – dogs as a possible source for zoonotic strongyloidiasis. PLoS Negl Trop Dis. https://doi.org/10.1371/journal.pntd.0005752
Janwan P, Intapan PM, Thanchomnang T et al (2011) Rapid detection of *Opisthorchis viverrini* and *Strongyloides stercoralis* in human fecal samples using a duplex real-time PCR and melting curve analysis. Parasitol Res 109:1593–1601
Kaminsky RG (1993) Evaluation of three methods for laboratory diagnosis *Strongyloides stercoralis* infection. J Parasitol 79:277–280
Kerepesi LA, Nolan TJ, Schad GA et al (2004) Human immunoglobulin G mediates protective immunity and identified protective antigens against larval *Strongyloides stercoralis* in mice. J Infect Dis 189:1282–1290

Kerepesi LA, Keiser PB, Nolan TJ et al (2005) DNA immunization with Na^+–K^+ATPase (*Sseat-6*) induces protective immunity to larval *Strongyloides stercoralis* in mice. Infect Immun 73:2298–2305

Kerepesi LA, Hess JA, Nolan TJ et al (2006) Complement component C3 is required for protective innate and adaptive immunity to larval *Strongyloides stercoralis* in mice. J Immunol 176:4315–4322

Kerepesi LA, Hess JA, Leon O et al (2007) Toll-like receptor 4 (TLR4) is required for protective immunity to larval *Strongyloides stercoralis* in mice. Microbes Infect 9:28–34

Kerry A, Little MD, Voge M (1976) Strongyloides fulleborni-like infections in man in Papua New Guinea. Am J Trop Med Hyg 25:694–699

Khieu V, Schär F, Marti H et al (2013) Diagnosis, treatment and risk factors of *Strongyloides stercoralis* in schoolchildren in Cambodia. PLoS Negl Trop Dis 7:e2035

Kikuchi T, Hino A, Tanaka T et al (2016) Genome-wide analyses of individual *Strongyloides stercoralis* (Nematoda: Rhabditoidea) provide insights to population structure and reproductive life cycles. PLoS Negl Trop Dis. https://doi.org/10.1371/journal.pntd.0005253

Kim AC, Lupatkin HC (2004) *Strongyloides stercoralis* infection as a manifestation of immune restoration syndrome. Clin Infect Dis 39:439–440

Kishimoto K, Hokama A, Hirata T et al (2008) Endoscopic and histopathological study on the duodenum of *Strongyloides stercoralis* hyperinfection. World J Gastroenteral 14:1768–1773

Korenaga M, Hitoshi Y, Yamaguchi N et al (1991) The role of interleukin-5 in protective immunity to *Strongyloides venezuelensis* infection in mice. Immunology 72:502–507

Korenaga M, Hitoshi Y, Takatu K et al (1994) Regulatory effect of anti-interleukin-5 monoclonal antibody on intestinal worm burden in a primary infection with *Strongyloides venezuelensis* in mice. Int J Parasitol 24:951–957

Koyasu S, Moro K (2012) Role of innate lymphocytes in infection and inflammation. Front Immunol 3:1–13

Kramme S, Nissen N, Soblik H et al (2011) Novel real-time PCR for the universal detection of *Strongyloides* species. J Med Microbiol 60:454–458

Krolewiecki AJ, Ramanathan R, Fink V et al (2010) Improved diagnosis of *Strongyloides stercoralis* using recombinant antigen-based serologies in a community-wide study in Northern Argentina. Clin Vaccine Immunol 17:1624–1630

Krolewiecki AJ, Lammie P, Jacobson J et al (2013) A public health response against *Strongyloides stercoralis*: time to look at soil-transmitted helminthiasis in full. PLoS Neg Trop Dis 7:e2165

Lanzafame M, Faggian F, Lattuada E (2005) Strongyloidiasis in an HIV-1–infected patient after highly active antiretroviral therapy–induced immune restoration. J Infect Dis 191:1027

Lawns D, Wilkinson RJ (2006) Immune reconstitution disease associated with parasitic infections following antiretroviral treatment. Parasite Immunol 28:625–633

Lewert RM, Lee C-L (1954) Studies on the passage of helminth larvae through host tissues. I. Histochemical studies on the extracellular changes caused by penetrating larvae. II. Enzymatic activity of larvae in vitro and in vivo. J Infect Dis 95:13–51

Ligas JA, Kerepesi LA, Galioto AM et al (2003) Specificity and mechanism of immunoglobulin M (IgM)- and IgG-dependent protective immunity to larval *Strongyloides stercoralis* in mice. Infect Immun 71:6835–6843

Little MD (1966) Comparative morphology of six species of *Strongyloides* (Nematoda) and redefinition of the genus. J Parasitol 52:69–84

Llenas-García J, Fiorante S, Salto E et al (2012) Should we look for *Strongyloides stercoralis* in foreign-born HIV-infected persons? J Immigr Minor Health 15:796–802

Lodh N, Caro R, Sofer S et al (2016) Diagnosis of *Strongyloides stercoralis*: detection of parasite-derived DNA in urine. Acta Trop 163:9–13

Lok JB, Shao H, Massey HC Jr et al (2017) Transgenesis in Strongyloides and related parasitic nematodes: historical perspectives, current functional genomic applications and progress towards gene disruption and editing. Parasitology 144:327–342

Loutfy MR, Wilson M, Keystone JS et al (2002) Serology and eosinophil count in the diagnosis and management of strongyloidiasis in a non-endemic area. Am Trop Med Hyg 66:749–752
Lucas (1990) Missing infections in AIDS. Trans R Soc Trop Med Hyg 84(Suppl 1):34–38
Machicado JD, Marcos LA, Tello R et al (2012) Diagnosis of soil-transmitted helminthiasis in an Amazonic community of Peru using multiple diagnostic techniques. Trans R Soc Trop Med Hyg 106:333–339
Maizels RM, Hewitson JP, Smith KA (2012) Susceptibility and immunity to helminth parasites. Curr Opin Immunol 24:459–466
Malpica L, White AC Jr, Leguia C et al (2019) Regulatory T cells and IgE expression in duodenal mucosa of *Strongyloides stercoralis* and human T lymphotropic virus type 1 co-infected patients. PLoS Neg Trop Dis. https://doi.org/10.1371/journal.pntd.0007415
Marcilla A, Garg G, Bernal D et al (2012) The transcriptome analysis of *Strongyloides stercoralis* L3i larvae reveals targets for intervention in a neglected disease. PLoS Negl Trop Dis 6:e1513
Marti H, Koella JC (1993) Multiple stool examinations for ova and parasites and rate of false-negative results. J Clin Microbiol 31:3044–3045
Mascarello M, Gobbi F, Angheben A et al (2011) Prevalence of *Strongyloides stercoralis* infection among HIV-positive immigrants attending two Italian hospitals, from 2000 to 2009. Ann Trop Med Parasitol 105:617–623
Masucci L, Graffeo R, Bani S et al (2011) Intestinal parasites isolated in a large teaching hospital, Italy, 1 May 2006 to 31 December 2008. Euro Surveill 16. pii=19891. http://www.eurosurveillance.org
McCarthy AE, Weld LH, Barnett ED et al (2013) Spectrum of illness in international migrants seen at GeoSentinel clinics in 1997–2009, Part 2: migrants resettled internationally and evaluated for specific health concerns. Clin Infect Dis 56:925–933
McKerrow JH, Brindley P, Brown M et al (1990) *Strongyloides stercoralis:* identification of a protease that facilitates penetration of skin by the infective larvae. Exp Parasitol 70:134–143
Mir A, Benahmed D, Igual R et al (2006) Eosinophil-selective mediators in human strongyloidiasis. Parasite Immunol 28:397–400
Montes M, Sanchez C, Verdonck K et al (2009) Regulatory T Cell expansion in HTLV-1 and strongyloidiasis co-infection is associated with reduced IL-5 responses to *Strongyloides stercoralis* antigen. PLoS Negl Trop Dis 3:e456
Montes M, Sawhney C, Barros N (2010) *Strongyloides stercoralis*: there but not seen. Curr Opin Infect Dis 23:500–504
Mori I, Ohshima Y (1995) Neural regulation of thermotaxis in *Caenorhabditis elegans*. Nature 376: 344–348
Moro K, Yamada T, Tanabe M et al (2010) Innate production of Th2 cytokines by adipose tissue-associated c-Kit+Sca-1+ lymphoid cells. Nature (London) 463:540–544
Nabha L, Krishna S, Ramananthan R et al (2012) Prevalence of *Strongyloides stercoralis* in an urban US AIDS cohort. Pathog Glob Health 106:238–244
Nagayasu E, Aung MPPTHH, Hortiwakul T et al (2017) A possible origin population of pathogenic intestinal nematodes, *Strongyloides stercoralis,* unveiled by molecular phylogeny. Sci Rep 7: 4844. https://doi.org/10.1038/s41598-017-05049-x
Nakada K, Kohakura M, Komoda H et al (1984) High incidence of HTLV antibody in carriers of *Strongyloides stercoralis*. Lancet 1:633
Nawa Y, Abe T, Imai J et al (1988) Impaired natural defence of beige (Chediak-Higashi syndrome) mice against tissue-migrating larvae of *Strongyloides ratti* and its reconstitution by bone marrow cells. Parasite Immunol 10:117–126
Neill DR, Wong SH, Bellosi A et al (2010) Nuocyte represent a new innate effector leukocyte that mediates type-2 immunity. Nature (London) 464:1367–1370
Nemetschke L, Eberhardt AG, Hertzberg H et al (2010) Genetics, chromatin diminution, and sex chromosome evolution in the parasitic nematode Genus *Strongyloides*. Curr Biol 20:1687–1696

Newton RC, Limpuangthip P, Greenberg S et al (1992) *Strongyloides stercoralis* hyperinfection in a carrier of HTLV-1 virus with evidence of selective immunosuppression. Am J Med 92:202–208

Nilforoushan MR, Mirhendi H, Rezaie S et al (2007) A DNA-based identification of *Strongyloides stercoralis* isolates from Iran. Iran J Public Health 36:16–20

Nishigori M (1928) On various factors influencing the development of *Strongyloides stercoralis* and autoinfection (in Japanese). Taiwan Igakkai Zassi 27:1–56. English edition: Nishigori (1978) In: Kean BH et al (eds) Tropical medicine and parasitology. Classic investigations, vol II. Cornell University Press, Ithaca, NY, pp 340–345

Nishihira J (2012) Molecular function of macrophage migration inhibitory factor and a novel therapy for inflammatory bowel disease. Ann N Y Acad Sci 1271:53–57

Nolan TJ, Megyeri Z, Bhopale VM et al (1993) *Strongyloides stercoralis*: the first rodent model for uncomplicated and hyperinfective strongyloidiasis, the Mongolian gerbil (*Meriones unguiculatus*). J Infect Dis 168:1479–1484

Nolan TJ, Rotman HL, Bhopale VM et al (1995) Immunity to a challenge infection of *Strongyloides stercoralis* third-stage larvae in the jird. Parasite Immunol 17:599–604

Nolan TJ, Bhopale VM, Rotman HL et al (2002) *Strongyloides stercoralis*: high worm population density leads to autoinfection in the jird (*Meriones unguiculatus*). Exp Parasitol 100:173–178

Nolan TJ, Brenes M, Ashton FT et al (2004) The amphidial neuron pair ALD controls the temperature-sensitive choice of alternative developmental pathways in the parasitic nematode, *Strongyloides stercoralis*. Parasitology 129:753–759

O'Connell AE, Hess JA, Santiago GA et al (2011a) Major basic protein from eosinophils and myeloperoxidase from neutrophils are required for protective immunity to *Strongyloides stercoralis* in mice. Infect Immun 79:2770–2778

O'Connell AE, Redding KM, Hess JA et al (2011b) Soluble extract from the nematode *Strongyloides stercoralis* induces CXCR2 dependent/IL-17 independent neutrophil recruitment. Microbes Infect 13:536–544

Olsen A, van Lieshout L, Marti H et al (2009) Strongyloidiasis- the most neglected of the neglected tropical diseases? Trans R Soc Trop Med Hyg 103:967–972

Ovington KS, Mckie K, Mattaei KI et al (1998) Regulation of primary *Strongyloides ratti* infections in mice: a role for interleukin-5. Immunology 95:488–493

Owhashi M, Abe T, Korenaga M et al (1986) Eosinophil chemotactic factor-release from Guinea Pig neutrophils after *in vitro* stimulation with *Strongyloides ratti* larvae. Jpn J Parasitol 35:121–126

Padigel UM, Lee JJ, Nolan TJ et al (2006) Eosinophils can function as antigen-presenting cells to induce primary and secondary immune responses to *Strongyloides stercoralis*. Infet Immun 74: 3232–3238

Padigel UM, Hess JA, Lee JJ et al (2007a) Eosinophils act antigen-presenting cells to induce immunity to *Strongyloides stercoralis* in mice. J Infect Dis 196:1844–1851

Padigel UM, Stein L, Redding K et al (2007b) Signaling through Gαi2 protein is required for recruitment of neutrophils for antibody-mediated elimination of larval *Strongyloides stercoralis* in mice. J Leukoc Biol 81:1120–1126

Pakdee W, Thaenkham U, DeKumyoy P et al (2012) Genetic differentiation of *Strongyloides stercoralis* from two different climate zone revealed by 18S ribosomal DNA sequence comparison. Southeast Asian J Trop Med Public Health 43:1333–1338

Pampiglione S, Ricciardi ML (1971) The presence of *Strongyloides fülleborni* von Linstow, 1905, in man in Central and East Africa. Parassitologia 13:257–269

Paula FM, Costa-Cruz JM (2011) Epidemiological aspects of strongyloidiasis in Brazil. Parasitology 38:1331–1340

Paula FM, Malta FM, Marques PD et al (2018) Molecular diagnosis of *Strongyloides stercoralis* among transplant candidates. Transpl Infect Dis. https://doi.org/10.1111/tid.12909

Pelletier LL Jr, Baker CB, Gam AA et al (1988) Diagnosis and evaluation of treatment of chronic strongyloidiasis in ex-prisoners of War. J Infect Dis 157:537–576

Porto AF, Neva FA, Bittencourt et al (2001) HTLV-1 decreases Th2 type of immune response in patients with strongyloidiasis. Parasite Immunol 23:503–507
Price AE, Liang H-E, Sullivan BM et al (2010) Systemically dispersed innate IL-13-expressing cells in type 2 immunity. Proc Natl Acad Sci U S A 107:11489–11494
Purtilo DT et al (1974) Fatal strongyloidiasis in immunosuppressed patients. Am J Med 56:488–493
Putland RA, Thomas SM, Grove DI et al (1993) Analysis of the 18S ribosomal RNA gene of *Strongyloides stercoralis*. Int J Parasitol 23:149–151
Ramanathan R, Burbelo PD, Groot S et al (2008) A luciferase immunoprecipitation systems assay enhances the sensitivity and specificity of diagnosis of *Strongyloides stercoralis* infection. J Infect Dis 198:444–451
Ramanathan R, Varma S, Ribeiro JMC et al (2011) Microarray-based analysis of differential gene expression between infective and noninfective larvae of *Strongyloides stercoralis*. PLoS Negl Trop Dis 5:e1039
Ravi V, Ramachandran S, Thompson RW et al (2002) Characterization of a recombinant immunodiagnostic antigen (NIE) from *Strongyloides stercoralis* L3-stage larvae. Mol Biochem Parasitol 125:73–81
Reche PA, Soumelis V, Gorman DM et al (2001) Human thymic stromal lymphopoietin preferentially stimulates myeloid cells. J Immunol 167:336–343
Rege AA, Dresden MH (1987) *Strongyloides* spp.: demonstration and partial characterization of acidic collagenolytic activity from infective larvae. Exp Parasitol 64:275–280
Reitz M, Brunn ML, Rodewald HR et al (2017) Mucosal mast cells are indispensable for the timely termination of *Strongyloides ratti* infection. Mucosal Immunol:481–492. https://doi.org/10.1038/mi.2016.56
Reitz M, Brunn ML, Voehringer D, Breloer M (2018) Basophils are dispensable for the establishment of protective adaptive immunity against primary and challenge infection with the intestinal helminth parasite *Strongyloides ratti*. PLoS Negl Trop Dis 12(11):e0006992. https://doi.org/10.1371/journal.pntd.0006992
Repetto SA, Duran PA, Lasala MB et al (2010) High rate of strongyloidosis infection, out of endemic area, in patients with eosinophilia and without risk of exogenous reinfection. Am J Trop Med Hyg 82:1088–1093
Repetto SA, Alba Soto CD, Cazorla SI et al (2013) An improved DNA isolation technique for PCR detection of *Strongyloides stercoralis* in stool samples. Acta Trop 126:110–114
Repetto SA, Ruybal P, Solana ME et al (2016) Comparison between PCR and larvae visualization methods for diagnosis of *Strongyloides stercoralis* out of endemic area: a proposed algorithm. Acta Trop 157:169–177
Repetto SA, Ruybal P, Batalla E et al (2018) Strongyloidiasis outside endemic areas: long-term parasitological and clinical follow-up after ivermectin treatment. Clin Infect Dis 66:1558–1564
Robson D, Welch E, Beeching NJ et al (2009) Consequences of captivity: health effects of far east imprisonment in World War II. Q J Med 102:87–96
Rotman HL, Yutanawiboonchai W, Brigandi RA et al (1996) *Strongyloides stercoralis*: eosinophil-dependent immune-mediated killing of third stage larvae in BALB/cByJ mice. Exp Parasitol 82:267–278
Rotman HL, Schnyder-Candrian S, Scott P et al (1997) IL-12 eliminates the Th-2 dependent protective immune response of mice to larval *Strongyloides stercoralis*. Parasite Immunol 19:29–39
Roxby AC, Gottlieb GS, Limaye AP (2009) Strongyloidiasis in transplant patients. Clin Infect Dis 49:1411–1423
Saenz SA, Siracusa MC, Perrigoue JG et al (2010) IL-25 elicits a multi-potent progenitor cell population that promotes Th2 cytokine responses. Nature (London) 464:1362–1366
Safer D, Brenes M, Dunipace S et al (2007) Urocanic acid is a major chemoattractant for the skin-penetrating parasitic nematode *Strongyloides stercoralis*. Proc Natl Acad Sci 104:1627–1630
Salazar SA, Gutierrez C, Berk SL (1995) Value of the agar plate method for the diagnosis of intestinal strongyloidiasis. Parasitology 23:141–145

Salles F, Bacellar A, Amorim M et al (2013) Treatment of strongyloidiasis in HTLV-1 and *Strongyloides stercoralis* coinfected patients is associated with increased TNFα and decreased soluble IL2 receptor levels. Trans R Soc Trop Med Hyg 107:526–529

Sato Y (2003) Strongyloidiasis. In: Otsuru M et al (eds) Progress of medical parasitology in Japan, vol 8. Meguro Parasitological Museum, Tokyo, pp 387–400

Sato Y, Inoue F, Kiuna S et al (1990) Immunoblot analysis of three antigen preparations from *Strongyloides stercoralis* larvae in human strongyloidiasis. Jpn J Parasitol 39:258–266

Sato Y, Kobayashi J, Toma H et al (1995) Efficacy of stool examination for detection of *Strongyloides* infection. Am J Trop Med Hyg 53:248–250

Satoh M, Toma H, Sato Y et al (2002a) Reduced efficacy of treatment of strongyloidiasis in HTLV-1 carriers related to enhanced expression of IFN-γ and TGF-β 1. Clin Exp Immunol 127:354–359

Satoh M, Toma H, Sugahara K et al (2002b) Involvement of IL-2/IL-2R system activation by parasite antigen in polyclonal expansion of CD4+25+ HTLV-1-infected T-cells in human carriers of both HTLV-1 and *S. stercoralis*. Oncogene 21:2466–2475

Schad (1989) Morphology and life history of *Strongyloides stercoralis*. In: Grove DI (ed) Strongyloidiasis – a major roundworm infection of man, 1st edn. Taylor & Francis, London, pp 85–104

Schad GA, Hellman ME, Muncey DW (1984) *Strongyloides stercoralis*: hyperinfection in immunosuppressed dogs. Exp Parasitol 57:287–296

Schär F, Odermatt P, Khier V et al (2013) Evaluation of real-time PCR for *Strongyloides stercoralis* and hookworm as diagnostic tool in asymptomatic schoolchildren in Cambodia. Acta Trop 126: 89–92

Seet RCS, Lau LG, Tambyah PA (2005) *Strongyloides* hyperinfection and hypogammaglobulinemia. Clin Diagn Lab Immunol 12:680–682

Segarra-Newnham M (2007) Manifestations, diagnosis, and treatment of *Strongyloides stercoralis* infection. Ann Pharmacother 41:1992–2001

Shikiya K, Kinjo N, Uehara T et al (1992) Efficacy of ivermectin against *Strongyloides stercoralis* in humans. Intern Med 31:310–312

Siddiqui AA, Berk SL (2001) Diagnosis of *Strongyloides stercoralis* infection. Clin Infect Dis 33: 1040–1047

Siddiqui AA, Stanley CS, Berk SL (2000a) A cDNA encoding the highly immunodominant antigen of *Strongyloides stercoralis*: γ-subunit of isocitrate dehydrogenase (NAD+). Parasitol Res 86: 279–283

Siddiqui AA, Stanley CS, Skelly PJ et al (2000b) A cDNA encoding a nuclear hormone receptor of the steroid/thyroid hormone-receptor superfamily from the human parasitic nematode *Strongyloides stercoralis*. Parasitol Res 86:24–29

Siddiqui AA, Genta RM, Berk SL (2006) Strongyloidiasis. In: Guerrant RL, Walker DH, Weller PF (eds) Tropical infectious diseases – principles, pathogens, & practice, 2nd edn. Churchill Livingstone, Philadelphia, pp 1274–1285

Sousa-Figueiredo JC, Day M, Betson M et al (2011) Field survey for strongyloidiasis in eastern Uganda with observations on efficacy of preventive chemotherapy and co-occurrence of soil-transmitted helminthiasis/intestinal schistosomiasis. J Helminthol 85:325–333

Stein LH, Redding KM, Lee JJ et al (2009) Eosinophils utilize multiple chemokine receptors for chemotaxis to the parasitic nematode *Strongyloides stercoralis*. J Innate Immun 1:618–630

Streit A (2008) Reproduction in *Strongyloides* (Nematoda): a life between sex and parthenogenesis. Parasitology 135:285–294

Suzuki T, Nara N, Miyake S et al (1989) Fatal strongyloidiasis latent over 42 years in the antineoplastic chemotherapy of a case with malignant lymphoma. Jpn J Med 28:96–99

Sweileh WM (2019) A bibliometric analysis of human strongyloidiasis research (1968–2017). Travel Med and Vacc 5:24. https://doi.org/10.1186/s40794-019-0100-1

Takagi M, Ishikawa G, Hori E (1973) An autopsy case of pemphigus vulgaris complicating a fatal strongyloidiasis. Acta Pathologica Japonica 23:173–181
Thamsborg SM, Ketzis J, Horii Y, Matthews JB (2017) *Strongyloides* spp. infections of veterinary importance. Parasitology 144:274–284
Thanchomnang T, Intapan PM, Sanpool O et al (2017) First molecular identification and genetic diversity of *Strongyloides stercoralis* and *Strongyloides fuelleborni* in human communities having contact with long-tailed macaques in Thailand. Parasitol Res 116:1917–1923
Toledo R, Munoz-Antoli C, Esteban J-G (2015) Strongyloidiasis with emphasis on human infections and its different clinical forms. Adv Parasitol 88:165–241
Tort J, Brindley PJ, Knox D et al (1999) Proteinases and associated genes of parasitic helminthes. In: Baker JR et al (eds) Advances in parasitol, vol 43, pp 161–266
Varatharajalu R, Parandaman V, Ndao M et al (2011) *Strongyloides stercoralis* excretory/secretory protein strongylastacin specifically recognized by IgE antibodies in infected human sera. Microbiol Immunol 55:115–122
Verweij JJ, Canales M, Polman K et al (2009) Molecular diagnosis of *Strongyloides stercoralis* in faecal samples using real-time PCR. Trans R Soc Trop Med Hyg 103:342–346
Viney ME (2006) The biology and genomics of *Strongyloides*. Med Microbiol Immunol 195:49–54
Viney ME, Lok JB (2015) The biology of *Strongyloides spp.* In:WormBook (ed) The *C. elegans* Research Community. WormBook. http://www.wormbook.org. https://doi.org/10.1895/wormbook.1.141.2
Viney ME, Brown M, Omoding NE et al (2004) Why does HIV infection not lead to disseminated strongyloidiasis? J Infect Dis 190:2175–2180
Wang C, Xu J, Zhou X et al (2013) Strongyloidiasis: an emerging infectious disease in China. Am J Trop med Hyg 88:420–425
Watanabe K, Noda K, Hamano S et al (2000) The crucial role of granulocytes in the early host defense against *Strongyloides ratti* infection in mice. Parasitol Res 86:188–193
Watanabe K, Sasaki O, Hamano S et al (2003) *Strongyloides ratti:* the role of interleukin-5 in protection against tissue migrating larvae and intestinal adult worms. J Helminthol 77:355–361
Werneck-Silva AL, Alvares EP, Gama P et al (2006) Intestinal damage in strongyloidiasis: the imbalance between cell death and proliferation. Dig Dis Sci 51:1063–1069
Wills-Karp M, Rani R, Dienger K et al (2012) Trefoil factor 2 rapidly induces interleukin 33 to promote type 2 immunity during allergic asthma and hookworm infection. J Exp Med 209:607–622
Winnicki W, Eder M, Mazal P et al (2018) Prevalence of *Strongyloides stercoralis* infection and hyperinfection syndrome among renal allograft recipients in Central Europe. Sci Rep 8:15406. https://doi.org/10.1038/s41598-018-33775-3
Wiria AE, Wammes LJ, Hamid F et al (2013) Relationship between carotid intima media thickness and helminth infections on Flores Island, Indonesia. PLoS One 8:e54855
Yamada M, Matsuda S, Nakazawa M et al (1991) Species-specific differences in heterogonic development of serially transferred free-living generations of *Strongyloides planiceps* and *Strongyloides stercoralis*. J Parasitol 77:592–594
Yasuda K, Muto T, Kawagoe T et al (2012) Contribution of IL-33–activated type II innate lymphoid cells to pulmonary eosinophilia in intestinal nematode-infected mice. Proc Natl Acad Sci U S A 109:3451–3456
Yoshimoto T, Yasuda K, Tanaka H et al (2009) Basophils contribute to Th2-IgE responses in vivo via IL-4 production and presentation of peptide-MHC class II complexes to CD4+ T cells. Nat Immunol 10:706–712

Younis AE, Soblik H, Ajonina-Ekoti I et al (2012) Characterization of a secreted macrophage migration inhibitory factor homologue of the parasitic nematode *Strongyloides* acting at the parasite-host cell interface. Microbes Infect 14:279–289

Zhou S, Harbecke, Streit A (2019) From the feces to the genome: a guideline for the isolation and preservation of *Strongyloides stercoralis* in the field for genetic and genomic analysis of individual worms. Parasit Vectors 12:496–506. https://doi.org/10.1186/s13071-019-3748-5

Ziegler SF, Artis D (2010) Sensing the outside world: TSLP regulates barrier immunity. Nat Immunol 11:289–293

Żukiewicz M, Kaczmarski M, Topczewska M et al (2011) Epidemiological and clinical picture of parasitic infections in the group of children and adolescents from north-east of Poland. Wiad Parazytol 57:179–187

Chapter 13
Anisakiasis

Simonetta Mattiucci, Marialetizia Palomba, Serena Cavallero, and Stefano D'Amelio

Abstract Anisakiasis refers to the zoonotic disease provoked in humans by the accidental ingestion of the larvae of *Anisakis* spp. infecting fish or squid and consumed raw or/and undercooked. These anisakid nematodes are heteroxenous parasites involving marine mammals (mainly cetaceans) as definitive hosts, while crustaceans (krill), fish, and squid act as intermediate/paratenic hosts in their life cycles. This chapter briefly describes the taxonomy of species of *Anisakis*, our knowledge of the definitive and intermediate/paratenic hosts involved in their life cycle, and their geographical distribution. Nine species have so far been detected genetically as belonging to the genus *Anisakis*. Among these, *A. simplex* (*sensu stricto*) and *A. pegreffii* are so far found to play a zoonotic role in humans. The ingestion of infected seafood can provoke gastric anisakiasis (GA), intestinal anisakiasis (IA), gastro-allergic anisakiasis (GAA), or extragastrointestinal anisakiasis. Pathological aspects and the diagnosis of human anisakiasis are also reviewed, including an overview of current knowledge of the *Anisakis* allergens involved in the human immunological response. Finally, literature on possible control measures involving the inactivation of *Anisakis* larvae in fish fillets, thus reducing transmission to humans, is reported.

13.1 Introduction

The family Anisakidae includes species of nematodes whose adult stages can be found in fish, fish-eating birds, and marine mammals, whereas the third-stage larvae (the infective stage) are commonly present in the body cavity and muscles of numerous fish and squid species. Anisakid nematodes are of both medical and economic concerns, due to their public health implications and their associated

S. Mattiucci (✉) · M. Palomba · S. Cavallero · S. D'Amelio
Department of Public Health and Infectious Diseases, Section of Parasitology, "Sapienza—University of Rome", Rome, Italy
e-mail: simonetta.mattiucci@uniroma1.it

F. Bruschi (ed.), *Helminth Infections and their Impact on Global Public Health*,
https://doi.org/10.1007/978-3-031-00303-5_13

effects on the marketability of fish products, which are often exacerbated by frequent warnings in the media.

Larval forms of anisakid nematodes, in particular those belonging to the genera *Anisakis* and *Pseudoterranova*, are in fact the main causative agents of human "anisakidosis," a fish-borne parasitic zoonosis caused by the ingestion of raw or undercooked fish or cephalopods, which are infected by these larvae.

This chapter deals with those species of *Anisakis* considered the major etiological agents of human "anisakiasis" (the term is related only to *Anisakis s*pp. as a causative agent) throughout the world. However, Mattiucci et al. (2017a) consider the pathogenetic aspects and occurrence of zoonoses related to the species of *Pseudoterranova* and *Contracaecum*.

Interest in *Anisakis s*pp. has been growing constantly since human anisakiasis was first reported in the Netherlands in the 1960s and subsequently gained increasing health and economic relevance, particularly in countries where the consumption of raw fish and/or squid is common. Human cases are increasingly reported in Japan, the United States, and many European countries (the United Kingdom, France, Spain, and Italy). This alarm has also raised an interest in gathering information on epizootiological data from anisakid infections in fish and cephalopods, with particular regard to those of commercial value that are commonly form part of the human diet worldwide. Official authorities and public bodies have been often involved in assessing a precise scenario for the prevalence and parasite burden infections of *Anisakis* spp. in edible fish, as well as depicting risk maps according to geographical area, season, fish size, and other parameters (EFSA 2010).

13.2 The Agent

Morphological characters of taxonomic significance in anisakid nematodes are very few (i.e., features such as the excretory system, the alimentary canal, the number and distribution of male caudal papillae, the position of the vulva, and the length of the spicules) and are applicable to adult specimens only. Furthermore, these are often only relevant to male individuals, making the identification of many worms at the species level difficult (Fagerholm 1989; Paggi et al. 1998a, b; Mattiucci et al. 2005, 2009, 2018). Indeed, in anisakid nematodes, cladogenetic events have been accompanied by minimal morphological differentiation, as ecological factors have led to a convergence of similar and well-adapted morpho-functional solutions. This has given rise to a large number of morphologically identical but reproductively isolated ("sibling") species. Therefore, morphological traits do not always provide definitive evidence for their identification. The small number of diagnostic characters in adult individuals is even more dramatically marked in larval forms, where the number of structural traits useful for diagnostic purposes is very limited. *Anisakis* spp. larvae can be identified only to the generic level; this is mainly on the basis of the morphology and length of the glandular part of the esophagus (i.e., the "ventriculus") and the presence/absence of a caudal spine ("mucron"). Based on these

differences, type I and type II larvae (*sensu* Berland 1961) have been described morphologically. Similarly, at higher taxonomic levels among anisakids, several morphological characters, even if apparently readily differentiating one group from another, can appear to be homoplastic and not always related to the phylogeny of the species or genera within the group.

Thus, the limited taxonomic significance of some morphological characters and the occurrence of speciation processes virtually devoid of morphological differentiation undoubtedly advocate the use of molecular approaches as reliable tools for inferring the systematic relationships and evolution of anisakid nematodes and, consequently, their correct identification at the species level, with obvious implications for their epidemiology.

Accurate epizootiological and epidemiological studies must necessarily rely on the correct identification of the etiological agent involved. Since the 1980s, pioneer studies on the genetic structure of anisakid nematodes have been carried out using multilocus allozyme electrophoresis (MAE). This tool revealed the existence of a high level of genetic heterogeneity within certain anisakid morphospecies, such as *Anisakis simplex* (s. l.) (e.g., Nascetti et al. 1986). The biological species concept (BSC) (Mayr 1963) was well supported by the application of allozyme markers for several *Anisakis* species. Indeed, the known diversity of species belonging to *Anisakis* quickly increased after the detection of several sibling species (i.e., species which are morphologically very similar but reproductively isolated) and led to the discovery and description of several new species. Reproductive isolation and the absence of gene flow have been demonstrated by allozymes between sympatric and allopatric sibling species, establishing their specific status (Nascetti et al. 1986; Mattiucci et al. 1997, 2001, 2002, 2005, 2009; Paggi et al. 1998a).

The introduction of polymerase chain reaction (PCR)-derived molecular methodologies subsequently confirmed the taxonomic assessment of species of *Anisakis* based on allozyme markers. Reference individuals initially characterized by allozymes have been used to develop and establish DNA-based approaches for species identification, such as PCR-RFLP and direct sequencing of ITS rDNA (D'Amelio et al. 2000) or mitochondrial DNA (Valentini et al. 2006).

At present, allozymes (Mattiucci and Nascetti 2006), PCR-RFLP of rDNA (D'Amelio et al. 2000; Cavallero et al. 2011), DNA sequence analysis of nuclear (ITS region of the rDNA) (Nadler et al. 2005; Cavallero et al. 2011) and mitochondrial genes (mtDNA *cox2* and *rrnS)* (Nadler et al. 2005; Valentini et al. 2006; Mattiucci et al. 2009; Mattiucci et al. 2014a; Cavallero et al. 2011) and DNA microsatellite loci (Mattiucci et al. 2019; Bello et al. 2020) have demonstrated that the genus *Anisakis* comprises distinct species. Actually, they are the following:

1. The three species included in the *A. simplex* (*sensu lato*) complex, i.e., *Anisakis simplex* (Rudolphi, 1809) (*sensu stricto*), *A. pegreffii* Campana-Rouget & Biocca, 1955 (= *A. simplex* A of Nascetti et al. 1986), and *A. berlandi* Mattiucci, Cipriani, Webb, Paoletti, Marcer, Bellisario, Gibson & Nascetti et al. 2014a (= *A. simplex* C of Mattiucci et al. 1997).

2. The two closely related taxa *A. ziphidarum* Paggi, Nascetti, Webb, Mattiucci, Cianchi & Bullini, 1998a, and *A. nascettii* Mattiucci, Paoletti & Webb, 2009.
3. The three closely related species *A. physeteris* (Baylis, 1920); *A. brevispiculata* Dollfus, 1966; and *A. paggiae* Mattiucci, Nascetti, Dailey, Webb, Barros, Cianchi & Bullini, 2005.
4. Finally, *A. typica* (Diesing, 1860).

The existence of two major clades (Clade I and Clade II) has been demonstrated by different phylogenetic inferences (Valentini et al. 2006; Mattiucci et al. 2009, 2014a, b). Clade I comprises one subclade formed by *Anisakis simplex* (*s. s.*), *A. pegreffii*, and *A. berlandi* and a second one which encompasses the two species (*Anisakis ziphidarum* and *A. nascettii*), whereas three species currently belong to the main Clade II, i.e., *Anisakis physeteris*, *A. brevispiculata*, and *A. paggiae*. The position of *A. typica* as forming a distinct phylogenetic lineage with respect to other species has also been demonstrated; its position in the phylogenetic tree, as representing a sister taxon to other *Anisakis* species, has been discussed in phylogenetic analyses based on different genetic data sets (Cavallero et al. 2011; Mattiucci et al. 2014a, b, 2018).

Palm et al. (2008) and Mattiucci and Nascetti (2008) have genetically detected the existence of one additional taxon, closely related to *A. typica*, which has been recovered at larval stage from nonmigratory fish species in Balinese, Javanese, and Malaysian waters of the Pacific Ocean. The preliminary results appear to indicate that this taxon (provisionally indicated as *Anisakis* sp. 1) may be a sibling species of *A. typica* occurring in central Pacific waters (Mattiucci and Nascetti 2008). A further gene pool, referred to as *Anisakis* sp. 2, has been genetically detected by means of allozyme markers and mtDNA *cox2* sequence analysis based on larvae of type II from swordfish in the equatorial area (Mattiucci et al. 2007; Garcia et al. 2011) and in commercial fish from the Southeastern Pacific Ocean off the Peru coast (Aco Alburqueque et al. 2020).

Interestingly, while those *Anisakis* spp. which have been included in Clade I exhibit larvae of morphotype I, those species comprising Clade II have larvae of morphotype II. This means that at present, five species of *Anisakis* have type I and three species have type II larval morphology. In other words, the larval stages of *Anisakis* spp. cannot be identified at species level by means of morphological features but only by using genetic/molecular markers.

However, despite the limited morphological characters available in adults of *Anisakis* spp., a "reconciliation" between genetic and morphological traits has been possible with the use of more detailed morphological and morphometric analyses of sibling species which has resulted in the finding of diagnostic features which can be used for species recognition at the adult stage. This is the case for *A. paggiae* with respect to the closely related taxa *A. brevispiculata* and *A. physeteris* (see Mattiucci et al. 2001), for *A. nascettii vs A. ziphidarum* (see Mattiucci et al. 2009), and for *A. pegreffii*, *A. simplex (s. s.)*, and *A. berlandi* (see Quiazon et al. 2009; Mattiucci et al. 2014a, b, 2018).

13.3 Current Methods Used for the Identification of *Anisakis* spp.

The limited value of morphological analyses makes use of genetic and molecular methods necessary for the identification of species of *Anisakis*. The most used molecular/genetic methods are briefly reported below.

13.3.1 Allozyme Markers

Allozyme markers (19–24 enzyme loci) have been used extensively to identify large number of *Anisakis* spp. populations sampled from many geographical regions in the Boreal and Austral hemispheres, detect "sibling species," discover new species, and address questions concerning population genetics, evolutionary biology, and the relationship between genetic variability and habitat disturbance (Mattiucci and Nascetti 2008). These genetic markers have proved to be a cheap, effective tool for the identification of large numbers of *Anisakis* spp. larvae; for example, they have been used to identify thousands of *Anisakis* spp. larvae used as biological tags in fish stock assessment (Mattiucci et al. 2004, 2007, 2008).

13.3.2 PCR-RFLP Analysis

Notwithstanding the huge amount of data which have been obtained from the application of MAE, the development of molecular markers for the accurate identification of related species using PCR-based approaches is in some cases preferable, especially as this approach requires only small amounts of fresh or ethanol-fixed parasite material for analysis. For example, PCR-based restriction fragment length polymorphism (PCR-RFLP) (D'Amelio et al. 2000) and sequence analyses of the ribosomal DNA (rDNA) internal transcribed spacers (ITS-1 and ITS-2) (Nadler et al. 2005; Cavallero et al. 2011; Mattiucci et al. 2014a, b) provide a useful approach for the specific identification of species of *Anisakis* from different definitive and intermediate/paratenic hosts.

13.3.3 DNA Sequencing of Nuclear and Mitochondrial Gene Loci

Direct DNA sequencing of some genes has proven to be a fruitful tool for the identification of the different sibling and morphospecies of *Anisakis*. Sequence

data are now available for almost all of the nine species recognized within the genus, the only exception being *A. schupakovi*. In particular, the sequences of all nine species available in GenBank represent both nuclear and mitochondrial genes.

As for the nuclear genes, the region of the nuclear ribosomal DNA, spanning the final part of the 18S subunit, the first internal transcribed spacer (ITS-1), the 5.8S subunit, the second internal transcribed spacer (ITS-2), and the very beginning of the 28S subunit, has been sequenced for all of the *Anisakis* spp. (Cavallero et al. 2011). The ITS region of the rDNA exhibits a significant degree of variation between closely related species and between the different morphospecies of *Anisakis*, and it is therefore useful for species discrimination. On the other hand, concerted evolution tends to minimize the intraspecific variation in this genomic region, thus allowing an unambiguous attribution of one specified sequence to one corresponding species.

In the case of the mitochondrial DNA, two regions have been sequenced for all of the *Anisakis* taxa; these are the mitochondrial gene *cox2* (cytochrome oxidase 2) (Valentini et al. 2006; Mattiucci et al. 2009, 2014a, b) and the *rrnS* (the small subunit of the ribosomal DNA in the mitochondrial genome) (Nadler et al. 2005; D'Amelio et al. 2012; Mattiucci et al. 2014a, b). These mitochondrial markers have been able to distinguish all of the taxa which have been characterized genetically as belonging to *Anisakis*. The mtDNA *cox2* region shows a high degree of polymorphism at the intraspecific level; this finding supports the possible use of this gene in further studies of population genetics and phylogeography of *Anisakis* spp., as recently suggested for some species by Baldwin et al. (2011).

In recent years, other diagnostic nuclear loci have been discovered and used for the identification of three members of the *A. simplex* (s. l.) species complex. They are the sequence analysis of the EF1 a-1 nDNA (Mattiucci et al. 2016), the *nas*10 nDNA. Based on the alternative nucleotide positions (i.e., diagnostic) found between the three taxa, the development of the amplification-refractory mutation system (ARMS) based on the *nas10* nDNA was successfully used in a large number of specimens (Palomba et al. 2020; Bello et al. 2021), can distinguish a single base sequence difference using one-step PCR. Following an ARMS reaction, the presence or absence of a PCR product is diagnostic for the presence or absence of the target diagnostic alleles existing between the three *Anisakis* species. Thus, ARMS-PCR revealed to be an easy, cheap, sensible, and rapid method for the identification of three species of the *A. simplex* (s. l.) complex, to be used in a multilocus genotyping approach (Palomba et al. 2020).

13.3.4 DNA Microsatellite Loci (SSRs)

The development of DNA microsatellite markers (SSRs) (Mladineo et al. 2017; Mattiucci et al. 2019) has permitted to find additional diagnostic markers between the three sibling species of *A. simplex* (s. l.) complex to be used in a multilocus

genotyping approach to correctly assign to parental taxa or hybrid categories several specimens of the three species of *A. simplex* (s.s.), *A. pegreffii*, and *A. berlandi* collected from both allopatric and sympatric areas (Mattiucci et al. 2019; Bello et al. 2021). In addition, they have allowed to discover in those *Anisakis* species several sex-linked loci (Mattiucci et al. 2019).

13.3.5 Multiplex and Species-Specific PCR

Umehara et al. (2008) have developed a method based on multiplex PCR which was able to recognize six different species of anisakids, including *A. simplex* (*s. s.*) and *A. pegreffii*. The specificity of these primers for discriminating between the two sibling species is increased due to the introduction of artificial mismatched bases. Recently, PCR-sequence-specific primers have been developed by Abe (2008) in order to establish a quick method for the discrimination of *A. simplex* (*s. s.*) from *A. pegreffii*.

Once genetically detected and characterized, species of *Anisakis* have proved to be ecologically different in terms of host, life cycle, and geographical distribution. These data are presented in the next section.

13.3.6 Real-Time PCR

The use of real-time PCR (RT-PCR), while extensively applied in gene expression analyses, has been quite limited for genotyping purposes. Cavallero et al. (2014) used high-resolution melting to investigate the genetic profiles of *A. pegreffii*, *A simplex* (*s.s.*), and their putative hybrids. Aligned, derivative melt curves and the different plot melt curves produced uniquely different plots easily distinguishable for the two species. In addition, a primer/probe system for the identification of five species of anisakid nematodes belonging to the genera *Anisakis* (i.e., *A. pegreffii* and *A. simplex* (*s. s.*)) and *Pseudoterranova* (i.e., *P. decipiens (s. s.)*, *P. krabbei*, and *P. bulbosa*) to be used in a RT-PCR with specific primers based on the mtDNA *cox2* diagnostic gene locus was developed and validated on a large number of nematodes belonging to those species (Paoletti et al. 2018). Because those anisakid species could be also found in coinfection in some fish species with the raphidascarid nematode *Hysterothylacium aduncum*, a species-specific primer probe system to be used in RT-PCR for this nematode species was also developed in the same primers/probe assay (Paoletti et al. 2018).

13.4 Life Cycle, Hosts, and Geographical Distribution of *Anisakis* spp.

Anisakis species have complex, indirect life cycles which involve various marine organisms at different levels of the trophic web in the marine ecosystem (Fig. 13.1). The adults live in the stomach of marine mammals, mainly cetaceans. The life cycle begins when female worms release eggs which are passed in the feces of their definitive host into the sea. According to some experimental studies, the full eggs are embryonated, and the larvae molt within the eggs, resulting in the third-stage larva (Køie et al. 1995; Højgaard 1998). The eggs are ingested by crustaceans, such as copepods and euphausiids (krill), in which they grow into their hemocoel. Fish or squid (Cephalopoda, Decapodiformes) become infected after eating an infected crustacean; the third-stage larva bores through the digestive tract wall of the fish or squid, passes into the visceral body cavity and undergoes to host-induced encapsulation (Levsen and Berland 2012). The life cycle is completed after the intermediate/paratenic host (fish, squid, or, directly, a crustacean) is predated by the definitive host. Inside the stomach or the intestine of its final mammalian host, *Anisakis* spp. undergo two final molts and develop into a sexually mature adult nematode.

Since *Anisakis* larvae do not undergo any development or molt inside the fish or squid, these hosts should be regarded as paratenic in terms of the nematode life cycle. Small fish and squid are frequently predated by larger fish species, which form an additional paratenic host in the cycle. This is important from an epidemiological point of view, because the repeated transmission of *Anisakis* larvae between hosts in the prey-predatory system enables an extensive bioaccumulation of infection in fish of a greater size. Several pelagic and demersal fish species show an increase in the prevalence and abundance of *Anisakis* larvae with age and size (Mattiucci et al. 2004; Levsen and Lunestand 2010; Levsen and Berland 2012). Such fish hosts can accumulate hundreds of *Anisakis* spp. larvae during life history. In contrast, an opposite trend has been observed in the fish species *Scomber scombrus* in the North Sea, where it has been suggested that infection levels could be influenced by host- and/or age-specific fish immunological characteristics (Levsen and Berland 2012).

In infected fish, the majority of the larvae are found in the visceral body cavity, typically encapsulated outside the organs; however, a certain number of larvae may migrate from the visceral cavity to the flesh of the fish, mostly to the belly flap of the fish, but also to the dorsal musculature. It has been demonstrated that in some fish hosts, migration occurs during the life of the fish (Karl et al. 2011) and not post-mortem as commonly believed. The occurrence of larvae in fish fillets does, however, represent a biological hazard to humans, following the consumption of raw or inadequately cooked fish. However, it has been suggested that different species of *Anisakis* larvae may have a different capacity to migrate and infect the fish fillets (Chou et al. 2010; Quiazon et al. 2011a).

While an extensive literature has been produced on the systematics and epidemiology of *Anisakis* spp., very little is known of the pathological significance of the

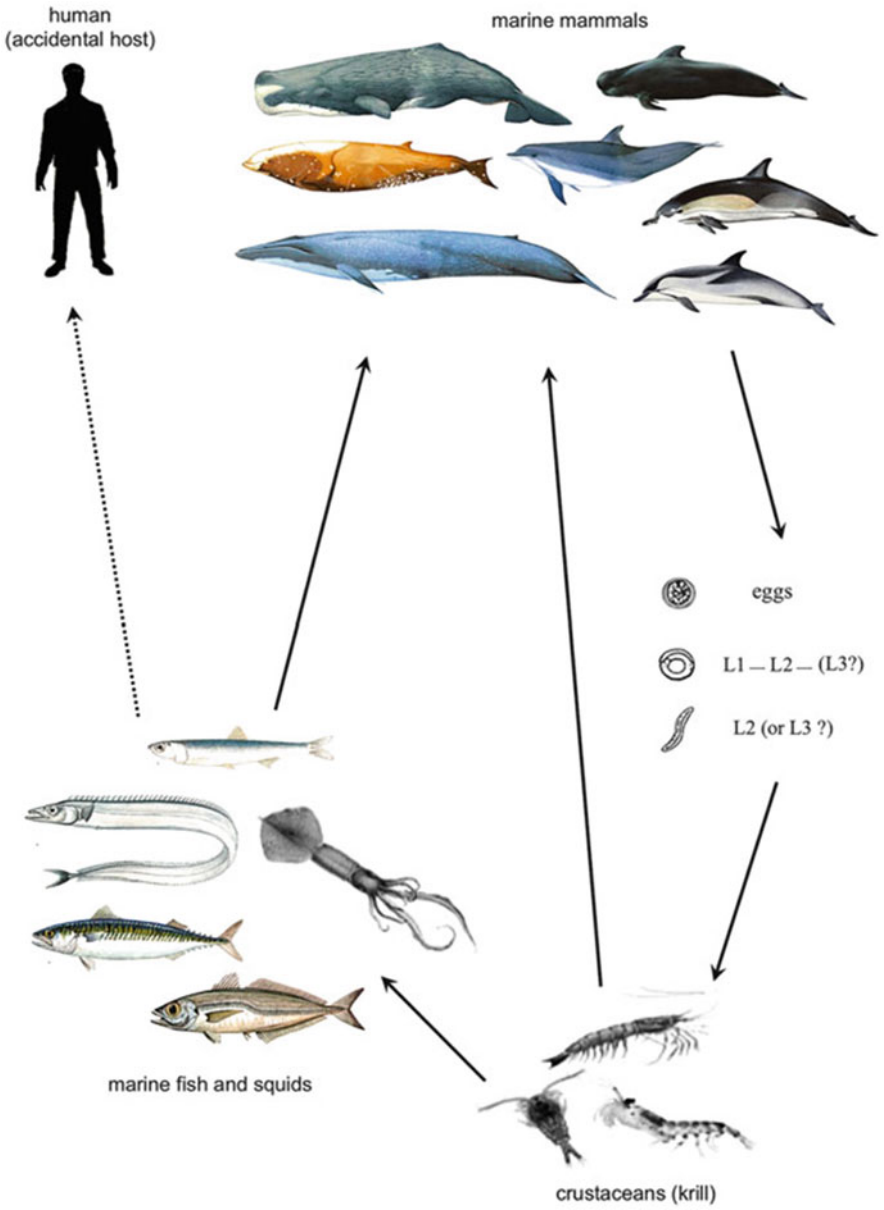

Fig. 13.1 Life cycle of *Anisakis* spp.

occurrence of the parasite in its fish host in relation to condition factor and general fitness. Some pathobiological changes have been reported in commercially important fish hosts in relation to *A. simplex* (*s. s.*), such as "stomach crater syndrome" in larger fish, e.g., cod (Berland 1961). This consists of a host-induced encapsulation in

the gastric mucosa of the fish, due to the fact that the thickness of the stomach wall impedes larval migration. This finding has been observed in other fish species, such as large swordfish (Mattiucci et al. submitted) and bluefin tuna (Mattiucci pers. obs.). "Red vent syndrome" (RVS, i.e., bleeding, swollen, and hemorrhagic vents) of wild Atlantic salmon has been correlated with large numbers of encapsulated *A. simplex* (*s. s.*) larvae in fish tissue at the level of vent and urogenital papilla (Beck et al. 2008; Noguera et al. 2009). In histopathological terms, the red vent exhibits gross lesions characterized by hemorrhages and moderate to severe inflammation dominated by eosinophilic granular cells and melanomacrophages around encapsulated *Anisakis* larvae in the host tissue (Noguera et al. 2009). It has been noted, however, that despite the severity of the infection of *Anisakis* spp. larvae in parasitized hosts, infected fish were generally in good overall condition.

13.4.1 Host Preference

Presented below is a synopsis of ecological factors, in terms of their definitive and intermediate/paratenic hosts and known geographical distribution, for the nine species of *Anisakis* which have been genetically determined.

Generally, *Anisakis* spp. larvae have been detected worldwide in Gadiformes, Perciformes, Clupeiformes, Pleuronectiformes, Scorpaeniformes, Zeiformes, Bericiformes, Lophiiformes, Anguilliformes, and Atheriniformes (Table 13.1). *Anisakis* spp. larvae have been also detected in a variety of cephalopods and, rarely, in elasmobranchs. Among the squid, they have been found mainly in the Ommastrephidae (Table 13.1). The known data on the occurrence of the species *A. pegreffii* and *A. simplex* (*s. s.*), i.e., those known as etiological agents of human anisakiasis, in fish and squid species from different fishing grounds have been reviewed (Mattiucci et al. 2018) and summarized in Table 13.1.

In the stomach of cetaceans, *Anisakis* spp. adults (Table 13.2) are often found free in the lumen and sometimes in clusters embedded in the mucosa and submucosa.

The presence of the two main clades (Clade I and Clade II), as presented above in the section on phylogenetic relationships between *Anisakis* spp., is also supported by ecological data relating to specific definitive host-*Anisakis* spp. relationships.

Sperm whales, *Physeter catodon*, *Kogia breviceps*, and *K. sima* (Table 13.2), are the main definitive hosts for *A. physeteris*, *A. brevispiculata*, and *A. paggiae*, respectively, which cluster in Clade II of the phylogenetic tree of *Anisakis* spp. (Mattiucci and Nascetti 2008; Mattiucci et al. 2009, 2014a, b; Cavallero et al. 2011). Several oceanic dolphins of the Delphinidae, Arctic dolphins of the Monodontidae, and porpoises of the Phocoenidae (Table 13.2) are hosts of the species *A. pegreffii*, *A. simplex* (*s. s.*), and *A. berlandi* (Mattiucci et al. 1997, 2014a, b, 2018; Mattiucci and Nascetti, 2008; Cavallero et al. 2011), which are the most derived group of species included in the main Clade I obtained in the *Anisakis* phylogenetic tree analysis.

Table 13.1 Intermediate/paratenic hosts so far detected, using molecular genetic markers, for *Anisakis* spp.

	A. simplex (*s. s.*)	*A. pegreffii*	*A. berlandi*	*A. typica*	*A. ziphidarum*	*A. nascettii*	*A. physeteris*	*A. brevispiculata*	*A. paggiae*
Cephalopods									
Sepiidae *Sepia officinalis*	IC	–	–	–	–	–	–	–	–
Ommastrephidae *Illex coindetii*	IC	WM	–	–	–	–	–	–	–
Moroteuthis ingens	–	–	MI, NZ	–	–	–	–	–	–
Nototodarus sloanii	–	NZ	.-	–	–	–	–	–	–
Todarodes pacificus	KYS	KYS	–	KYS	–	–	–	–	–
Todarodes sagittatus	IC, NEA	NAM	–	–	–	–	–	–	
Todaropsis angolenis	–	SA	SA						
Todaropsis eblanae	IC	IC, SA	–	–	–	–	–	–	–
Histiotheutidae									
Histioteuthis bonnellii	–	CM	–	–	–	–	–	–	–
Fishes									
Anguillidae *Synapobranchus kaupii*	–	–	–	–	–	–	CSA	–	–
Anoplogastridae *Anoplogaster cornuta*	–	–	–	–	–	–	–	–	IRS

(continued)

Table 13.1 (continued)

	A. simplex (*s. s.*)	*A. pegreffii*	*A. berlandi*	*A. typica*	*A. ziphidarum*	*A. nascettii*	*A. physeteris*	*A. brevispiculata*	*A. paggiae*
Anoplopomatidae *Anoplopoma fimbria*	NEP	–	NEP	–	–	–	–	–	–
Belonidae									
Belone belone	IC	IC	–	–	–	–	–	–	–
Berycidae *Beryx splendens*	–	–	–	–	–	–	JA	JA	JA
Bothidae *Arnoglossus laterna*	PC	–	–	–	–	–	–	–	–
Arnoglossus imperialis	–	PC	–	–	–	–	–	–	–
Bramidae									
Brama brama	–	SA	–	–	–	–	–	–	–
Carangidae *Decapterus macarellus*	–	–	–	PNG	–	–	–	–	–
Trachurus capensis	–	SA	–	–	–	–	–	–	–
Trachurus mediterraneus	MOR	CM, MOR	–	–	–	–	–	–	–
Trachurus picturatus	AZ, MD	AZ, MD	–	AZ, MD	–	–	–	–	–
Trachurus trachurus	NEA, IC, MA, WM	CM, EM, WM, IC, MA, NZ, MOR	–	EM	–	AZ	CM	–	–

Selar crumenophthalmus	–	–	–	CHS, HWA, CSA	–	–	–	–	–
Caesionidae *Caesio cuning*	–	–	–	IND	–	–	–	–	–
Centrophoridae *Deania profundorum*	AZ	–	–	–	–	–	–	–	–
Citharidae *Citharus linguatula*	PC	PC	–	–	–	–	–	–	–
Clupeidae									
Clupea harengus	NEA, BS, NEP, BAL, NS	–	–	–	–	–	–	–	–
Etrumeus whiteheadi	–	SA	–	–	–	–	–	–	–
Sardinops sagax	NEP	NEP	NEP	–	–	–	–	–	–
Congridae									
Conger conger	IC	CM	–	–	–	–	–	–	–
Astroconger myriaster	–	CHS	–	–	–	–	–	–	–
Coryphaenidae									
Coryphaena hippurus	–	–	–	SC	–	–	–	–	–
Emmelichthydae									
Emmelichthys nitidus nitidus	–	SA	–	–	–	–	–	–	–
Engraulidae									

(continued)

Table 13.1 (continued)

	A. simplex (*s. s.*)	*A. pegreffii*	*A. berlandi*	*A. typica*	*A. ziphidarum*	*A. nascettii*	*A. physeteris*	*A. brevispiculata*	*A. paggiae*
Engraulis encrasicolus	–	CM, WM	–	–	–	–	–	–	–
Etmopteridae *Etmopterus spinax*	AZ	–	–	–	–	–	AZ	–	–
Etmopterus pusillus	AZ	–	–	--	--	--	–	–	–
Etmopterus princeps	AZ	–	–	–	–	–	–	–	–
Gadidae									
Boreogadus saida	IC	–	–	–	–	–	–	–	–
Gadus macrocephalus	–	CHS	–	–	–	–	–	–	–
Gadus morhua	BS, IC	–	–	–	–	–	–	–	–
Melanogrammus aeglifinus	BS	–	–	–	–	–	–	–	–
Micromesistius poutassou	IC	CM, IC, NAM	–	–	–	–	–	–	–
Theragra chalcogramma	NEP, JA, BE	JA	–	–	–	–	–	JA	JA
Trisopterus luscus	IC	–	–	–	–	–	–	–	–
Gempylidae									
Thyrsites atun	–	–	SA	–	–	–	–	–	–
Gerreidae									
Gerres oblongus	–	–	–	PNG	–	–	–	–	–
Hexagrammidae									

Pleurogrammus azonus	NEA, JA	–	–	–	–	–	–	–	–
Lampridae									
Lampris guttatus	–	WM	–	–	–	–	CM, WM	–	–
Lophiidae									
Lophius piscatorius	IC	NAM, MOR	–	–	–	–	–	–	–
Lophius vomerinus	–	SA	–	–	–	–	–	–	–
Lotidae									
Molva dypterygia	IC	–	–	–	–	–	–	–	–
Brosme brosme	NEA	–	–	–	–	–	–	–	–
Lutjanidae *Pinjalo lewisi*	–	–	–	PNG	–	–	–	–	–
Pinjalo pinjalo	–	–	–	PNG	–	–	–	–	–
Macrouridae *Macruronus novaezelandiae*	–	–	NZ	–	–	–	–	–	–
Trachyrincus scabrus	MOR	MOR	–	–	–	–	–	–	–
Merluccidae									
Merluccius capensis	–	SA	–	–	–	–	–	–	–
Merluccius hubbsi	–	FA	–	–	–	–	–	–	–
Merluccius merluccius	NEA, IC, MA, NAM, MOR	CM, EM, WM, IC, NEA, MA, NAM, MOR	–	MA, EM, NAM, MOR	MA, MOR	IC	CM, MA, WM, IC, EM, NAM, MOR	MA	IC

(continued)

Table 13.1 (continued)

	A. simplex (*s. s.*)	*A. pegreffii*	*A. berlandi*	*A. typica*	*A. ziphidarum*	*A. nascettii*	*A. physeteris*	*A. brevispiculata*	*A. paggiae*
Myctophydae *Myctophum punctatum*	MAR	–	–	–	–	–	–	–	–
Gymnoscopelus nicholsi	–	SHI	SHI	–	–	–	–	–	–
Electrona carlsbergi	–	–	SHI	–	–	–	–	–	–
Muraenidae *Muraena helena*	–	NAM	–	–	–	–	–	–	–
Moridae									
Pseudophycis bachus	–	NZ	NZ	–	–	–	–	–	–
Dicentrarchus labrax	NEA, BB	CM	–	–	–	–	–	–	–
Nemipteridae *Nemipterus virgatus*	–	–	–	CHS	–	–	–	–	–
Nemipterus bathybius	–	–	–	CHS	–	–	–	–	–
Notothenidae *Notothenia coriiceps*	–	–	SHI	–	–	–	–	–	–
Notothenia rossii	–	–	SHI	–	–	–	–	–	–
Ophidiidae									
Genypterus capensis	–	SA	–	–	–	–	–	–	–

Osmeridae *Hypomesus pretiosus japonicus*	JA	–	–	–	–	–	–	–	–
Phycidae *Phycis phycis*	–	NAM	–	NAM	–	–	NAM	–	–
Phycis blennoides	–	NAM	–	–	–	–	NAM	–	–
Pinguipedidae									
Parapercis colias	–	NZ	NZ	–	–	–	–	–	–
Pleuronectidae									
Hippoglossus hippoglossus	BE	–	–	–	–	–	–	–	–
Platichthys flesus	–	–	–	PC	–	–	–	–	–
Rachycentridae *Rachycentron canadum*	TW	–	–	–	–	–	–	–	–
Petromyzontidae *Petromyzon marinus*	IC	–	–	–	–	–	–	–	–
Salmonidae									
Oncorhynchus gorbuscha	SI, ALA	–	–	–	–	–	–	–	–
Oncorhynchus keta	SI, KYS, ALA	–	–	–	–	–	–	–	–
Oncorhynchus nerka	ALA	–	–	–	–	–	–	–	–
Salmo salar	NWA	–	–	–	–	–	–	–	–
Schophtalmidae									
Lepidorhombus boscii	IC	IC, NAM	–	–	–	–	–	–	–

(continued)

Table 13.1 (continued)

	A. simplex (*s. s.*)	*A. pegreffii*	*A. berlandi*	*A. typica*	*A. ziphidarum*	*A. nascettii*	*A. physeteris*	*A. brevispiculata*	*A. paggiae*
Scomberesocidae									
Scomberesox saurus	NWA	–	–	–	–	–	–	–	–
Scombridae *Auxis rochei*	–	–	–	IND	–	–	–	–	–
Auxis thazard	–	–	–	BR, IND	–	–	–	–	–
Euthynnus affinis	–	–	–	SC	–	–	–	–	–
Euthynnus alletteratus	MOR	MOR	–	–	–	–	–	–	–
Katsuwonus pelamis	–	–	–	HWA, CSA	–	–	–	–	–
Rastrelliger kanagurta				THA					
Sarda orientalis	–	–	–	SC	–	–	–	–	–
Scomber australasicus	–	TW	–	TW	–	–	–	–	–
Scomber japonicus	AZ, MD, JA, MOR	AZ, MD, JA, KYS, NAM, MOR	–	AZ, MD, MOR	AZ, MD, MOR	–	MD, JA	–	–
Scomber scombrus	NEA, IC, NAM, MOR	CM, IC, NAM, MOR	–	NAM, MOR	MOR	–	NAM, JA	–	–
Scomberomorus commerson	–	–	–	SC	–	–	–	–	–
Scomberomorus maculatus	–	–	–	PNG	–	–	–	–	–
Scomberomorus niphonius	–	CHS							

Thunnus albacares	–	–	–	PNG	–	–	–	–	–
Thunnus thynnus	JA	CM, BR, NAM	–	BR, CM	–	–	–	–	–
Scorpaenidae									
Scorpaena scrofa	IC	IC	–	–	–	–	–	–	–
Sebastidae									
Helicolenus dactylopterus	–	CM, SA	–	–	–	–	–	–	–
Sebastolobus alascanus	–	–	NEP	–	–	–	–	–	–
Soleidae									
Dicologoglossa cuneata	–	PC	–	–	–	–	–	–	–
Solea senegalensis	PC	–	–	–	–	–	–	–	–
Sparidae									
Spondyliosoma cantharus	IC	–	–	–	–	–	–	–	–
Sternoptychidae									
Maurolicus muelleri	NEA	–	–	–	–	–	–	–	–
Trachichthyidae									
Hoplostethus atlanticus	–	–	TA	–	–	–	–	–	–
Hoplostethus mediterraneus	–	CM	–	–	–	–	–	–	–
Trachinidae *Echiichthys vipera*	–	NAM	–	–	–	–	–	–	–

(continued)

Table 13.1 (continued)

	A. simplex (*s. s.*)	*A. pegreffii*	*A. berlandi*	*A. typica*	*A. ziphidarum*	*A. nascettii*	*A. physeteris*	*A. brevispiculata*	*A. paggiae*
Trachinus draco	–	NAM	–	–	–	–	–	–	–
Trichiuridae									
Lepidopus caudatus	–	CM, SA	–	–	–	–	–	–	–
Aphanopus carbo	MD	MD	–	–	MD	AZ, MD	AZ	AZ	AZ
Trichiurus lepturus	JA	NAM, JA, KYS	–	BR, TW, JA, KYS, IND	–	–	–	–	–
Lepturacanthus savala	–	–	–	IND	–	–	–	–	–
Triglidae									
Eutrigla gurnardus	IC	–	–	–	–	–	–	–	–
Xiphiidae									
Xiphias gladius	NEA, NWA, CNA	CM, NAM	–	–	–	–	CM, IC, NEA, NWA, CNA, TEQ, CSA	NEA, NWA, TEQ, CSA	NEA, TEQ, CSA

Sampling locality codes: ALA, Alaska; AZ, Azores Islands; BB, Biscay Bay; BE, Bering Sea; BR, Brazilian Atlantic coast; BS, Barents Sea; CHS, China Sea; CM, Central Mediterranean Sea; CNA, Central North Atlantic Ocean; CS, Caribbean Sea; CSA, Central South Atlantic Ocean; EM, East Mediterranean Sea; FA, Falkland Islands; FL, Florida coast; HAW, Hawaii; IC, Iberian Atlantic coast; IND, Indian Sea; IRS, Irminger Sea (Greenland); JA, Japan Sea; KYS, Korea Yellow Sea; MA, Mauritanian coast; MAR, Mid-Atlantic Ridge; MI, Macquarie Island; MD, Madeira; MOR, Morocco; NAM, North African Mediterranean coast; NEA, Northeast Atlantic; NEP, Northeast Pacific; NS, North Sea; NWA, Northwest Atlantic; NZ, New Zealand; PC, Portuguese coast; PNG, Papua New Guinea; SWA, Southwest Atlantic; SA, South Africa; SC, Somali coast; SHI, Shetland Islands; SI, Sakhalin Islands; TA, Tasman Sea; TEQ, Tropical Equatorial Atlantic; THA, Thailand; TW, Taiwan; WM, West Mediterranean (data from Bao et al. 2013; Bernardi et al. 2011; Cavallero et al. 2012; Chou et al. 2011; Dzido et al. 2009; Farjallah et al. 2008a, b; Garcia et al. 2011; Kuhn et al. 2011; Mattiucci et al. 1986, 1997, 2001, 2002, 2004, 2005; Mladineo et al. 2012; Nascetti et al. 1986; Orecchia et al. 1986; Paggi et al. 1998a, b; Abollo et al. 2001; Pontes et al. 2005; Marques et al. 2006; Mattiucci and Nascetti 2006, 2007; Serracca et al. 2013; Setyobudi et al. 2010; Shih et al. 2010; Cipriani et al. 2019, 2021; Palomba et al. 2021)

Table 13.2 Definitive hosts so far detected, using molecular genetic markers, for *Anisakis* spp.

	A. simplex (*s. s.*)	*A. pegreffii*	*A. berlandi*	*A. typica*	*A. ziphidarum*	*A. nascettii*	*A. physeteris*	*A. brevispiculata*	*A. paggiae*
Cetaceans									
Balenopteridae									
Balaenoptera acutorostrata	NEA	–	–	–	–	–	–	–	–
Delphinidae *Delphinus capensis*	–	NZ	–	–	–	–	–	–	–
Delphinus delphis	IC	IC	–	–	–	–	–	–	–
Globicephala melas	IC, SA, NEA	NZ	SA, NZ	–	–	–	–	–	–
Globicephala macrorhynchus	FL	–	–	FL	–	–	–	–	–
Lagenodelphis hosei	–	FL	–	FL	–	–	–	–	–
Lagenorhynchus albirostris	NEA	–	–	–	–	–	–	–	–
Lissodelphis borealis		–	NEP	–	–	–	–	–	–
Orcinus orca	NEP	–	–	–	–	–	–	–	–
Peponocephala electra	–	–	–	BR	–	–	–	–	–
Pseudorca crassidens	NEP	–	NEP		–	–	–	–	–
Sotalia fluviatilis	–	–	–	BR	–	–	–	–	–
Sotalia guianensis	–	–	–	BR	–	–	–	–	–
Stenella clymene	–	–	–	BR	–	–	–	–	–

(continued)

Table 13.2 (continued)

	A. simplex (*s. s.*)	*A. pegreffii*	*A. berlandi*	*A. typica*	*A. ziphidarum*	*A. nascettii*	*A. physeteris*	*A. brevispiculata*	*A. paggiae*
Stenella coeruleoalba	IC	SA, MS	–	EM, FL	–	–	–	–	–
Stenella attenuata	–	–	–	FL, CS	–	–	–	–	–
Stenella longirostris	–	–	–	BR, FL	–	–	–	–	–
Steno bredanensis	FL	–	–	CS, BR, FL	–	–	–	–	–
Tursiops truncatus	FL	CM, SA, WM	–	FL, CS	–	–	–	–	–
Kogiidae									
Kogia breviceps	FL, NWA	–	–	BR	–	–	FL, NWA	SA, IC, FL, NWA	SA, FL, NWA
Kogia sima	–	–	–	NWA, FL	–	–	–	CS, NWA, FL	FL, CS, NWA
Monodontidae									
Delphinapterus leucas	NWA	–	–	–	–	–	–	–	–
Neobalenidae									
Caperea marginata	–	SA	–	–	–	–	–	–	–
Phocoenidae									
Phocoena phocoena	NEP, NS	–	–	–	–	–	–	–	–
Physeteridae									
Physeter macrocephalus	–	–	–	–	–	–	CM, FL	–	–
Ziphiidae									
Mesoplodon bowdoini	–	–	–	–	NZ	NZ	–	–	–

Mesoplodon densirostris	–	–	–	–	SA	NZ	–	–	–
Mesoplodon europaeus	–	–	–	–	FL, CS	–	–	–	–
Mesoplodon grayi	–	–	–	–	–	SA	–	–	–
Mesoplodon layardii	–	–	–	–	SA	NZ	–	–	–
Mesoplodon mirus	–	–	–	–	–	NZ	–	–	–
Ziphius cavirostris	–	–	–	–	CM, SA, CS	NZ	–	–	–
Pinnipeds									
Phocidae									
Mirounga augustirostris	–	–	NEP	–	–	–	–	–	–
Mirounga leonina	–	–	SHI	–	–	–	–	–	–

Sampling locality codes: AN, Antarctica; AZ, Azores; BE, Bering Sea; BR, Brazilian Atlantic coast; BS, Barents Sea; CM, Central Mediterranean Sea; CS, Caribbean Sea; EM, East Mediterranean Sea; FA, Falkland Islands; FL, Florida coast; IC, Iberian Atlantic coast; JA, Sea of Japan; MA, Mauritanian coast; MD, Madeira; MS, Mediterranean Sea; NAM, North African Mediterranean coast; NEA, Northeast Atlantic; NEP, Northeast Pacific; NS, North Sea; NWA, Northwest Atlantic; NZ, New Zealand; PC, Portuguese coast; SA, South Africa; SC, Somali coast; SI, Sakhalin Islands; TA, Tasman Sea; WM, West Mediterranean (data from Carvalho et al. 2010; Cavallero et al. 2011, 2012; Colom-Llavina et al. 2009; Klimpel et al. 2011; Quiazon et al. 2009; Mattiucci and Nascetti 2006, 2007; Mattiucci et al. 1986, 1997, 2001, 2002, 2004, 2005; Nascetti et al. 1986; Orecchia et al. 1986; Paggi et al. 1998a, 1998b)

Beaked whales, *Ziphius cavirostris*, *Mesoplodon layardii*, *M. mirus*, *M. grayi*, *M. densirostris*, and *M. europeus*, are hosts of *A. ziphidarum* (Table 13.2) (Paggi et al. 1998a, b; Mattiucci et al. 2009; Cavallero et al. 2011) and *A. nascettii* (Mattiucci et al. 2009, 2014a, b, 2018; Pontes et al. 2005) which are separated as a subclade and included in the main Clade I of the *Anisakis* phylogenetic tree. Thus, the phylogenetic relationships (Mattiucci and Nascetti 2008) of *Anisakis* species mirror those proposed for their cetaceans definitive hosts (Milinkovitch 1995; Cassens et al. 2000; Nikaido et al. 2001; Arnason et al. 2004). Elaboration of these empirical results in order to assess the global congruence of the co-phylogenetic relationship between the host and parasite trees determined by ParaFit (Legendre et al. 2002) was statistically significant ($P < 0.05$) (Mattiucci and Nascetti 2008). Individual host-parasite associations which contributed more to the co-phylogenetic cetacean-*Anisakis* spp. mapping were represented by those between *A. physeteris* and *Physeter catodon*, *A. brevispiculata* and *Kogia breviceps*, and *A. ziphidarum* and *Mesoplodon* spp., suggesting host-parasite co-speciation events, whereas a less significant contribution to the total test was that formed by the host-parasite association *A. simplex* (*s. s.*) and *Balaenoptera acutorostrata*, suggesting a possible host-switching event (Mattiucci and Nascetti 2008).

13.4.2 Geographical Distribution of Nine Species of Anisakis

Despite the fact that only *A. simplex* (*s. s.*) and *A. pegreffii* are known causative agents of human anisakiasis (see Sect. 13.5), a pathogenic role for other species belonging to the genus cannot be excluded. This is also due to the presence of the above species in a wide range of intermediate/paratenic hosts of commercial importance and to the fact that the molecular identification to the species level of the etiological agent causing human anisakiasis has been possible only in recent years. Therefore, we consider it useful to summarize below the geographical distribution of all *Anisakis* species which have been detected genetically.

Anisakis simplex (Rudolphi, 1809) (*sensu stricto*) is widespread between 35°N and the Arctic Circle; it is present in both the western and eastern Atlantic and Pacific Oceans (Mattiucci et al. 1997, 1998; Abollo et al. 2001; Nadler et al. 2005; Umehara et al. 2006, 2008; Abe et al. 2005, 2006; Quiazon et al. 2009, 2011b) . The southern limit of this species in the Northeast Atlantic Ocean is the waters around the Gibraltar region. *A. simplex* (*s. s.*) is also occasionally present in western Mediterranean waters due to the migration of pelagic fish species into the Alboran Sea from the Atlantic (Mattiucci et al. 2004, 2007; Mattiucci and Nascetti 2008). It has so far been genetically recognized as occurring in several species of cetacean hosts (Table 13.1). Several squid and fish species have been found harboring larvae of this species throughout its geographical range. A sympatric area between *A. simplex* (*s. s.*) and *A. pegreffii* has been identified along the Spanish and Portuguese Atlantic coasts (Mattiucci et al. 1997, 2004, 2007, 2008; Abollo et al. 2001; Pontes et al. 2005; Marques et al. 2006; Hermida et al. 2012), in the Alboran Sea (Mattiucci et al.

2004, 2007), and in the Sea of Japan (Umehara et al. 2006; Quiazon et al. 2009, 2011b). In areas of sympatry, few F1 hybrid individuals between *A. pegreffii* and *A. simplex* (*s. s.*) have been recognized using allozymes (Mattiucci et al. 2004); however, several individuals exhibiting recombinant genotypes at the ITS of the ribosomal DNA between *A. pegreffii* and *A. simplex* (*s. s.*) have been recorded using PCR-RFLP markers, but their status as hybrid forms between the two species has not so far been confirmed (Abollo et al. 2003), and recent studies have evidenced that studies on ITS may overestimate the real extent of hybridization (Roca-Geronès et al. 2021). *A. simplex* (*s. s.*) also occurs in sympatry with *A. berlandi* in the Eastern Pacific Ocean, where it has been identified in definitive and intermediate/paratenic hosts (Mattiucci et al. 1997, 1998, 2018; Paggi et al. 1998a, b). Recently, the first demonstration of the occurrence of hybridization events between *A. pegreffii* and *A. berlandi* in a contact zone in the Southern Pacific Ocean waters (from off New Zealand waters) was provided by using a multilocus nuclear genotyping approach, including both SSR loci and ARMS at *nas10* nDNA gene locus (Bello et al. 2021).

Anisakis pegreffii Campana-Rouget & Biocca, 1955 is the dominant species of *Anisakis* in the Mediterranean Sea, being widespread in many fish species. Indeed, it is presently the most important anisakid nematode in several pelagic and demersal fish from Mediterranean waters (Paggi et al. 1998b; Mattiucci and Nascetti 2008; Farjallah et al. 2008a; Chaligiannis et al. 2012; Cavallero et al. 2012; Mladineo et al. 2012; Serracca et al. 2013; Cipriani et al. 2017a, b). It is also widely distributed at both adult and larval stages in the Austral Region between 35°N and 55°S (Mattiucci et al. 1997, Mattiucci et al. 2014a, b, 2018). In Atlantic waters, the northerly limit of its geographical range is represented by the Iberian and Portuguese coasts (Mattiucci et al. 1997, 2004, 2007; Abollo et al. 2001; Pontes et al. 2005; Marques et al. 2006; Hermida et al., 2012). It has been detected, at the larval stage, in some fish hosts from Japanese marine waters (Abe et al. 2006; Umehara et al. 2006, 2008; Quiazon et al. 2009, 2011b) and in Chinese marine waters (Zhu et al. 2007).

Anisakis berlandi Mattiucci, Cipriani, Webb, Paoletti, Marcer, Bellisario, Gibson & Nascetti in press (= *A. simplex* C of Mattiucci et al. 1997) exhibits a discontinuous range, including the Canadian and Chilean Pacific coasts, New Zealand waters, and South African Atlantic coast (Mattiucci et al. 1997, unpubl. Obs.; Nadler et al. 2005). This species has been identified at the adult stage in cetaceans as occurring syntopically with *A. pegreffii* (Irigoitia et al. 2020) and as a larva in some fish species (Table 13.1). It has also occasionally been identified in the seals *Mirounga leonina* from the sub-Antarctic area (Mattiucci and Nascetti 2008) and in *M. angustirostris* from Northeast Pacific Ocean (Nadler et al. 2005).

Anisakis typica (Diesing, 1860) has a range extending from 30°S to 35°N in warmer temperate and tropical waters (Mattiucci et al. 2002; Cavallero et al. 2012). In these areas, it has been found as an adult in species of dolphin and as a larva in several fish species (Tables 13.1 and 13.2). *A. typica* has also been identified in cetaceans and fish from the eastern Mediterranean Sea (off Cyprus). Its presence in these waters could be the result of the "Lessepsian migration" (through the Suez Canal) (Mattiucci et al. 2004) of its intermediate/paratenic hosts from the Indian

Ocean. It has also been recognized in flatfishes captured in central Portuguese waters of the Northeast Atlantic (Marques et al. 2006) and only rarely in some fish species caught along the North African coast (Tunisia and Lybia) of the Mediterranean Sea (Farjallah et al. 2008a). *A. typica* larvae have also been identified in fish from Chinese marine waters (Zhu et al. 2007).

Anisakis ziphidarum Paggi, Nascetti, Webb, Mattiucci, Cianchi & Bullini, 1998 was first described, both genetically and morphologically, as an adult in beaked whales from the South Atlantic Ocean (off the South African coast) and in the Mediterranean Sea. This species has also been found in the Central Atlantic, including the Caribbean Sea (Colom-Llavina et al. 2009; Cavallero et al. 2011), and in the South Pacific waters (off the New Zealand coast). Thus, its geographical range appears to be wide and related to that of its definitive hosts. Only very limited data are available concerning its infection in fish and/or squid, but it is responsible for a low prevalence of infection in some fish species in Central Atlantic waters (Mattiucci et al. 2004; Pontes et al. 2005; Hermida et al. 2012). However, it seems that this species may involve other intermediate hosts, such as squid, rather than fish in its life cycle, as these represent the main food source of beaked whales (Mattiucci and Nascetti 2008).

Anisakis nascettii Mattiucci, Paoletti & Webb, 2009 has been detected at the adult stage in beaked whales from New Zealand waters and from off the South African coast. It has also been identified at L4 stage in ziphiid cetaceans from the Central Atlantic Ocean (Iglesias et al. 2008). This species has been identified genetically, at the larval stage, as heavily infecting the squid *Moroteuthis ingens* in the Tasman Sea. This appears to support the hypothesis that this species involves squids rather than fish in its life cycle (Mattiucci et al. 2009).

Anisakis physeteris (Baylis, 1920) was first genetically characterized in its main definitive host, the sperm whale *Physeter macrocephalus*, from Mediterranean waters (Mattiucci et al. 1986). Genetically identified adults have also been recorded in the Central Atlantic Ocean (Mattiucci and Nascetti 2008; Cavallero et al. 2011).

The infection has been also recorded in the pygmy sperm whale, *Kogia* spp., from the Caribbean Sea and Gulf of Mexico (Cavallero et al. 2011) and in the Mediterranean Sea (Santoro et al. 2018). Its type II larvae have been found rarely occurring in pelagic and demersal fish species (Mattiucci et al. 2018), while squid species belonging to the family Histioteuthidae from deep waters of the Mediterranean Sea have been found infected by larvae of *A. physeteris* (Palomba et al. 2021).

Anisakis brevispiculata Dollfus, 1966 has been characterized genetically using allozymes (Mattiucci et al. 2001), the mtDNA *cox2* gene (Valentini et al. 2006), and ITS rDNA sequence analysis (D'Amelio et al. 2000; Nadler et al. 2005) based on material from a pygmy sperm whale, *Kogia breviceps*, in South African and Northeast Atlantic waters (off Iberian coast). Type II *Anisakis* larvae corresponding to *A. brevispiculata* have been recognized using allozyme markers as a rare parasites of the fish *Merluccius merluccius* (see Mattiucci et al. 2004) and heavily infecting the swordfish *Xiphias gladius* in tropical-equatorial Atlantic waters (Garcia et al. 2011).

Anisakis paggiae Mattiucci, Nascetti, Dailey, Webb, Barros, Cianchi & Bullini, 2005 was first genetically characterized and described morphologically as an adult

parasite of the pygmy sperm whale and the dwarf sperm whale off both Florida and the South African Atlantic coast (Mattiucci et al. 2005). It has also been identified in kogiids from the Caribbean Sea (Cavallero et al. 2011). In recent years, this parasite species was also identified in *Kogia sima* from the Sea of Japan (Quiazon et al. 2013a).

13.5 The Human Response to the Parasite

Third-stage larvae of *Anisakis* spp. infecting the flesh of marine fish or squid, if ingested alive by humans, can cause the zoonotic disease "anisakiasis." The transmission of this fish-borne pathogen is particularly associated with the tradition of consumption of raw or undercooked fish. A number of fish dishes are considered to be of high risk for human anisakiasis. They include, among others, the Scandinavian gravlax, Dutch salted and marinated herring, Japanese sushi and sashimi, Spanish boquerones and anchovies, and Italian marinated anchovies. First reported in the Netherlands, anisakiasis has acquired an increasing health and economic relevance especially in countries such as Japan, where the consumption of raw fish and squid is frequent, although human cases are increasingly reported from many European countries (Spain, Italy, the United Kingdom, and France). In recent years, due to the popularity and increased consumption of Japanese dishes, as well as the consumption of small, pickled fish, more and more cases of anisakiasis have been reported worldwide (Audicana and Kennedy 2008). However, notification of human anisakiasis is not mandatory, and consequently, despite several reported cases over time, it remains an underestimated zoonosis.

As indicated above, of the nine species of *Anisakis* which have been characterized genetically, only two, i.e., *A. simplex* (*s. s.*) and *A. pegreffii*, have so far been reported as causative agents of human anisakiasis (D'Amelio et al. 1999; Moschella et al. 2004; Umehara et al. 2008; Fumarola et al. 2009; Mattiucci et al. 2011, 2013b).

13.5.1 Immunopathological Processes

The pathological changes occurring within the gastrointestinal tract during an infection by *Anisakis* app. Larvae are the combined result of the direct invasive capacity of the larva and the interaction between the host's immune response and the antigens released by the infective larvae during the invasion. *Anisakis* larva release proteolytic enzymes in order to invade the gastrointestinal mucosa. These antigenic proteins have been isolated and characterized as excretory-secretory antigens (E/S) (Moneo et al. 2000; Shimakura et al., 2004; Rodriguez-Perez et al. 2008; Caballero et al. 2011; Kobayashi et al. 2011). Humoral and cellular responses are also involved

in the infections with *Anisakis* larvae. Th2 cytokine production and the resulting mastocytosis, IgE response, and eosinophilia characterize local inflammatory lesions produced by *Anisakis* spp. larvae. Eosinophilic infiltration in the tissues surrounding the parasite has been reported in both acute and chronic infections. Eosinophilic cell concentration in damaged areas is related not only to the production of chemotactic factors released by T lymphocytes, mast cells, and basophils but also to some chemotactic substances produced directly by *Anisakis* larvae. Eosinophilic infiltration is the most effective process in the destruction of larvae at the local level (gastrointestinal tract). The presence of eosinophilic cells characterizes a late stage of type I immune hypersensitivity in response to *Anisakis* infection.

13.5.2 Histopathology of Anisakiasis

From the histopathological point of view, anisakiasis may be classified into the following four sequential stages. The first stage is "phlegmon formation," and the second is the "abscess formation," which is rather frequent in gastric anisakiasis and is characterized by abundant necrotic tissue around the larvae and by a rich population of eosinophils. The third stage is "abscess-granuloma formation," which corresponds to the flogistic evolution of the disease at least 6 months after the ingestion of the larva; at this stage, the larva is in the form of few remnants which are invaded by eosinophils, surrounded by giant cells and abundant inflammatory parvicellular infiltrate. Finally, the most advanced stage is "granuloma formation," characterized by a further decrease in the presence of eosinophils, but with abundance of lymphocytes, giant cells and significant collagenization (Kikuchi et al. 1990).

13.6 Clinical Manifestations of Anisakiasis

On the basis of the site reached by the live ingested *Anisakis* larva, the disease could be subdivided into in gastric anisakiasis (GA) and intestinal anisakiasis (IA). The acute form appears to be gastric and is characterized by nausea, vomiting, and epigastric pain. These symptoms appear 1–6 hours after the ingestion of the infected fish. In intestinal anisakiasis, acute signs start to appear about 7 days after infection in the form of abdominal pain, nausea, vomiting, fever, diarrhea, and fecal occult blood. Several, rarely occurring, extragastrointestinal sites have been also documented (i.e., oro-pharyngeal, abdominal cavity, mesenteries, and omentum). Anisakiasis of the digestive tract is classified, from the clinical point of view, as "acute" or "moderate" and "invasive" or "not invasive," depending on the location

reached by the larva, if it has remained in the gastric and/or intestinal lumen, and by its capacity to invade or not the submucosal layer of the gastric or intestinal wall.

13.6.1 Gastric Anisakiasis (GA)

Epigastric pain is the most frequent sign of acute GA. Other symptoms are nausea, vomiting, abdominal fullness or distension, anorexia, and chest pain. High fever is not a classical sign of GA, although it is quite frequently slightly higher than normal (37.5 °C). As for the localization in the gastric mucosa, Shibata et al. (1989) divided the stomach into four main parts, namely, anterior wall, lesser curvature, posterior wall, and greater curvature. The majority of larvae were found in the greater curvature, followed by the posterior wall. These authors also described several endoscopic findings as tissue events, such as edematous hypertrophic gastric folds, increase in gastric secretion and peristalsis, and mucosal lesions, including edema, redness, coagulation, hemorrhage, and ulceration. Cases of GA described recently from Italy (Mattiucci et al. 2013a, b) were characterized clinically by epigastric pain after 2 hours following the ingestion of raw seafood, vomiting, and other digestive symptoms. Endoscopic findings have showed that in most of the cases, the *Anisakis* larvae were mainly located in the lumen of the stomach and had not invaded the submucosal layer of the gastric wall.

13.6.2 Intestinal Anisakiasis (IA)

Most of the cases of intestinal infections with *Anisakis* are characterized by symptoms such as nausea, vomiting, and abdominal bulging, although numerous cases of asymptomatic intestinal anisakiasis have also been observed. The “mild form” of IA is characterized by eosinophilic granulomas forming “tumor-like” formations in the intestinal wall, whereas the “fulminant form” has the symptoms of acute ileus, acute appendicitis, acute abdomen, or regional ileitis. Cases of intestinal anisakiasis reported from Spain (Rosales et al. 1999) and from Italy (Moschella et al. 2004; Mattiucci et al. 2011) were characterized by a clinical picture of acute abdominal pain, acute appendicitis, or acute abdomen. Although difficult to evaluate, some biochemical tests based on leucocyte counts or percentage of eosinophils, as well as of enzymes GOT and GPT, can be of help in suspected cases of intestinal anisakiasis (Ishikura and Kikuchi 1990). Radiography or ultrasonographic images, showing the thickness of the intestinal wall, a marked dilatation of the intestine, the so-called keyboard sign and ascites pooling between the dilated intestines, have been reported as characterizing both “mild” and “fulminant” forms of IA (Ishikura and Kikuchi 1990).

13.6.3 Gastro-allergic Anisakiasis (GAA)

This is an acute allergic reaction in the context of an acute gastric presence of an *Anisakis* larva, when the live parasite attempts to invade the submucosal layer of the gastric wall (Daschner et al. 2011). GAA is characterized by urticaria, angioedema, and anaphylaxis; it consists of an acute IgE-mediated, generalized reaction. In this type of anisakiasis, the allergic reactions take place starting from 2 or 3 hours up to 2 or 3 days after the ingestion of an infected fish (Daschner et al. 2011; Mattiucci et al. 2013b). Recently, two GAA cases, characterized by urticaria and edema of the oral mucosa, due to *A. pegreffii*, were recognized by molecular methods in Italian patients after they had consumed "marinated anchovies" (Mattiucci et al. 2013b). In those cases, the endoscopic findings showed *Anisakis* larva invading the submucosal layer of the gastric wall. In addition, the serum samples from the patients showed IgE reactivity in WB analysis against *Ani s 1* antigen of *A. pegreffii* (see Table 13.3 and Sect. 13.6.4).

Table 13.3 Allergens of *Anisakis* (*Ani s*) so far characterized, with the percentage of IgE reactivity in human sera

Allergen	MW (kDa)	Location of the products	Major allergen	Panallergen	IgE reactivity (%)
Ani s 1	*24*	E/S	Yes		85
Ani s 1 isoform	21	E/S			?
Ani s 2	97	S	Yes	Yes	88
Ani s 3	41	S	Yes	Yes	4
Ani s 4	9	E/S			27
Ani s 5	15	E/S			25–49
Ani s 6	*7*	E/S			18
Ani s 7	139	E/S	Yes		83–100
Ani s 8	15	E/S			25
Ani s 9	14	E/S			13
Ani s 10	22	S (?)			39
Ani s 11	55	S (?)			47
Ani s 11-li	(?)	S (?)			?
Ani s 12	(?)	(?)	Yes		57
Ani s 13	37	E/S	Yes		
Ani s 14	27	(?)			

E/S, excretory-secretory products; S, somatic; (?) indicates unknown location in the larva or molecular weight

13.6.4 Anisakis *Allergy*

Live or dead anisakid larvae ingested with fish can lead to the onset of allergic reactions which are reported as frequently associated with high levels of the immunoglobulin IgE (Audicana and Kennedy 2008). "*Anisakis* allergy" was described for the first time in Japan (Ishikura and Kikuchi 1990) but was followed by a plethora of contradictory publications and research (for reviews, see Daschner et al. 2011; Nieuwenhuizen and Lopata 2013). In Spain, since 1995, more than 150 cases of allergy due to *Anisakis* have been reported (Del Pozo et al. 1997; Audicana and Kennedy 2008); more than 50% of the *Anisakis* allergic patients required emergency treatment, with 5 of 64 being hospitalized due to respiratory failure (Fernández de Corres et al. 2001). Although the usual signs of anisakiasis are characterized by urticaria, anaphylactic shock, and respiratory failure due to edema, Kikuchi et al. (1990) also referred to a possible association between *Anisakis* and rheumatic pathology. Additionally, cases of occupational *Anisakis* allergy were described in fishmongers or were related to exposure (either by contact or inhalation) to fish meal in chicken feed (Anibarro and Seoane 1998; Armentia et al. 1998). Clinical symptoms of allergic anisakiasis range from urticaria to anaphylactic shock. The diagnosis of anisakiasis can be complicated, as infections with helminths, in general, are associated with high levels of IgE and other immunoglobulins. Additionally, cross reactivity occurs with other parasite antigens.

To date, 14 *Anisakis (Ani s)* allergens have been characterized, numbered from *Ani s 1* to *Ani s 12* (Table 13.3), according to the Allergen Nomenclature designated by the WHO and IUIS. They include both somatic (S) and excretory/secretory (E/S) antigens, whereas some remain not well defined (Table 13.3). Purified allergens have been proved to be useful in the diagnosis of *Anisakis* allergy, especially in combination (Moneo et al. 2007).

Ani s 2 and *Ani s 3* allergens have a somatic (muscular) location in the *Anisakis* larva and have been shown to be paramyosin and tropomyosin. These are considered as "panallergens." A phylogenetic comparison of tropomyosin amino acid sequences, within and between different invertebrates, demonstrates that nematode tropomyosins of *Anisakis* and *Ascaris* are closely related to those of insect, crustaceans, and mites (Nieuwenhuizen and Lopata 2013). This indicates a possible immunological cross reactivity. Indeed, *Anisakis* muscle proteins paramyosin and tropomyosin are thought to be responsible for the cross reactivity between *Anisakis* and other invertebrates and for the IgE hypersensitivity detected in blood sera often reported in allergic patients (Asturias et al. 2000; Guarneri et al. 2007; Mattiucci et al. 2017b).

The major allergens of *Anisakis*, which are recognized at a high percentage level by IgE and IgG in serum samples of patients, are *Ani s1* (24 kDa) and *Ani s7* (139 kDa) and *Ani s13* (37 kDa); they are located in excretory/secretory glands (ES) (Audicana and Kennedy 2008; Mattiucci et al. 2017b). *Ani s1* was recognized by IgE in sera of GAA patients (Moneo et al. 2000; Mattiucci et al. 2013b, 2017b). *Ani s 1* has also been detected at a high percentage in patients from Morocco

sensitized to *Anisakis* (Abattouy et al. 2012). *Ani s 1* has been recognized both by IgE and IgG in patients with *Anisakis* allergy (Mattiucci and Bruschi pers.obs.; Mattiucci et al. 2017b). *Ani s 1* also seems to be a heat-stable allergen (Moneo et al. 2000); therefore, allergic reactions could occur not only after the consumption of undercooked fresh fish but also from infected fish which have been cooked or frozen. An isoform of *Anis 1* also exists at 21 kDa (Shimakura et al. 2004). It has been suggested that the "mild" form of GA and the allergic condition in Spain are related to the isoform of *Ani s 1* (Moneo et al. 2000; Shimakura et al. 2004).

Ani s 7, a glycoprotein, is also considered as major allergen, having being recognized in up to 100% of sera samples in patients with *Anisakis* allergy (Rodríguez et al. 2008). The amino acid sequence similarity of *Ani s 1*, *Ani s 7*, and *Ani s 12* in *A. pegreffii* and *A. simplex* (*s. s.*) has recently been described and compared by Quiazon et al. (2013b).

Other minor allergens are represented by *Ani s 4*, a heat-stable protein, which has been recognized in 27–30% of patients (Moneo et al. 2005), and *Ani s 5*, *Ani s 8*, and *Ani s 9*, all heat-stable E/S proteins, but less frequently recognized in patient sera (Caballero et al. 2008; Kobayashi et al. 2007; Rodriguez-Perez et al. 2008). However, their role could be relevant as in terms of an allergic reaction after the ingestion of cooked, frozen, or canned fish products (Rodriguez-Perez et al. 2008). These allergens can be considered as food allergens.

Additional allergens have been identified and named *Ani s 10-12*; however, little knowledge is so far available concerning their function and location in *Anisakis* larvae. Furthermore, a hemoglobin from *A. pegreffii* was recently characterized, which seems to be responsible for a high immunoactivity in hypersensitive patients, and it has been shown to have a phylogenetic similarity with other invertebrate hemoglobins (Nieuwenhuizen and Lopata 2013).

Finally, healthy individuals can have high levels of anti-*Anisakis* IgE in their serum without the development of allergic symptoms. On the other hand, individuals with low levels of specific IgE antibodies may show clinical manifestations of anisakiasis. For example, minimal symptoms of allergy have been observed in a patient shown by endoscopy to have a heavy parasite burden (200 *Anisakis* larvae) in the stomach (Jurado-Palomo et al. 2010). Here, the specific humoral response to *Anisakis* was weak, a finding congruent with a previous experimental model (Amano et al. 1995) in which it was observed that a high parasite load could lead to a poor IgE response, suggesting a possible immunomodulation role for *Anisakis* spp. larvae. Finally, other immunoglobulins, such as IgA, IgG1, IgG2b, and IgG2c, can also be detected in allergic reactions due to *Anisakis* (Anadón et al. 2009).

13.6.5 Anisakis *and Cancer*

It's known that some helminth infections are causative factor for human cancer, most likely by eliciting chronic inflammation or by tumorigenic effect from parasitic secretions (Liao et al. 2018; Arora et al. 2019). In particular, helminth extracellular

vesicles have immunomodulatory effects and may contribute to pathogenesis, even promoting tumorigenesis as demonstrated for parasitic flatworms (Chaiyadet et al. 2015). Available in vitro studies using human fibroblasts (Messina et al. 2016), human dendritic cells (Napoletano et al. 2018), and human epithelial colonic cancer cells (Speciale et al. 2017) described a modulatory activity exerted by *Anisakis* parasitic products as upregulation of oxidative stress, inhibition of apoptosis-related biomarkers, and inflammatory induction. A pilot study aimed at exploring the tumorigenic potential of *Anisakis* using hamster ovary cells and Sprague Dawley rats revealed increasing cell proliferation, decreasing apoptosis, and changes in the expression of serum cancer-related miRNAs in rats (Corcuera et al. 2018). Further studies are needed to assess the tumorigenic potential of *Anisakis* and other parasite-derived molecules.

13.7 Diagnosis of Human Anisakiasis

13.7.1 Histological Diagnosis

Histological sections of hematoxylin-eosin stained granulomas, removed after the surgical treatment at both gastric and intestinal levels, often revealed the presence of worms with the morphological features characteristic of *Anisakis* larvae. This happened when the nematode in the removed nodule is in a very good state of preservation. The following characters in particular, when visible at the microscopic level, enable identification at the generic level:

1. In transverse section: a thin cuticle lacking lateral alae; polymyarian muscle cells, separated into four quadrants by chords with two winglike distal lobes; intestine circular with a triangular lumen and 50–70 tall columnar epithelial cells; and excretory cell (renette cell) banana-shaped and situated ventrally to the intestine.
2. In sagittal section: the muscular part of the esophagus followed by the glandular part (ventriculus) and the absence of a ventricular appendix and/or intestinal caecum.

These microscopic findings permitted the identification of *Anisakis* larva in granulomas removed from the gastric wall in the first documented case of GA in Italy (Stallone et al. 1996), in several cases of IA and GA reported later (Pampiglione et al. 2002), in a granuloma surgically removed from near the ileocecal valve (Moschella et al. 2004), in an extragastrointestinal case of anisakiasis (Cancrini et al. 1997), and, finally, in a granuloma lesion provoked by *A. pegreffii* in a case of IA in Italy (Mattiucci et al. 2011). Unfortunately, identification to the specific levels of the etiological agent in these cases was not possible, except for the last one reported by Mattiucci et al. (2011) (see also Sect. 13.7.2).

13.7.2 Molecular Diagnosis

The very limited specific diagnostic features of individual *Anisakis* spp. larvae available on the basis of morphological examination means that it is impossible to identify them as etiological agents of anisakiasis using microscopy. Furthermore, when larvae infect humans, they can become spoiled or fragmented. This often happens, for instance, when they are removed by endoscopy, making it impossible to identify them morphologically, even at the generic level. Likewise, in histological sections of granuloma examined after intestinal surgery, it is sometimes very hard even to recognize the etiological agent as a nematode.

In contrast, our knowledge of the causative agents of human anisakiasis was greatly advanced by the application of molecular methodologies. The first molecular identification of a larva recovered following a gastric endoscopy was reported by D'Amelio et al. (1999) in Italy. The larva was identified as *A. pegreffii* by PCR amplification of the entire ITS and subsequent RFLP analysis, as expected since this is the most frequent species in fishes from Italian marine waters. The same approach was applied by Farjallah et al. (2008a), who recognized a larva recovered from the esophagus of a patient as belonging to *A. pegreffii*. On the basis of the same molecular method, further two cases of GA in Italy caused by *A. pegreffii* were reported by Fumarola et al. (2009). Six additional cases of gastric anisakiasis (GA) and two cases of gastro-allergic anisakiasis (GAA), removed by endoscopy from eight Italian patients, have been diagnosed as belonging to the same species, i.e., *A. pegreffii*, by PCR amplification and sequencing of the ITS region of the rDNA and the mitochondrial mtDNA*cox2* gene by Mattiucci et al. (2013b).

The widest survey was conducted by Umehara et al. (2007) in Japan, where 99 larvae from human patients were identified as *A. simplex* (*s. s.*), and one case had *A. pegreffii* as the etiological agent, using the same method described by D'Amelio et al. (2000). Similarly, Lim et al. (2012) emphasize the importance of genotyping human cases of anisakiasis in Korea, where *A. pegreffii* was widely reported as causative agent of the diseases.

The first molecular identification of *A. pegreffii* in a paraffin-embedded granuloma as the etiological agent of an IA case was performed by Mattiucci et al. (2011). Later on, several cases of invasive anisakiasis by the parasite *A. pegreffii* found in granuloma, by biopsy or larval fragments, were successfully diagnosed in Italy by the use of Rt-PCR hydrolysis probe system (see Sect. 11.3.6) (Mattiucci et al. 2017c).

Thus, according to the molecular identification of cases of human anisakiasis undertaken so far, both of the sibling species *A. pegreffii* and *A. simplex* (*s. s.*) have been shown to be causative agents of human anisakiasis. No data are so far available for the third species of the *A. simplex c*omplex, i.e., *A. berlandi* (= *A. simplex* C). We may also assume that *A. pegreffii* is able to provoke in humans gastric, intestinal, and gastro-allergic anisakiasis (Mattiucci et al. 2013b).

However, although human infection is highest in countries where eating raw fish is widespread, the molecular identification of human cases remains very limited,

especially in those European countries where allergic symptoms and hypersensitivity associated with the parasite are frequently reported. Yet, surprisingly, in Europe, the obligatory notification of the human anisakiasis does not exist.

13.7.3 Sero-diagnosis

Currently, most sero-diagnostic tests for *Anisakis* reactivity include the use of ImmunoCAP systems, immunoblotting (WB), ELISA, and Skin Prick Test (SPT) (Audicana and Kennedy 2008). All of these methods use partially purified antigens and crude extract of *Anisakis* larvae. This gives these methodologies a poor specificity value, due to cross reactivity with antigens from many other parasites and allergens. It should especially be noted that specific IgE detection using ImmunoCAP assay can overestimate the number of human cases sensitized to *Anisakis* allergens. In other words, the sensitivity of these tests can be exaggerated and result in false-positive results. Some authors have preferred to use IgE and IgG detection via western blotting (WB) to differentiate, for instance, between anisakiasis or *Anisakis* allergy and asymptomatic *Anisakis* IgE-sensitized patients (Del Pozo et al. 1997; García et al. 1997; Moneo et al. 1997). In recent years, purified *Anisakis* allergens have proven to be useful in diagnosis using WB, especially when combined antigens are used (Moneo et al. 2007). WB assay to study the *Anisakis*-specific immune response in several cases of anisakiasis (GA, IA, GAA) due to *A. pegreffii* revealed the antibody sera response against *Anis 1*, *Anis 7*, and *Ani s13* (Mattiucci et al. 2017b).

13.8 "*Omic*" Technologies Applied to Anisakid Nematodes

Advancements in technologies employed in high-throughput next-generation sequencing (NGS) methods are supporting the spread of studies that, combined with advances in computational biology and bioinformatics, have greatly accelerated discoveries within basic and biomedical research for many parasitic diseases. The most updated "omic" studies performed on anisakid nematodes have been recently reviewed (D'Amelio et al. 2020).

Among anisakids, the draft genome of the species *A. simplex* was the first available on the website WormBase ParaSite elaborated by the Parasite Genomics group at the Wellcome Trust Sanger Institute, in the framework of the 50 Helminth Genomes project (PRJEB496 project).

Comparative RNA-seq analyses on different developmental stages can help in understanding adaptive processes, such as molecular pathways linked to parasitic survival and discovery of stage-enriched gene expression (Kim et al. 2018; Cavallero et al. 2020; Nam et al. 2020).

Proteomic studies (Arcos et al. 2014; Fæste et al. 2014) showed that *A. simplex* proteins were homologous to allergens already characterized in other nematodes, insects, and shellfishes, which may be the source of possible cross reactivity, as also confirmed by comparative genomic approaches. Recently, Stryiński et al. (2019) analyzed the global proteome of *A. simplex* L3 and L4, allowing them to detect sets of modulated proteins that provided a stage-specific proteomic signature.

13.9 Treatment

The endoscopic removal of larvae in gastric anisakiasis and the surgical treatment of intestinal granulomas appear to remedy the disease. On the other hand, the effective use of anthelminthic drugs is not supported by large surveys, although recent studies have highlighted a significant success of albendazole against cultured *Anisakis* larvae in vitro (Arias-Diaz et al. 2006). This suggests the possible use of this chemical in treating clinical manifestations of human anisakiasis, at least when the *Anisakis* larvae are still in the stomach, a short time after ingestion of the infected fish.

13.10 Prevention and Control

The consumption of fish infected by *Anisakis* larvae is a biological hazard that can be prevented by control measures under the supervision of health authorities and by the use of proper storage and processing methods that enable the inactivation of the larvae. EU (European Community) Regulation Nos. 853/2004 and 1276/2011, regarding treatment to kill viable parasites in fishery products intended for human consumption, state that "all wild caught seawater or freshwater fish must be considered at risk of containing viable parasites of human health hazard if these products are to eaten raw or almost raw. . . ." Cooking at 70 °C is used to kill the larvae within a short time. However, it needs to be considered that some allergens (e.g., *Ani s 1*, *Ani s 4*, and *Ani s 9*) have been demonstrated to be thermostable and allergens released from the larvae into the surrounding tissue have retained their allergenicity even after the larvae have been killed by heat treatment (Vidaček et al. 2011). With regard to deep-freezing, Adams et al. (1999) reported a low survival of live *Anisakis* larvae per fillet (0–3%) after 6 hours at −40 °C, but up to 30% of them survived after 48 hours at 0 °C. Similarly, Wharton and Aalders (2002) demonstrated that larvae can survive at temperatures down to −10 °C. Whereas deep-freezing and cooking for sufficiently long periods are retained as the most effective methods, cold smoking and marinating procedures are unable to safety kill the larvae, unless high food-grade acetic acid concentrations are used (Sánchez-Monsalvez et al. 2005). Dry salting can devitalize the parasite, provided that the salt is widely distributed in all parts of the muscle and is used at correct concentrations (>20° Baumé; see ICMSF 1996).

Recently, Brutti et al. (2010) demonstrated a complete inactivation of *A. simplex* larvae in raw fish using high hydrostatic pressure treatments, and the effects of microwave treatments have been reported by Adams et al. (1999), Tejada et al. (2006), and Vidaček et al. (2011).

Finally, the same EU regulation (1276/2011) states that "...in the case that epidemiological data show that the fishing grounds do not represent a health hazard with regard to the presence of parasites, the competent authority may adopt national measures which authorise an exemption from the required freezing treatment on fishery products derived from the wild catches." In this context, it should be emphasized that these control measures should be established in the future, when (i) they can be based on data on infection levels of genetically identified *Anisakis* spp. larvae in food fish, (ii) the fish are from a defined fishing ground, (iii) the location and percentage infection of larvae in fish fillets (the edible part) are known, (iv) the pathogenicity to humans of different species of *Anisakis* is fully established, and (v) the risk of *Anisakis* allergy due to antigenic proteins released by these parasites in fish products is fully clarified.

Acknowledgments We are very grateful to Dr. David I. Gibson for reading the chapter. We wish to thank Michela Paoletti for her help in preparing tables and the figure.

References

Abattouy N, Valero A, Martín-Sánchez J, Peñalver MC, Lozano J (2012) Sensitization to *Anisakis simplex* Species in the Population of Northern Morocco. J Investig Allergol Clin Immunol 22(7):514–519

Abe N (2008) Application of the PCR-sequence-specific primers for the discrimination among larval *Anisakis simplex* complex. Parasitol Res 102:1073–1075

Abe N, Ohya N, Yanagiguchi R (2005) Molecular characterization of *Anisakis pegreffii* larvae in pacific cod in Japan. J Helminthol 79:303–306

Abe N, Tominaga K, Kimata I (2006) Usefulness of PCR-restriction fragments length polymorphism analysis of the internal transcribed spacer region of rDNA for identification of *Anisakis simplex* complex. Jpn J Infect Dis 59:60–62

Abollo E, Gestal C, Pascual S (2001) Anisakid infection in the European shag *Phalacrocorax aristotelis aristotelis*. J Helminthol 75:209–214

Abollo E, Paggi L, Pascual S, D'Amelio S (2003) Occurrence of recombinant genotypes of *Anisakis simplex* s.s. and *Anisakis pegreffii* (Nematoda: Anisakidae) in an area of sympatry. Infection Genetics and Evolution 3:175–181

Aco Alburqueque R, Palomba M, Santoro M, Mattiucci S (2020) Molecular identification of zoonotic parasites of the genus *Anisakis* (Nematoda: Anisakidae) from fish of the Southeastern Pacific Ocean (off Peru coast). Pathogens:9:910

Adams AM, Miller KS, Wekell MM, Dong FM (1999) Survival of *Anisakis simplex* in microwave-processed arrowtooth flounder (*Atheresthes stomias*). J Food Prot 62(4):403–409

Amano T, Nakazawa M, Sugiyama H, Secor WE, Oshima T (1995) Specific antibody patterns of Winstar rats inoculated with third stage larvae of *Anisakis simplex*. J Parasitol 81:536–542

Anadón AM, Romarís F, Escalante M, Rodríguez E, Gárate T, Cuéllar C, Ubeira FM (2009) The *Anisakis simplex* Ani s 7 major allergen as an indicator of true *Anisakis* infections. Clin Exp Immunol 156(3):471–478

Anibarro B, Seoane FJ (1998) Occupational conjunctivitis caused by sensitization to *Anisakis*. J Allergy Clin Immunol 102(2):331–332

Arcos SC, Ciordia S, Roberston L, Zapico I, Jimenez-Ruiz Y, Gonzalez-Munoz M, Moneo I, Carballeda-Sangiao N, Mahillo AR, Albar JP, Navas A (2014) Proteomic profiling and characterization of differential allergens in the nematodes *Anisakis simplex* sensu stricto and *A. pegreffii*. Proteomics 14:1547–1568

Arias- Diaz J, Zuloaga J, Vara E, Balibrea J, Balibrea JL (2006) Efficacy of albendazole against simplex larvae in vitro. Digest Liver Dis (Alimentary tract) 38:24–26

Armentia A, Lombardero M, Callejo A, Martín Santos JM, Martin Gil FJ, Vega JM, Arranz ML, Martínez C (1998) Occupational asthma by *Anisakis simplex*. J Allergy Clin Immunol 102:831–834

Arnason U, Gullberg A, Janke A (2004) Mitogenomic analyses provide new insights into cetacean origin and evolution. Gene 333:27–34

Arora N, Kaur R, Anjum F, Tripathi S, Mishra A, Kumar R, Prasad A (2019) Neglected agent eminent disease: linking human helminthic infection, inflammation, and malignancy. Front Cell Infect Microbiol 9:402

Asturias JA, Eraso E, Moneo MA (2000) Is tropomyosin an allergen in *Anisakis*? Allergy 55(9):898

Audicana MT, Kennedy MW (2008) *Anisakis simplex*: from obscure infectious worm to inducer of immune hypersensitivity. Clin Microbiol Rev 21:360–379

Baldwin RE, Rew MB, Johansson ML, Banks MA, Jacobson KC (2011) Population structure of three species of *Anisakis* nematodes recovered from Pacific sardines (*Sardinops sagax*) distributed throughout the California Current System. J Parasitol 97:545–554

Bao M, Garci ME, Antonio JM, Pascual S (2013) First report of *Anisakis simplex* (Nematoda, Anisakidae) in the sea lamprey (*Petromyzon marinus*). Food Control 33(1):81–86

Beck M, Evans R, Feist SW, Stebbing P, Longshaw M, Harris E (2008) *Anisakis simplex* sensu lato associated with red vent syndrome in wild adult Atlantic salmon *Salmo salar* in England and Wales. Dis Aquat Org 82:61–65

Bello E, Paoletti M, Webb S, Nascetti G, Mattiucci S (2020) Cross-species utility of microsatellite loci for the genetic characterisation of *Anisakis berlandi* (Nematoda: Anisakidae). Parasite 27:9

Bello E, Palomba M, Webb SC, Paoletti M, Cipriani P, Nascetti G (2021) Investigating the genetic structure of the parasites *Anisakis pegreffii* and *A. berlandi* (Nematoda: Anisakidae) in a sympatric area of the southern Pacific Ocean waters using a multilocus genotyping approach: First evidence of their interspecific hybridization. Infect Genet Evol 92:104887

Berland B (1961) Nematodes from some Norwegian marine fishes. Sarsia 2:1–50

Bernardi C, Gustinelli A, Fioravanti ML, Caffara M, Mattiucci S, Cattaneo P (2011) Prevalence and mean intensity of *Anisakis simplex* (*sensu stricto*) in European sea bass (*Dicentrarchus labrax*) from Northeast Atlantic Ocean. Int J Food Microbiol 148(1):55–59

Brutti A, Rovere P, Cavallero S, D'Amelio S, Danesi P, Arcangeli G (2010) Inactivation of *Anisakis simplex* larvae in raw fish using high hydrostatic pressure treatments. Food Control 21:331–333

Caballero ML, Moneo I, Gomez-Aguado F, Corcuera MT, Casado I, Rodrıguez-Perez R (2008) Isolation of Ani s 5, an excretory-secretory and highly heat-resistant allergen useful for the diagnosis of Anisakis larvae sensitization. Parasitol Res 103:1231–1233

Caballero ML, Umpierrez A, Moneo I, Rodriguez-Perez R (2011) Ani s 10, a new *Anisakis* simplex allergen: cloning and heterologous expression. Parasitol Int 60:209–212

Cancrini G, Magro G, Giannone G (1997) Primo caso di anisakiosi extragastrointestinale nell'uomo diagnosticato in Italia. Parassitologia 39:13–17

Cassens I, Vicario S, Waddell V, Balchowsky H, Van Belle D, Ding W, Fan C, Lal Mohan R, Simoes-Lopes P, Bastida R, Meyer A, Stanhope M, Milinkovitch M (2000) Independent adaptation to riverine habitats allowed survival of ancient cetacean lineages. Proc Natl Acad Sci 97:11343–11347

Cavallero S, Nadler SA, Paggi L, Barros NB, D'Amelio S (2011) Molecular characterization and phylogeny of anisakid nematodes from cetaceans from southeastern Atlantic coasts of USA, Gulf of Mexico, and Caribbean Sea. Parasitol Res 108:781–792

Cavallero S, Ligas A, Bruschi F, D'Amelio S (2012) Molecular identification of *Anisakis* spp. from fish collected in the Tyrrhenian Sea (NW Mediterranean). Vet Parasitol 187(3-4):563–566

Cavallero S, Costa A, Caracappa S, Gambetta B, D'Amelio S (2014) Putative hybrids between two *Anisakis* cryptic species: Molecular genotyping using High Resolution Melting. Exp Parasitol 146:87–93

Cavallero S, Lombardo F, Salvemini M, Pizzarelli A, Cantacessi C, D'Amelio S (2020) Comparative transcriptomics reveals clues for differences in pathogenicity between *Hysterothylacium aduncum*, *Anisakis simplex* sensu stricto and *Anisakis pegreffii*. Genes 11:321

Chaiyadet S, Sotillo J, Smout M, Cantacessi C, Jones MK, Johnson MS, Turnbull L, Whitchurch CB, Potriquet J, Laohaviroj M, Mulvenna J, Brindley PJ, Bethony JM, Laha T, Sripa B, Loukas A (2015) Carcinogenic liver fluke secretes extracellular vesicles that promote cholangiocytes to adopt a tumorigenic phenotype. J Infect Dis 212:1636–1645

Chaliggiannis I, Lalle M, Pozio E, Sotiraki S (2012) Anisakidae infection in fish of the Aegean Sea. Vet Parasitol 184:362–366

Chou YY, Wang CS, Chen HG, Chen HY, Chen SN, Shih HH (2010) Parasitism between *Anisakis simplex* (Nematoda: Anisakidae) third-stage larvae and the spotted mackerel *Scomber australasicus* with regard to the application of stock identification. Vet Parasitol 177(3–4): 324–331

Cipriani P, Palomba M, Giulietti L, Bellisario B, Buselic I, Mladineo I, Cheleschi R, Nascetti G, Mattiucci S (2017a) *Anisakis pegreffii* (Nematoda: Anisakidae) in European anchovy *Engraulis encrasicolus* from the Mediterranean Sea: fishing ground as a predictor of parasite distribution. Fish Res 202:59–68

Cipriani P, Sbaraglia GL, Paoletti M, Giulietti L, Bellisario B, Palomba M, Buselic I, Mladineo I, Nascetti G, Mattiucci S (2017b) The Mediterranean European hake, *Merluccius merluccius*: Detecting drivers influencing the *Anisakis* spp. larvae distribution. Fish Res 202:79–89

Colom-Llavina MM, Mignucci-Giannoni AA, Mattiucci S, Paoletti M, Nascetti G, Williams EHJr (2009) Additional records of metazoan parasites from Caribbean marine mammals, including genetically identified anisakid nematodes. Parasitol Res 5:1239-1252

Corcuera MT, Rodríguez-Bobada C, Zuloaga J, Gomez-Aguado F, Rodriguez-Perez R, Mendizabal A, Gonzalez P, Arias-Diaz J, Caballero ML (2018) Exploring tumourigenic potential of the parasite *Anisakis*: a pilot study. Parasitol Res 117:3127–3136

D'Amelio S, Mathiopoulos KD, Brandonisio O, Lucarelli G, Doronzo F, Paggi L (1999) Diagnosis of a case of gastric anisakidosis by PCR-based restriction fragment length polymorphism analysis. Parassitologia 41:591–593

D'Amelio S, Mathiopoulos K, Santos CP, Pugachev ON, Webb SC, Picanço M, Paggi L (2000) Genetic markers in ribosomal DNA for the identification of members of the genus *Anisakis* (Nematoda: Ascaridoidea) defined by polymerase chain reaction-based restriction fragment length polymorphism. Int J Parasitol 30:223–226

D'Amelio S, Cavallero S, Busi M, Ingrosso S, Giuffra E, Farjallah S, Costa G (2012) *Anisakis* (Chapter 45). In: Molecular detection of human parasitic pathogens. (eds: Dongyou Liu, RCPA Biosecurity QAP, New South Wales, Australia)

D'Amelio S, Lombardo F, Pizzarelli A, Bellini I, Cavallero S (2020) Advances in omic studies drive discoveries in the biology of anisakid nematodes. Genes 11:801. https://doi.org/10.3390/genes11070801

Daschner A, Rodero M, De Frutosi C, Valls A, Vega F, Blanco C, Cuéllar C (2011) Different serum cytokine levels in chronic vs. acute *Anisakis simplex* sensitization-associated urticarial. Parasite Immunol 33(6):357–362

Del Pozo MD, Audicana M, Diez JM, Muñoz D, Ansotegui IJ, Fernández E, García M, Etxenagusia M, Moneo I, Fernández de Corres L (1997) *Anisakis simplex*, a relevant etiologic factor in acute urticaria. Allergy 52(5):576–579

Dzido J, Kijewska A, Rokicka M, Świątalska-Koseda A, Rokicki J (2009) Report on anisakid nematodes in polar regions – Preliminary results. Pol Sci 3(3):207–211

EFSA Panel on Biological Hazards (BIOHAZ) (2010) Scientific Opinion on risk assessment of parasites in fishery products. EFSA Journal 8: 1543. Available on line: wwwefsa.europa.eu

Fæste CK, Jonscher KR, Dooper MM, Egge-Jacobsen W, Moen A, Daschner A, Egaas E, Christians U (2014) Characterisation of potential novel allergens in the fish parasite *Anisakis simplex*. EuPA Open Proteom 4:140–155

Fagerholm HP (1989) Intra-specific variability of the morphology in a single population of the seal parasite *Contracaecum osculatum* (Rudolphi) (Nematoda, Ascaridoidea), with a redescription of the species. Zool Scr 18:33–41

Farjallah S, Busi M, Mahjoub MO, Slimane BB, Paggi L, Saidn K, D'Amelio S (2008a) Molecular characterization of larval anisakid nematodes from marine fish off the Moroccan and Mauritanian coasts. Parasitol Int 57:430–436

Farjallah S, Slimane BB, Busi M, Paggi L, Amor N, Blel H, Said K, D'Amelio S (2008b) Occurrence and molecular identification of *Anisakis* spp. from the North African coasts of Mediterranean Sea. Parasitol Res 102:371–379

Fernández de Corres L, Del Pozo MD, Aizpuru F (2001) Prevalencia de la sensibilización a *Anisakis simplex* en tres áreas españolas, en relación a las diferentes tasas de consumo de pescado. Relevancia de la alergia a *Anisakis simplex*. J Invest Allergol Clin Immunol 16:337–346

Fumarola L, Monno R, Ierardi E, Rizzo G, Giannelli G, Lalle M, Pozio E (2009) *Anisakis pegreffii* etiological agent of gastric infections in two Italian women. Foodborne Pathog Dis 6:1157–1159

García M, Moneo I, Audícana MT, del Pozo MD, Muñoz D, Fernández E, Díez J, Etxenagusia MA, Ansotegui IJ, Fernández de Corres L (1997) The use of IgE immunoblotting as a diagnostic tool in *Anisakis simplex* allergy. J Allergy Clin Immunol 99(497):501

Garcia A, Mattiucci S, Damiano S, Santos MN, Nascetti G (2011) Metazoan parasites of swordfish, *Xiphias gladius* (Pisces: Xiphiidae) from the Atlantic Ocean: implications for host stock identification. ICES J Mar Sci 68:175–182

Guarneri F, Guarneri C, Benvenga S (2007) Cross-reactivity of *Anisakis simplex*: possible role of *Ani s 2* and *Ani s 3*. Int J Dermatol 46(2):146–150

Hermida M, Mota R, Pacheco CC, Santos CL, Cruz C, Saraiva A, Tamagnini P (2012) Infection levels and diversity of anisakid nematodes in blackspot seabream, *Pagellus bogaraveo*, from Portuguese waters. Parasitol Res 110:1919–1928

Højgaard D (1998) Impact of temperature, salinity and light on hatching of eggs of *Anisakis simplex* (Nematoda: Anisakidae), isolated by a new method, and some remark on survival of larvae. Sarsia 83:21–28

Iglesias R, D'Amelio S, Ingrosso S, Farjallah S, Martínez-Cedeira JA, García-Estévez JM (2008) Molecular and morphological evidence for the occurrence of *Anisakis* sp. A (Nematoda, Anisakidae) in the Blainville's beaked whale *Mesoplodon densirostris*. J Helminthol 82:305–308

International Commission on Microbiological Specifications for Foods (ICMSF) (1996) Microorganisms in Foods. 5. Characteristics of microbial pathogens. London: Blackie Academic & Professional

Irigoitia M, Palomba M, Braicovich PE, Lanfranchi AL, Denuncio P, Gana JCM, Mattiucci S, Timi JT (2020) Genetic identification of Anisakis spp. (Nematoda: Anisakidae) from cetaceans of the Southwestern Atlantic Ocean: ecological and zoogeographical implications. Parasitol Res 120: 1–13

Ishikura H, Kikuchi K (1990) Intestinal anisakiasis: infected fish, sero-immunological diagnosis, and prevention. Tokyo, (eds.) Springer: 1–265

Jurado-Palomo J, López-Serrano MC, Moneo I (2010) Multiple Acute Parasitization by *Anisakis simplex*. J Investig Allergol Clin Immunol 20(5):437–441

Karl H, Baumann F, Ostermeyer U, Kuhn T, Klimpel S (2011) Anisakis simplex (s.s.) larvae in wild Alaska salmons: no indication of post-mortem migration from viscera into flesh. Dis Aquatic Org 94:201–209

Kikuchi Y, Ishikura H, Kikuchi K, Hayasaka H (1990) Pathology of gastric anisakiasis. In: Ishikura H, Namiki M (eds) Gastric anisakiasis. Springer, Tokyo, pp 117–131

Kim JH, Kim JO, Jeon CH, Nam UH, Subramaniyam S, Yoo SI, Park JH (2018) Comparative transcriptome analyses of the third and fourth stage larvae of *Anisakis simplex* (Nematoda: Anisakidae). Mol Biochem Parasitol 226:24–33

Klimpel S, Kuhn T, Busch MW, Karl H, Palm HW (2011) Deep-water life cycle of *Anisakis paggiae* (Nematoda: Anisakidae) in the Irminger Sea indicates kogiid whale distribution in north Atlantic waters. Polar Biol 34:899–906

Kobayashi Y, Shimakura K, Ishizaki S, Nagashima Y, Shiomi K (2007) Purification and cDNA cloning of a new heat-stable allergen from *Anisakis simplex*. Mol Biochem Parasitol 155(2): 138–145

Kobayashi Y, Ohsaki K, Ikeda K, Kakemoto S, Ishizaki S, Shimakura K, Nagashima Y, Shiomi K (2011) Identification of novel three allergens from *Anisakis simplex* by chemiluminescent immunoscreening of an expression cDNA library. Parasitol Int 60:144–150

Køie M, Berland B, Burt MDB (1995) Development to third-stage larva occurs in eggs of *Anisakis simplex* and *Pseudoterranova decipiens* (Nematoda, Ascaridoidea, Anisakidae). Can J Fish Aquat Sci 52(1):134–139

Legendre P, Desdevises Y, Bazin E (2002) A statistical test for host-parasite coevolution. Syst Biol 51:217–234

Levsen A, Berland B (2012) *Anisakis* species, in Fish Parasites: pathobiology and protection (Woo PTK and Buchmann K Eds). 298–309

Levsen A, Lunestand BT (2010) *Anisakis simplex* third stage larvae in Norwegian spring spawning herrin (*Clupea harengus* L.) with emphasis on larval distribution in the flesh. Vet Parasitol 171: 247–253

Liao C, Cheng X, Liu M, Xuelin W, Boireau P (2018) *Trichinella spiralis* and tumors: cause, coincidence, or treatment? Anti Cancer Agents Med Chem 18:8

Lim H, Jung BK, Cho J, Yooyen T, Shin EH, Chai JY (2012) Molecular diagnosis of cause of anisakiasis in humans, South Korea. Emerg Infect Dis 21(2):342–344

Marques JF, Cabral HN, Busi M, D'Amelio S (2006) Molecular identification of *Anisakis* species from Pleuronectiformes off the Portuguese coast. J Helminthol 80:47–51

Mattiucci S, Nascetti G (2006) Molecular systematics, phylogeny and ecology of anisakid nematodes of the genus *Anisakis* Dujardin, 1845: an update. Parasite 13:99

Mattiucci S, Nascetti G (2008) Advances and trends in the molecular systematics of Anisakid nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes. Adv Parasitol 66:47–148

Mattiucci S, Nascetti G, Bullini L, Orecchia P, Paggi L (1986) Genetic structure of *Anisakis physeteris*, and its differentiation from the *Anisakis simplex* complex (Ascaridida: Anisakidae). Parasitology 93:383–387

Mattiucci S, Nascetti G, Cianchi R, Paggi L, Arduino P, Margolis L, Brattey J, Webb S, D'Amelio S, Orecchia P, Bullini L (1997) Genetic and ecological data on the *Anisakis simplex* complex, with evidence for a new species (Nematoda, Ascaridoidea, Anisakidae). J Parasitol 86: 401–416

Mattiucci S, Paggi L, Nascetti G, Ishikura H, Kikuchi K, Sato N, Cianchi R, Bullini L (1998) Allozyme and morphological identification of *Anisakis*, *Contracaecum* and *Pseudoterranova* from Japanese waters (Nematoda, Ascaridoidea). Syst Parasitol 40:81–92

Mattiucci S, Paggi L, Nascetti G, Abollo E, Webb SC, Pascual S, Cianchi R, Bullini L (2001) Genetic divergence and reproductive isolation between *Anisakis brevispiculata* and *Anisakis physeteris* (Nematoda: Anisakidae). Int J Parasitol 31:9–14

Mattiucci S, Paggi L, Nascetti G, Portes Santos C, Costa G, Di Benedetto AP, Ramos R, Argyrou M, Cianchi R, Bullini L (2002) Genetic markers in the study of *Anisakis typica* (Diesing, 1860): larval identification and genetic relationships with other species of *Anisakis* Dujardin, 1845 (Nematoda: Anisakidae). Syst Parasitol 51:159–170

Mattiucci S, Abaunza P, Ramadori L, Nascetti G (2004) Genetic identification of *Anisakis* larvae in European hake from Atlantic and Mediterranean waters for stock recognition. J Fish Biol 65: 495–510

Mattiucci S, Nascetti G, Dailey M, Webb SC, Barros NB, Cianchi R, Bullini L (2005) Evidence for a new species of *Anisakis* Dujardin, 1845: morphological description and genetic relationships between congeners (Nematoda: Anisakidae). Syst Parasitol 61:157–171

Mattiucci S, Abaunza P, Damiano S, Garcia A, Santos MN, Nascetti G (2007) Distribution of *Anisakis* larvae, identified by genetic markers, and their use for stock characterization of demersal and pelagic fish from European waters: an update. J Helminthol 81:117–127

Mattiucci S, Farina V, Campbell N, Mackenzie K, Ramos P, Pinto AL, Abaunza P, Nascetti G (2008) *Anisakis* spp. larvae (Nematoda: Anisakidae) from Atlantic horse mackerel: their genetic identification and use as biological tags for host stock identification. Fish Res 89:146–151

Mattiucci S, Paoletti M, Webb SC (2009) *Anisakis nascettii* n. sp. (Nematoda: Anisakidae) from beaked whales of the southern hemisphere: morphological description, genetic relationships between congeners and ecological data. Syst Parasitol 74:199–217

Mattiucci S, Paoletti M, Borrini F, Palumbo M, Macarone Palmieri R, Gomes V, Casati A, Nascetti G (2011) First molecular identification of the zoonotic parasite *Anisakis pegreffii* (Nematoda: Anisakidae) in a paraffin-embedded granuloma taken from a case of human intestinal anisakiasis in Italy. BMC Infect Des 11:82

Mattiucci S, Paoletti M, Webb CS, Nascetti G (2013a) *Pseudoterranova* and *Contracaecum*. Invited chapter In: Molecular detection of human parasitic pathogens. (Don Liu Editor). CRC Press, Boca Raton, FL, 645–656

Mattiucci S, Fazii P, De Rosa A, Paoletti M, Salomone Megna A, Glielmo A, De Angelis M, Costa A, Meucci C, Calvaruso V, Sorrentini I, Palma G, Bruschi F, Nascetti G (2013b) Anisakiasis and gastroallergic reactions associated with *Anisakis pegreffii* infection. Italy Emerg Infect Dis 19(3). https://doi.org/10.3201/eid1903.121017

Mattiucci S, Cipriani P, Webb SC, Paoletti M, Marcer F, Bellisario B, Gibson DI, Nascetti G (2014a) Genetic and morphological approaches distinguishing the three sibling species of the *Anisakis simplex* species complex, with a species designation as *Anisakis berlandi* n. sp. for *A. simplex* sp. C (Nematoda: Anisakidae). J Parasitol 100:199–214

Mattiucci S, Garcia A, Cipriani P, Neves Santos M, Nascetti G, Cimmaruta R (2014b) Metazoan parasite infection in the swordfish, *Xiphias gladius* L. from the Mediterranean Sea, and comparison with Atlantic populations: implications for its stocks characterization. Parasite 21: 35

Mattiucci S, Acerra V, Paoletti M, Cipriani P, Levsen A, Webb SC, Canestrelli D, Nascetti G (2016) No more time to stay 'single' in the detection of *Anisakis pegreffii*, *A. simplex* (s.s.) and hybridization events between them: a multi-marker nuclear genotyping approach. Parasitology 143:998–1011

Mattiucci S, Cipriani P, Paoletti M, Levsen A, Nascetti G (2017a) Reviewing biodiversity and epidemiological aspects of anisakid nematodes from the North-east Atlantic Ocean. J Helminthol 91:422–439

Mattiucci S, Colantoni A, Crisafi B, Mori-Ubaldini F, Caponi P, Fazii G, Nascetti G (2017b) IgE sensitization to *Anisakis pegreffii* in Italy: comparison of two methods for the diagnosis of allergic anisakiasis. Parasite Immunol 39:e12440

Mattiucci S, Paoletti M, Colantoni A, Carbone A, Gaeta R, Proietti A, Frattaroli S, Fazii P, Bruschi F, Nascetti G (2017c) Invasive anisakiasis by the parasite *Anisakis pegreffii* (Nematoda: Anisakidae): diagnosis by real-time PCR hydrolysis probe system and immunoblotting assay. BMC Infect Dis 17:530

Mattiucci S, Cipriani P, Paoletti M, Nascetti G (2018) Molecular epidemiology of *Anisakis* and Anisakiasis: an ecological and evolutionary road map. Adv Parasitol 99:93–263

Mattiucci S, Bello E, Paoletti M, Webb S, Timi JT, Levsen A, Cipriani P, Nascetti G (2019) Novel polymorphic microsatellite loci in *Anisakis pegreffii* and *A. simplex* (s.s.) (Nematoda:

Anisakidae): implications for species recognition and population genetic analysis. Parasitology 146:1–51
Mayr E (1963) Animal species and evolution. Belknap Press, Harvard University Press, Cambridge, MA, p 797
Messina CM, Pizzo F, Santulli A, Buselic I, Boban M, Orhanovic S, Mladineo I (2016) *Anisakis pegreffii* (Nematoda: Anisakidae) products modulate oxidative stress and apoptosis-related biomarkers in human cell lines. Paras Vectors 9:607
Milinkovitch MC (1995) Molecular phylogeny of cetaceans prompts revision of morphological transformations. Trends Ecol Evol 10:328–334
Mladineo I, Šimat V, Miletić J, Beck R, Poljak V (2012) Molecular identification and population dynamic of *Anisakis pegreffii* (Nematoda: Anisakidae Dujardin, 1845) isolated from the European anchovy (*Engraulis encrasicolus* L.) in the Adriatic Sea. Int J Food Microbiol 157(2):224–229
Mladineo I, Trumbić Ž, Radonić I, Vrbatović A, Hrabar J, Bušelić I (2017) *Anisakis simplex* complex: ecological significance of recombinant genotypes in an allopatric area of the Adriatic Sea inferred by genome-derived simple sequence repeats. Int J Parasitol 47:215–223
Moneo I, Audícana MT, Alday E, Curiel G, Del Pozo MD, García M (1997) Periodate treatment of *Anisakis simplex* allergens. Allergy 52:565–569
Moneo I, Caballero ML, Gómez F, Ortega E, Alonso MJ (2000) Isolation and characterization of a major allergen from the fish parasite *Anisakis simplex*. J Allergy Clin Immunol 106(1):177–182
Moneo I, Caballero ML, Gonzalez-Muñoz M, Rodriguez-Mahillo AI, Rodriguez-Perez R, Silva A (2005) Isolation of a heat-resistant allergen from the fish parasite *Anisakis simplex*. Parasitol Res 96:285–289
Moneo I, Caballero ML, Rodriguez-Perez R, Rodriguez-Mahillo AI, Gonzalez-Muñoz M (2007) Sensitization to the fish parasite *Anisakis simplex*: clinical and laboratory aspects. Parasitol Res 101:1051–1055
Moschella CM, Mattiucci S, Mingazzini P, DeAngelis G, Assenza M, Lombardo F, Monaco S, Paggi L, Modini C (2004) Intestinal anisakiasis in Italy: case report. J Helminthol 78:271–273
Nadler SA, D'Amelio S, Dailey MD, Paggi L, Siu S, Sakanari JA (2005) Molecular phylogenetics and diagnosis of *Anisakis*, *Pseudoterranova*, and *Contracaecum* from northern pacific marine mammals. J Parasitol 91:1413–1429
Nam UH, Kim JO, Kim JH (2020) De novo transcriptome sequencing and analysis of *Anisakis pegreffii* (Nematoda: Anisakidae) third-stage and fourth stage larvae. J Nematol 52:1–16
Napoletano C, Mattiucci S, Colantoni A, Battisti F, Zizzari IG, Rahimi H, Nuti M, Rughetti A (2018) *Anisakis pegreffii* impacts differentiation and function of human dendritic cells. Parasite Immunol 40:e12527
Nascetti G, Paggi L, Orecchia P, Smith JW, Mattiucci S, Bullini L (1986) Electrophoretic studies on the *Anisakis simplex* complex (Ascaridida: Anisakidae) from the Mediterranean and North-East Atlantic. Int J Parasitol 16:633–640
Nieuwenhuizen NE, Lopata AL (2013) *Anisakis* – a food-borne parasite that triggers allergic host defences. Int J Parasitol 43(12–13):1047–1057
Nikaido M, Mtsumo F, Hamilton H, Brownell RL, Cao Y, Ding W, Zuoyan Z, Shedlock AM, Fordyce RE, Hasegawa M, Okada N (2001) Retroposon analysis of major cetaceans lineages: the monophyly of toothed whales and the paraphyly of river dolphins. PNAS 98:7384–7389
Noguera P, Collins C, Bruno D, Pert C, Turnbull A, McIntosh A, Lester K, Bricknell I, Wallace S, Cook P (2009) Red vent syndrome in wild Atlantic salmon *Salmo salar* in Scotland is associated with *Anisakis simplex sensu stricto* (Nematoda: Anisakidae). Dis Aquat Org 87:199–215
Orecchia P, Paggi L, Mattiucci S, Smith JW, Nascetti G, Bullini L (1986) Electrophoretic identification of larvae and adults of *Anisakis* (Ascaridida: Anisakidae). J Helminthol 60:331–339
Paggi L, Nascetti G, Webb SC, Mattiucci S, Cianchi R, Bullini L (1998a) A new species of *Anisakis* Dujardin, 1845 (Nematoda: Anisakidae) from beaked whale (Ziphiidae): allozyme and morphological evidence. Syst Parasitol 40:161–174

Paggi L, Mattiucci S, D'Amelio S, Nascetti G (1998b) Nematodi del genere *Anisakis* in pesci, cefalopodi e cetacei del Mar Mediterraneo e dell'Oceano Atlantico e Pacifico. Biol Mar Mediterr 5:585–1592

Palm HW, Damriyasa IM, Linda IB, Oka M (2008) Molecular genotyping of *Anisakis* Dujardin, 1845 (Nematoda: Ascaridoidea: Anisakidae) larvae from marine fish of Balinese and Javanese waters, Indonesia. Helminthologia 45:3–12

Palomba M, Paoletti M, Webb SC, Nascetti G, Mattiucci S (2020) A novel nuclear marker and development of an ARMS-PCR assay targeting the metallopeptidase 10 (*nas* 10) locus to identify the species of the *Anisakis simplex* (s. l.) complex (Nematoda, Anisakidae). Parasite 27:39

Palomba M, Mattiucci S, Crocetta F, Osca D, Santoro M (2021) Insights into the role of deep-sea squids of the genus *Histioteuthis* (Histioteuthidae) in the life cycle of ascaridoid parasites in the Central Mediterranean Sea waters. Sci Rep 11:7135

Pampiglione S, Rivasi F, Criscuolo M, De Benedettis A, Gentile A, Russo S, Testini M, Villani M (2002) Human anisakiasis in Italy: a report of eleven new cases. Pathol Res Pract 198:429–434

Paoletti M, Mattiucci S, Colantoni A, Levsen A, Gay M, Nascetti G (2018) Species-specific real time-PCR primers/probe systems to identify fish parasites of the genera *Anisakis*, *Pseudoterranova* and *Hysterothylacium* (Nematoda: Ascaridoidea). Fish Res 202:38–48

Pontes T, D'Amelio S, Costa G, Paggi L (2005) Molecular characterization of larval anisakid nematodes from marine fishes of Madeira by a PCR-based approach, with evidence for a new species. J Parasitol 91:1430–1434

Quiazon KMA, Yoshinaga T, Santos MD, Ogawa K (2009) Identification of larval *Anisakis* spp. (Nematoda: Anisakidae) in Alaska Pollock (*Theragra chalcogramma*) in Northern Japan using morphological and molecular markers. J Parasitol 95(5):1227–1232

Quiazon KMA, Yoshinaga T, Ogawa K (2011a) Experimental challenge of *Anisakis simplex* sensu stricto and *Anisakis pegreffii* (Nematoda: Anisakidae) in rainbow trout and olive flounder. Parasitol Int 60(2):126–131

Quiazon KMA, Yoshinaga T, Ogawa K (2011b) Distribution of *Anisakis* species larvae from fishes of the Japanese waters. Parasitol Int 60(2):223–226

Quiazon KMA, Santos MD, Yoshinaga T (2013a) *Anisakis* species (Nematoda: Anisakidae) of Dwarf Sperm Whale *Kogia sima* (Owen, 1866) stranded off the Pacific coast of southern Philippine archipelago. Vet Parasitol 197(1–2):221–230

Quiazon KMA, Zenke K, Yoshinaga T (2013b) Molecular characterization and comparison of four *Anisakis* allergens between *Anisakis simplex* sensu stricto and *Anisakis pegreffii* from Japan. Mol Biochem Parasitol 190(1):23–26

Roca-Geronès X, Alcover MM, Godínez-González C, Montoliu I, Fisa R (2021) Hybrid genotype of *Anisakis simplex* (s.s.) and *A. pegreffii* identified in third- and fourth-stage larvae from sympatric and allopatric Spanish marine waters. Animals 11(8):2458

Rodríguez E, Anadón AM, García-Bodas E, Romarís F, Iglesias R, Gárate T, Ubeira FM (2008) Novel sequences and epitopes of diagnostic value derived from the Anisakis simplex Ani s 7 major allergen. Allergy 63(2):219–225

Rodriguez-Perez R, Moneo I, Rodriguez-Mahillo A, Caballero ML (2008) Cloning and expression of Ani s 9, a new *Anisakis simplex* allergen. Mol Biochem Parasitol 159(2):92–97

Rosales MJ, Mascaró C, Fernandez C, Luque F, Moreno MS, Parras L, Cosano A, Muñoz JR (1999) Acute intestinal anisakiasis in Spain: a fourth-stage *Anisakis simplex* larva. Memorias do Instituto Oswaldo Cruz 94(6):823–826

Sánchez-Monsalvez I, de Armas-Serra C, Martínez J, Dorado M, Sánchez A, Rodríguez-Caabeiro F (2005) A new procedure for marinating fresh anchovies and ensuring the rapid destruction of *Anisakis* larvae. J Food Prot 68:1066–1072

Santoro M, Di Nocera F, Iaccarino D, Cipriani P, Guadano Procesi I, Maffucci F, Hochscheid S, Blanco C, Cerrone A, Galiero G, Nascetti G, Mattiucci S (2018) Helminth parasites of the dwarf sperm whale *Kogia sima* (Cetacea: Kogiidae) from the Mediterranean Sea, with implications on host ecology. Dis Aquat Org 129:175–182

Serracca L, Cencetti E, Battistini R, Rossini I, Prearo M, Pavoletti E, Fioravanti ML, Righetti M, Di Donfrancesco B, Ercolini C (2013) Survey on the presence of *Anisakis* and *Hysterothylacium* larvae in fishes and squids caught in Ligurian Sea. Vet Parasitol 196(3-4):547–551

Setyobudi E, Jeon CH, Lee CH, Seong KB, Kim JH (2010) Occurrence and identification of *Anisakis* spp. (Nematoda: Anisakidae) isolated from chum salmon (*Oncorhynchus keta*) in Korea. Parasitol Res 108:585–592

Shibata O, Uchida Y, Furusawa T (1989) Acute Gastric Anisakiasis with special analysis of the location of the worms penetrating the gastric mucosa. Gastric Anisakiasis in Japan 53–57

Shih HH, Ku CC, Wang CS (2010) *Anisakis simplex* (Nematoda: Anisakidae) third-stage larval infections of marine cage cultured cobia, *Rachycentron canadum* L., in Taiwan. Vet Parasitol 171:277–285

Shimakura K, Miura H, Ikeda K, Ishizaki S, Nagashima Y, Shirai T, Kasuya S, Shiomi K (2004) Purification and molecular cloning of a major allergen from *Anisakis simplex*. Mol Biochem Parasitol 135:69–75

Speciale A, Trombetta D, Saija A, Panebianco A, Giarratana F, Ziino G, Minciullo PL, Cimino F, Gangemi S (2017) Exposure to *Anisakis* extracts can induce inflammation on *in vitro* cultured human colonic cells. Parasitol Res 116:2471–2477

Stallone O, Paggi L, Balestrazzi A, Mattiucci S, Montinari M (1996) Gastric Anisakiasis in Italy: case report. Medit J Surg Med 4:13–16

Stryiński R, Mateos J, Pascual S, González ÁF, Gallardo JM, Łopieńska-Biernat E, Medina I, Carrera M (2019) Proteome profiling of L3 and L4 *Anisakis simplex* development stages by TMT-based quantitative proteomics. J Proteome 201:1–11

Tejada M, Solas MT, Navas A, Mendizábal A (2006) Scanning electron microscopy of *Anisakis* larvae following different treatments. J Food Prot 69(6):1379–1387

Umehara A, Kawakami Y, Matsui T, Araki J, Uchida A (2006) Molecular identification of *Anisakis simplex* sensu stricto and *Anisakis pegreffii* (Nematoda: Anisakidae) from fish and cetacean in Japanese waters. Parasitol Int 55:267–271

Umehara A, Kawakami Y, Araki J, Uchida A (2007) Molecular identification of the etiological agent of the human anisakiasis in Japan. Parasitol Int 56(3):211–215

Umehara A, Sugiyama H, Kawakami Y, Uchida A, Araki J (2008) Identification of *Anisakis simplex* larvae from fish in Japanese market at the sibling species level. Clin Parasitol 19: 114–117

Valentini A, Mattiucci S, Bondanelli P, Webb SC, Mignucci-Giannone A, Colom-Llavina MM, Nascetti G (2006) Genetic relationships among *Anisakis* species (Nematoda: Anisakidae) inferred from mitochondrial *cox*-2 sequences, and comparison with allozyme data. J Parasitol 92:156–166

Vidaček S, De Las Heras C, Solas MT, García ML, Mendizábal A, Tejada M (2011) Viability and antigenicity of *Anisakis simplex* after conventional and microwave heating at fixed temperatures. J Food Prot 74(12):2119–2126

Wharton DA, Aalders O (2002) The response of *Anisakis* larvae to freezing. J Helminthol 76:363–368

Zhu XQ, Podolska M, Liu JS, Yu HQ, Chen HH, Lin ZX, Luo CB, Song HQ, Lin RQ (2007) Identification of anisakid nematodes with zoonotic potential from Europe and China by single-strand conformation polymorphism analysis of nuclear ribosomal DNA. Parasitol Res 101: 1703–1707

Chapter 14
Lymphatic and Tissue Filariasis

Marc P. Hübner, Laura E. Layland, and Achim Hoerauf

Abstract The range and burden of neglected tropical diseases, many of which are helminth-derived, remains enormous. Infections are rampant in poor districts, and although efforts have been implemented over the years, there remain insufficient networks for disease control. It is estimated that more than 200 million people are infected with filarial nematodes. During coevolution, filariae have developed tactics to modulate the host's immune system so that they can persist for many years. Therefore, most individuals remain asymptomatic and mansonellosis and loiasis are primarily thought of as nuisance infections. Nevertheless, pathology can develop into elephantiasis during lymphatic filariasis (LF) and *Onchocerca volvulus* infections can lead to vision loss or skin pathology. Due to this severe pathology, the WHO road map for neglected tropical diseases 2021–2030 declared to target the elimination of transmission for onchocerciasis and elimination as public health problem for LF in 80% of the endemic countries by 2030. Most filarial species require the endosymbiotic *Wolbachia* bacteria for development and maturation. Indeed, targeting *Wolbachia* via antibiotic therapy has provided an alternative therapeutic approach which, in contrast to drugs currently employed in mass drug administration programs, is highly macrofilaricidal, i.e., kills the adult filariae. This chapter provides an overview about filarial agents drawing upon both their similarities and differences with regard to host immune reactions, ensuing pathologies and how infections alter response to vaccines and other diseases. All of these aspects have to be considered when implementing therapy, especially when adverse side effects may occur. These effects are synopsized in the final section alongside current success stories in terms of elimination and future strategies to control these public health problems.

M. P. Hübner · L. E. Layland · A. Hoerauf (✉)
Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany
e-mail: hoerauf@uni-bonn.de

F. Bruschi (ed.), *Helminth Infections and their Impact on Global Public Health*,
https://doi.org/10.1007/978-3-031-00303-5_14

Abbreviations

ADL	Acute filarial lymphatic disease
ALB	Albendazole
CFA	Circulating filarial antigen
DEC	Diethylcarbamazine
EN	Endemic normals
GEO	Generalized onchocerciasis
Ig	Immunoglobulin
IVM	Ivermectin
LF	Lymphatic filariasis
MDA	Mass drug administration
Mf	Microfilaria
SAE	Serious side effects
TPE	Tropical pulmonary eosinophilia

14.1 The Agents

Lymphatic and tissue filariasis are caused by filarial nematodes that belong to the family of Onchocercidae. There are eight filarial species that utilize humans as definite hosts, and each species has developed its own evasion strategies to avoid overt responses by the host's immune system. Whereas *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori* cause lymphatic filariasis, *Onchocerca volvulus*, *Loa loa*, *Mansonella perstans*, *M. streptocerca*, and *M. ozzardi* are responsible for eliciting tissue filariasis. The life cycle of these nematodes is relatively uniform and requires specific blood-feeding arthropod vectors for transmission and development (Fig. 14.1).

14.2 Lymphatic Filariasis

To laymen, elephantiasis requires no introduction since the disfiguring pathology remains engraved in the minds of all who have ever viewed such images (Fig. 14.2). However, these unforgettable features have provided historians with the tools to follow how the disease emerged in epidemiological regions (Otsuji 2011). Indeed, it appears that ancient Greek and Roman writers were already able to associate certain areas with the disease and could distinguish between the similar symptoms of leprosy "elephantiasis graecorum" and LF "elephantiasis arabum." Moreover, although verifiable documentation about the disease does not appear before the sixteenth century, there are hints in artifacts from ancient Egypt. These include a statue of pharaoh Mentuhotep II around 2,000 B.C. which is depicted with swollen

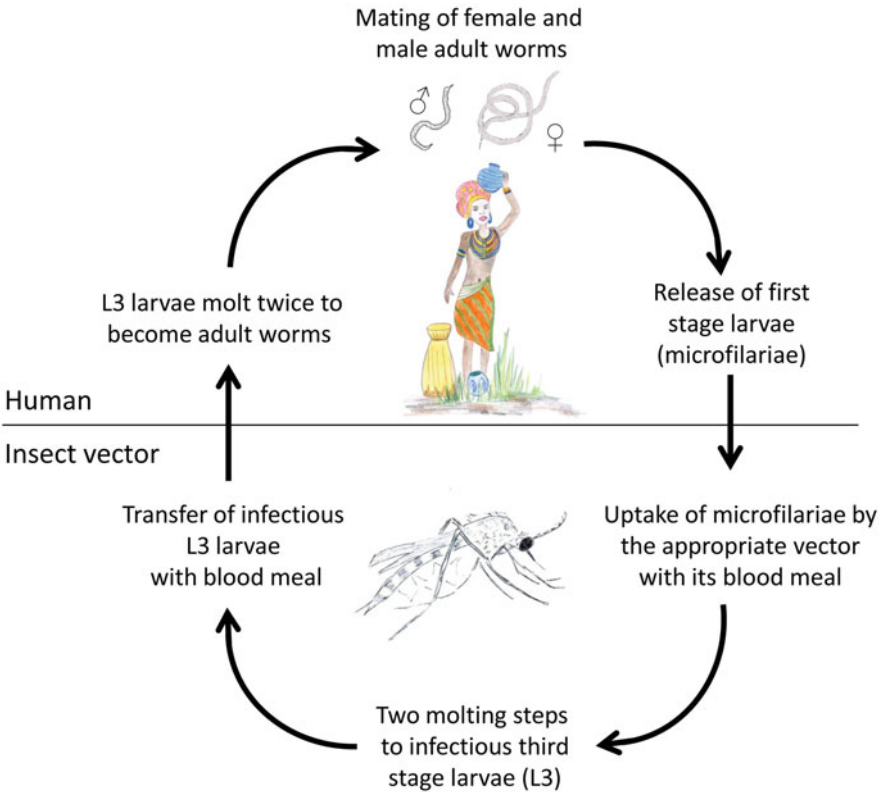

Fig. 14.1 Schematic life cycle of filariae. Infectious third stage larvae (L3) are transmitted by a blood-feeding arthropod vector to the human host. During the next 6–12 months, L3 larvae molt twice into adult female and male worms. Following mating adult female worms release first stage larvae (microfilariae), and according to the filarial species, they can be found in the skin or peripheral blood. Following uptake by the appropriate vector microfilariae undergo two molting steps to become infectious L3 larvae

legs and a limestone relief on Queen Hatshepsut's temple at EL-Deir Bahari memorializes a trading expedition and clearly shows a woman suffering from elephantiasis. Other indications include the millennium old "strange disease picture scroll" from Japan which portrays elephantiasis and hydrocele, and from Africa, a sculpture of Nok (ca. 500 A.C.) is also modeled with hydrocele.

14.2.1 Life Cycle, Vectors, and Morphology

Today, *W. bancrofti* accounts for 90% of lymphatic filarial infections which is ranked as the second most common cause of physical disability causing social, sexual, psychological, and economical problems (Michael et al. 1996). To date, no

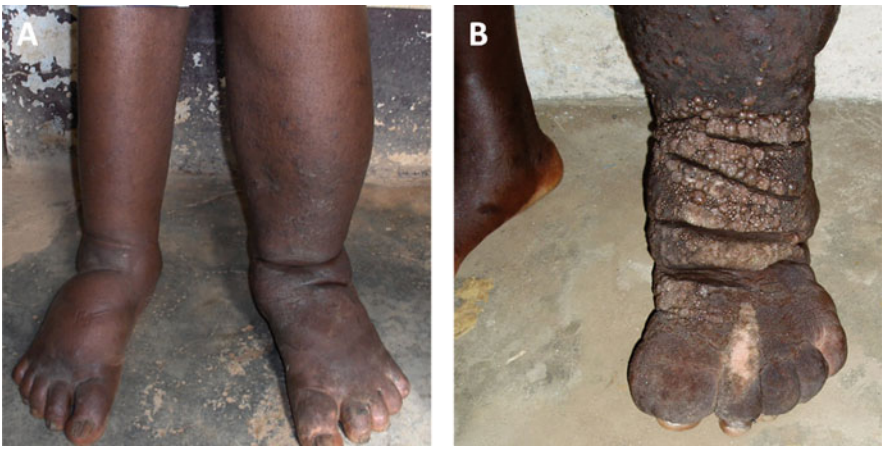

Fig. 14.2 Lymphatic filariasis. Lymphedema with (**a**) skin folds and knobs (stage 5) and (**b**) additional mossy lesions (stage 6)

reservoir hosts have been revealed for this species, and the disease is transmitted to its preferential human hosts by mosquito species within the genera *Culex*, *Anopheles*, *Mansonia*, and *Aedes* (Manguin et al. 2010). The more focal regions of *B. malayi* and *B. timori* have been determined by their mosquito vectors. For example, *B. timori*, which was not identified until 1977, is restricted to the Lesser Sunda Islands of Indonesia since its vector, *Anopheles barbirostris*, breeds in rice fields (Atmosoedjono et al. 1977). *B. malayi* is transmitted via *Mansonia* species although several *Anopheles* species transmit the infection in towns. Development of these filarial nematodes occurs in both the mammalian and vector hosts (Fig. 14.1).

Perhaps one of the most important milestones in tropical medicine was Sir Patrick Manson's discovery that mosquitoes transmitted filariasis. Vector uptake of microfilariae (Mf) from the host's blood initiates the beginning of a new life cycle which encompasses considerable sexual dimorphism (Nutman and Kazura 2011). Mf were first observed in the hydrocele fluid of a Cuban immigrant by the Parisian-based surgeon Demarquay in 1863. Three years later Wucherer also noted Mf in the urine of Brazilian patients with hematuria and chyluria. After another decade Bancroft discovered adult worms in a patient's abscess, and following consultation with Cobbold gave credence to the nematode we now recognize as *W. bancrofti* (Grove 1990; Theodorides 1994). During another decade, parallel studies from Lewis in Calcutta and Manson in Amoy, China, established disease manifestations. In addition, Manson tracked the metamorphosis of Mf inside the vector and obtained his material by letting mosquitoes bite his gardener Hin Lo. Five days after their blood meal, he dissected the flies and observed in their stomachs "a simple animal, with no structure, which, after a series of very interesting metamorphoses, increases exponentially in size and develops a digestive tract, becoming suitable for independent life." Nowadays we understand that the Mf shed their sheaths penetrating the midgut and upon migrating to the thoracic muscles develop into infective third stage

larvae (L3, approx. 1.5 mm) over a period of up to 3 weeks (Nutman and Kazura 2011) (Fig. 14.1). They then proceed to migrate to the salivary glands and enter the host when the mosquito feeds. No sexual reproduction or replication occurs within the vector host. Over a period of 6 months, L3 reside in lymphatics (groins, axillae) and develop into adult worms. Female worms produce several thousand Mf per day which remain in the lymphatics or migrate to blood vessels under the skin where they can survive for >1 year. Manson also noted the nocturnal periodicity of Mf since these life-forms migrate to the skin capillaries between 10 pm and 2 am to coincide with vector activity. In 1927, Lichtenstein observed that Mf in parts of Indonesia were different from *W. bancrofti* and sent the specimens to Brug, in the Netherlands, for classification. In 1940, Rao and Mapleston found Mf and adult worms of the same species, but it took another two decades before Buckley's suggestion to create a new genus, *Brugia*, was adopted. New species of *Brugia* were then found throughout the world in animals.

14.2.2 Epidemiology of Infection

Since 1950, urban populations in sub-Saharan Africa have risen about 25%, and by 2030 it is estimated that every other individual will live in urbanized districts (Simonsen and Mwakitalu 2013). Although research on LF has mainly focused on rural areas, it has the potential for urban transmission, and this aspect is now a major future challenge in the elimination of LF as a public health problem (Addiss 2010). Currently, 858 million people live in 50 endemic countries in Asia, Africa, Central and South America, and the Pacific (WHO 2020a,b). In 2018, 51 million people were infected, which represents a decline of 74% in comparison to 2000. Although mortality is uncommon, there is a considerable degree of morbidity. As mentioned above, *W. bancrofti* is the most widespread lymphatic filarial parasite accounting for 90% of LF infections, and a third of those are seriously incapacitated and disfigured by the disease. Approximately 65% of infected individuals live in South and Southeast Asia, 30% in Africa, and the remainder in other tropical areas. Whereas *B. timori* is restricted to islands in Eastern Indonesia, individuals infected with *B. malayi* are spread throughout Southern China, India, Indonesia, Thailand, Vietnam, Malaysia, the Philippines, and South Korea (Local Burden of Disease Neglected Tropical Diseases Collaborators 2020; Ramachandran 1981; Rebollo and Bockarie 2013).

14.2.3 The Host Response to the Parasite

To ensure long-term survival within the host, helminths have become masters of immune-regulation (Babu and Nutman 2012, 2014; Dreyer et al. 2000; Hoerauf et al. 2005; Joardar et al. 2021; King et al. 1993; Nutman et al. 1987a). Therefore,

although the socioeconomic impact of elephantiasis and hydrocele designate this infection as a major public health concern, only a fraction of the infected individuals present such severe symptoms (Hoerauf et al. 2005; Keiser and Nutman 2002; Otabil and Tenkorang 2015; Yimer et al. 2015). Indeed, the majority remain asymptomatic which in essence means that although viable adult worms and circulating Mf are present within the host, a balance has emerged which prevents overt pathology (Babu et al. 2006; Ottesen 2008; Simonsen 2009). Moreover, a test specific for filarial antigens revealed that there are amicrofilaremic individuals whose numbers are roughly equal to the proportion of asymptomatic Mf^+ individuals (Simonsen and Dunyo 1999; Turner et al. 1993). Thus, this latent group has remained largely neglected in immunological profiling, but since these individuals are a dead end for transmission, differences in their responses may provide information about how Mf are prevented from developing or travelling to the periphery and aid in the development of new prevention strategies (Arndts et al. 2012). Interestingly, Schroeder et al. found that live B. *malayi* microfilariae inhibited migration of neutrophils and monocytes but not of lymphocytes, even though vascular parameters were largely untouched showing that live MF are quite inert (Schroeder et al. 2012).

The immune system of individuals living in endemic areas is permanently exposed to a multitude of active and decaying infection-related material which leads to a low but constant triggering of innate and adaptive immune cascades. Responses of *W. bancrofti*-infected individuals are directly related to antigens released during the different stages of infection which elicit diverse patterns of immune factors in acute and chronic phases (Steel et al. 1994). Indeed, triggering innate pathways such as TLR or Trif has revealed an impact on both the expansion of cell populations and filarial-specific $CD4^+$ T-cell responses (Rodrigo et al. 2016; Wiszniewsky et al. 2019). TLR4 has been actively investigated, and both immunomodulatory molecules such as cystatin (Das et al. 2021) or sheath proteins (Mukherjee et al. 2017) can ligate to TLR4 causing activation or polarization of DC, macrophages, and T cells including regulatory T cells (Mukherjee et al. 2017, 2019). The frequency and intensity of such responses have also been associated with symptoms and pathology (Dreyer et al. 2000; Hoerauf et al. 2011; Pfarr et al. 2009). Using murine models, researchers have elucidated that many aspects of the immune system are involved in developing protective immune responses: Th2 responses (Al-Qaoud et al. 2000; Babu et al. 2000; Frohberger et al. 2019; Martin et al. 2000a, b; Specht et al. 2006; Volkmann et al. 2001, 2003), Th1 responses (Muhsin et al. 2018; Saeftel et al. 2001), $CD4^+$ T cells (Al-Qaoud et al. 1997; Rodrigo et al. 2016), neutrophils (Ajendra et al. 2016; Al-Qaoud et al. 2000; Frohberger et al. 2020; Pionnier et al. 2016; Saeftel et al. 2001), eosinophils (Ehrens et al. 2021; Frohberger et al. 2019; Martin et al. 2000a; b; Pionnier et al. 2020; Turner et al. 2018), B cells and antibodies (Al-Qaoud et al. 1998), and cytokines/chemokines (Bouchery et al. 2012; Gentil et al. 2014; Hong et al. 2019; Martin et al. 2000a; b; Ritter et al. 2017; Specht et al. 2011). Correlations between different subsets have also revealed that expression of FasL on the surface of peripheral B-1 cells from filarial patients was higher than EN and positively correlated with peripheral apoptotic T-helper cells. Expanding the pool of potential mediators immune anergy in

lymphedema to include FasL-expressing B-1 cells (Mishra et al. 2017). With regard to innate responses, in vitro studies have demonstrated that live parasites or extracts thereof influence dendritic cells or epidermal Langerhans cells, inducing apoptosis or altered phenotypes which results in skewed T-cell activation and responses (Babu and Nutman 2003; Rodrigo et al. 2016; Semnani et al. 2001, 2004, 2003). An early manifestation in LF patients is acute filarial lymphatic disease (ADL) which is characterized by a sudden high fever, painful inflammation of lymph nodes and lymphatics, and transient local edema. Immunological comparisons between ADL and Mf^+ individuals or those with chronic pathology have demonstrated that they have increased levels of TNF in their sera, a cytokine associated with the severity of the acute disease (Das et al. 1996; Nutman and Kumaraswami 2001).

With regard to adaptive immunity, a hallmark of patent infections is reduced proliferation to filarial antigens but not bystander or mitogenic stimuli (Mahanty and Nutman 1995) and such T-cell hyporesponsiveness is a feature of asymptomatic individuals (King et al. 1992; Semnani and Nutman 2004). However, both $CD4^+$ and $CD8^+$ T cells develop during infection (Kroidl et al. 2019) expressing IL-10 superfamily cytokine members such as IL-19 and IL-24 which were shown to regulate immune responses during active infection (Anuradha et al. 2014b) and potentially protect against the development of pathology. Decreased Th1 responses in asymptomatic Mf^+ patients are counterbalanced by dominant Th2 profiles, including elevated IL-4 and IL-5 (Nutman and Kumaraswami 2001), but they also have increased regulatory networks such as IL-10, TGF-β, and regulatory cell populations (Babu and Nutman 2014). More in-depth studies have revealed that IL-5(+) and IL-5(-) Th2 cells regulate immune responses during filarial infections and that these two Th2 subpopulations are controlled by different cytokine receptor-mediated processes (Anuradha et al. 2014a). Regulatory cell populations are induced during infection, and O'Regan et al. showed that IL-10+/PDL1+ monocyte/macrophages could be induced by Mf and had a suppressive nature (O'Regan et al. 2014a). The latter include $Foxp3^+$ regulatory T cells, considered part of the network which induced hyporesponsiveness, which have been identified in both *B. timori*- and *W. bancrofti*-infected populations (Wammes et al. 2012). Treg populations from Mf+ *B. timori*-infected individuals but not EN or pathology groups were shown to suppress proliferation and possibly Th2 cytokine responses to BmA (Wammes et al. 2012). In contrast, Treg and Breg populations were induced in individuals with an ongoing *W. bancrofti* infection but receded to levels found in the EN population after the infection cleared. Moreover, IL-10-producing $CD19^+CD24^{high}CD38d^{high}$ Breg were specifically increased in patently infected (CFA+Mf+) individuals (Ritter et al. 2019). In fact, PBMC from patent individuals spontaneously secrete higher levels of IL-10 when compared to individuals with chronic pathology; a characteristic also reflected upon restimulation of PBMC with filarial but not bystander stimuli (Mahanty and Nutman 1995; Mahanty et al. 1996). This immunosuppressive profile in Mf^+ patients is also characterized by high IgG4 production which is in line with patients infected with the generalized form of *O. volvulus*. Elevated levels of IgG4 limit IgE-mediated worm attack, and such host protective responses are reduced in chronic LF patients (Kurniawan et al. 1993). In fact, the proportion of filarial-

specific IgE differs in the various clinical states of LF: the highest levels are found in patients with tropical pulmonary eosinophilia (TPE) and the lowest in Mf^+ asymptomatic individuals (Hussain et al. 1981). Therefore, in asymptomatic individuals the induction of IgG4, elevated IL-10, and a skewed balance between Th1 and Th2 immunity and regulatory T-cell networks represent the major mechanisms used by filarial parasites to evade destruction and prevent the onset of severe pathology (Adjobimey and Hoerauf 2010). Interestingly, research showed that asymptomatic amicrofilaremic individuals had elevated filarial-specific immune responses when compared to Mf^+ individuals and these findings were independent of age. Further studies also showed that asymptomatic individuals had lower levels of disialylated IgG compared to EN (O'Regan et al. 2014b), but IgG4 antibodies were able to inhibit the binding of IgG1 and IgG2 to C1q in a Fc-Fc-dependent manner (Prodjinotho et al. 2019). Other studies also showed that IgG4 antibodies from Mf+, Mf-, and EN, in contrast to those with lymphedema, natively exhibit FcγRI/II-dependent suppressive properties on granulocytes showing again the development of distinctive patterns within the different cohorts in endemic areas (Prodjinotho et al. 2017). Such data provides the platform to deciphering how immune mechanisms may hinder the release of Mf into the circulation (Arndts et al. 2012).

In contrast, individuals with chronic pathology mount strong Th1 immune responses (IL-6 and IL-8), present elevated filarial-specific IgG1, and even increased Th17 production when compared to Mf^+ patients (Babu et al. 2009; Nutman et al. 1987a; Ottesen et al. 1977; Satapathy et al. 2006). These pro-inflammatory cytokines and their receptors are associated with the induction of vascular endothelial growth factors (VEGF) (Numasaki et al. 2004; Ristimaki et al. 1998) which has been further linked to lymphangiogenesis and vascular permeability (Debrah et al. 2006). In fact, investigations revealed that a single nuclear polymorphism (SNP) in VEGF-A is significantly higher in hydrocele patients than in Mf^+ or lymphedema individuals (Debrah et al. 2007). Moreover, patients with severe pathology have a different SNP for TGF-β than asymptomatic individuals indicating that genetic traits are also responsible for these overt reactions (Debrah et al. 2011a). EN are subjects that are free of demonstrable filarial infection and do not present any manifestations found in acute or chronic phases (Ravindran et al. 2003). PBMCs of such individuals usually proliferate significantly more in response to filarial antigens and secrete higher levels of IL-2 and IFN-γ compared to infected patients (Dimock et al. 1996). Furthermore these individuals contain higher levels of IgG1 and IgG2 but decreased levels of IgG4 in their sera compared to Mf^+ patients (Steel et al. 1996).

In recent years, isolation of proteins or molecules from either filarial worms or *Wolbachia* has been used to investigate responses in both preclinical and clinical settings. For example, immunization of phosphoglycerate mutase in mice generated a Th1/Th2 type immune responses and nearly 60% protection following infection (Singh et al. 2014). Jha and colleagues revealed that EN individuals had been exposed to and had specific Ig profiles to both *B. malayi* and *Wolbachia* proteins (Jha et al. 2017). A major group of immune-dominant proteins produced by filarial worms are ALT (abundant larval transcript) which were mapped to develop a multiple antigenic peptide (Madhumathi et al. 2017), and their ability to activate

innate pathways contributes to protective immunity (Ramanathan et al. 2015). Research into other molecules include small RNA exosome like vesicles (Zamanian et al. 2015), cuticular collagen (COL-4) (Arunkumar et al. 2014), MIF-2 homologue derived from *W. bancrofti* (Chauhan et al. 2015), nanochitosan (Malathi et al. 2015), tetraspanin (Dakshinamoorthy et al. 2013), the Shp-1 sheath protein (Jawaharlal et al. 2014), venom allergen-like protein-1 (BmVAL-1) (Darwiche et al. 2018), troponin-1 (Kushwaha et al. 2019), pepsin inhibitor (rBm33) from *B. malay* (Sreenivas et al. 2017), and *Wolbachia*-derived translation initiation factor-1 (Nag et al. 2013). All studies aim to understand or provoke anti-Mf immunity to reduce transmission, antifilarial immunity to decipher ways to block parasite development (Immanuel et al. 2017), or potential vaccine candidates (Kalyanasundaram et al. 2020; Hotez et al. 2016; Khatri et al. 2018; Sahoo et al. 2018).

14.2.4 Immunopathological Processes and Disease

LF is a chronic and persistent disease, and aside from lymphedema or the severe disfigurement to limbs (Fig. 14.2) or genitalia (only in *W. bancrofti* infections), infected individuals can suffer from a broad spectrum of clinical manifestations (Dreyer et al. 1999). In the acute phase, these include the abovementioned ADL and TPE and in endemic areas DLA (dermatolymphangioadenitis) which presents as edematous inflammatory plaques, vesicles, ulcers, and hyperpigmentation and is often associated with trauma. TPE can induce paroxysmal cough, nocturnal wheezing (due to the periodicity) weight loss, low-grade fever, adenopathy, and of course extremely high levels of eosinophils (Chitkara and Krishna 2006). Levels of total IgE and filarial-specific Igs are also high, and patients are usually Mf^- as strong immune responses against Mf are associated with TPE (King and Nutman 1991). The development of TPE has been linked to the presence of filarial gamma-glutaryl transpeptidase homologues that induce cross-reactive antibodies against the human pulmonary epithelium (Lobos et al. 2003). A rare complication of LF is chyluria, which is an obstruction of the renal lymphatics and may cause malnutrition of the infected individual due to the large loss of fat and proteins in the urine.

Although the majority of infected individuals remain asymptomatic, nearly all present some degree of subclinical disease such as microscopic hematuria/proteinuria and dilated lymphatics, and in men with *W. bancrofti* infection, there is often the presence of scrotal lymphangiectasia. With regard to subclinical symptoms in asymptomatic Mf^--infected individuals, it was shown that around worm nests, there were no differences in the level of lymph dilation between asymptomatic Mf^+ and Mf^- *W. bancrofti*-infected males although the Mf^+ group did have more scrotal worm nests (Arndts et al. 2012). Dilation of the lymphatics has also been noted in children infected with brugian parasites, and the damage to lymph vessels is thought to be induced by adult worms that are dying following drug administration or natural death (Jungmann et al. 1991; Pfarr et al. 2009). The parasite debris is thought to be absorbed or partially calcified inside affected tissues, and it is thought

that these structures provoke changes that induce dilation of the lymphatics and thickening of the lymphatic vessel wall as well as fibrosis and lymphatic obstruction (Nutman and Kumaraswami 2001; Ottesen 2008). Granuloma formation has also been demonstrated in and around these infected vessels (Nutman and Kumaraswami 2001). These early stages predispose to lymphatic dysfunction, and once established it is basically irreversible even after treatment (Freedman et al. 1995). If such symptoms and reactions are not limited, they can lead to different clinical manifestations such as lymphedema which may progress to the most severe disease form, elephantiasis (nonreversible edema, with skin thickening and nodular or warty excrescences), to urogenital disorders or to hydroceles (Shenoy 2008). However, the occurrence of lymphedema and hydrocele is not mutually exclusive, and both are characterized by dilation of the lymphatic vessels and extravasation of fluid from the vessels into the surrounding tissues. The enlargement of the lymph vessels results in less efficient lymph flow which is always orientated against gravity in the legs. Lymphedema evolves over many years and is often associated with acute attacks of ADLA due to skin injuries and ensuing bacterial infections (Suma et al. 1997). Whereas *W. bancrofti* affects limbs, genitals, and breasts, *B. malayi* occurs below the knees and elbows. Lymphedema of the limbs is graded according to Dreyer (Dreyer et al. 1999) as follows: (1) swelling is reversible overnight, (2) swelling is not reversible overnight, (3) shallow skin folds are visible, (4) occurrence of knobs, (5) knobs and deep skin folds are present, (6) additional mossy lesions, and (7) patients are unable to perform daily tasks. The WHO has a similar grading system reviewed in (Kumaraswami 2000). These final stages witness the skin thickening into folds often with hypertrichosis, blackening, intertrigo in the webs of toes, and nonhealing ulcers (Burri et al. 1996). The swelling can become so large that the person is incapacitated and in need of daily care. In contrast to hydrocele patients, lymphedema-affected individuals become more vulnerable to opportunistic microorganisms that may enter the lymphatics through smaller wounds (Pfarr et al. 2009; Shenoy 2008).

14.2.5 Diagnosis

As with other tropical-related infections, a correct diagnosis requires epidemiological history of the patient as well as physical findings and laboratory tests. In endemic areas, individuals presenting lymphedema in the extremities or disease of the male genitalia are usually a sign of filarial infection providing there are no injuries or evidence of congestive heart failure. Although radionuclide lymphoscintigraphy imaging can highlight widespread abnormalities in the lymphatics, verification and/or identification of the filarial species can only be determined through analysis of the parasite itself (Freedman et al. 1994). Thus, traditionally, ongoing infections were determined by the presence of Mf in blood although occasionally they are also found in hydrocele or other fluids. Standard detection employs thin blood smears stained with Giemsa, but for greater sensitivity Mf can be concentrated by filtering

(3–5μm pore) (Palumbo 2008), Knott's centrifugation, or a gradient centrifugation which also allows the collection of living Mf. As mentioned above, due to the periodicity of Mf, the timing of blood sampling is critical and should be based on the knowledge about the infection in the endemic area (nocturnal periodicity in most areas). Asymptomatic Mf^- individuals are detected with the rapid-format immunochromatographic card test for circulating filarial antigen (CFA) and specific ELISA both of which are highly sensitive (96–100%) and commercially available for *W. bancrofti* (Weil et al. 2013). CFA is field-applicable and even allows the identification of individuals with low parasitemia which could occur, for example, after treatment with microfilaricidal drugs. Importantly, the CFA test does not require sample collection at a specific time. Thus, the CFA test is mainly used for mapping of LF endemic areas. False-positive results that may occur in heavily infected loiasis patients (Pion et al. 2016) are hereby not a major problem, as in general the habitats of the transmitting vectors for LF and loiasis do not overlap. However, the CFA test does not detect brugian infections. So other serological assays analyzing *Brugia*-specific Igs in ELISAs (Haarbrink et al. 1999; Kurniawan et al. 1993) or a diagnostic dipstick test based on IgG4 serology have to be used to diagnose *Brugia* spec. infections. In contrast to the CFA test, antibody-based tests are not able to differentiate between active and past infections and are therefore not recommended for individuals from endemic areas but rather for travellers. Should worms be available, the different species can be identified by size and distinct traits (Table 14.1). An additional diagnostic parameter for *W. bancrofti* infection is the visualization of active adult worms via high-frequency ultrasonography with Doppler techniques since they show characteristic pattern movements termed filarial dance sign (FDS) (Dreyer et al. 1994; Mand et al. 2003). This technique is extremely sensitive in the area of the male scrotum, where worm nests are stable but less so in lymphatics of women as there is no predilection site. Unfortunately, in patients infected with *B. malayi*, only a fraction of the worm nests can be detected by ultrasonography since they migrate around the body (Mand et al. 2006; Shenoy et al. 2000). A big improvement for filarial diagnostics may be polymerase chain reaction (PCR)-based assays which detect filarial DNA in blood samples or the transmitting vectors (Pilotte et al. 2013; Rao et al. 2006). However, such diagnostic methods are expensive for rapid field testing and would only be feasible if samples could be easily transported to a centralized laboratory with appropriate equipment and there were specialists in the field trained for vector control. Molecular LAMP assays are a field-applicable method that are now also available to diagnose Mf from peripheral blood or the transmitting vectors with specificity and sensitivity that are comparable to PCR (Poole et al. 2017). However, both molecular assays do also require the sample collection during the periodicity of the Mf.

Table 14.1 Key facts on human pathogenic filariae

Species and date of discovery	Geographical distribution	Worldwide infection rate	Major severe forms of pathology	Microfilariae	Residency of adult worms	Size of adult worms	Life time in host	Presence of *Wolbachia*	Treatment	Vector	Animal reservoir
Wuchereria bancrofti 1863–1900	Sub-Saharan Africa, Southern and Southeast Asia Caribbean, South America, Pacific Islands	51 million	Lymphangitis, elephantiasis, hydrocele	Blood, nocturnal, sheathed, 260 μm	Lymphatic vessels and lymph nodes; Men: scrotal tissue	Males 4 cm, Females 8–10 cm	<10 years	Yes	Ivermectin, DEC, albendazole, doxycycline	*Culex*, *Anopheles*, *Mansonia*, and *Aedes* mosquitoes	No
Brugia malayi 1927	India, Southeast Asia	6 million	Lymphangitis, elephantiasis	Blood, nocturnal, sheathed, 260 μm	Lymphatic vessels and lymph nodes	Males 2 cm, Females 4–5 cm	5–15 years	Yes	Ivermectin, DEC, albendazole, doxycycline	*Mansonia, Anopheles* mosquitoes	Kra monkey, felines
Brugia timori 1960–1970	Indonesia: Lesser Sunda Islands	?	Lymphangitis, elephantiasis	Blood, nocturnal, sheathed, 260 μm	Lymphatic vessels and lymph nodes	Males 2 cm, females 4–5 cm	?	Yes	Ivermectin, DEC, albendazole, doxycycline	*Anopheles barbirostris* mosquitoes	No
Loa loa 1770	West and Central Africa	3–13 million	Eye worm, Angioedema, Calabar swelling	Blood, diurnal, sheathed, 230–300 μm	Subcutaneous tissue	Males 3–3.5 cm, females 5–7 cm	<17 years	No	DEC[a], ivermectin[a], albendazole	*Chrysops* tabanid flies	No
Onchocerca volvulus 1875/1893	Sub-Saharan Africa, Yemen, foci	21 million	Blindness, dermatitis, Sowda	Skin (upper dermis), unsheathed, 300 μm	Subcutaneous nodules	Males 2–5 cm, females	<14 years	Yes	Ivermectin, moxidectin, doxycycline	*Simulium* black flies	No

	in Latin America					35–70 cm					
Mansonella streptocerca 1922/1972	West and Central Africa	?	Mild dermatitis (mainly following treatment)	Skin (upper dermis), unsheathed, 180–240 μm	Dermal skin layer	Males 1.7 cm, females 2.7 cm	?	Not yet verified	DEC, ivermectin	*Culicoides* midges	Chimpanzees
Mansonella perstans 1890/1898	Sub-Saharan Africa, Central and South America	114 million	Mainly asymptomatic	Blood, unsheathed, 200 μm	Peritoneal, pleural, and pericardial cavity	Males 3.5–4.5 cm, females 7–8 cm	?	Yes	DEC, albendazole, doxycycline	*Culicoides* midges	Chimpanzees and gorillas
Mansonella ozzardi 1897/1898	Central and South America, the Caribbean	?	Mainly asymptomatic	Blood and skin, unsheathed, 220 μm	Peritoneal and pleural cavity	Males 2.6 cm, females 5 cm	?	Yes	Ivermectin[a], doxycycline?	*Culicoides* midges, *Simulium amazonicum*	Nonhuman primates, other mammals, birds, amphibians

[a]may cause severe adverse reactions

14.3 Tissue Filariasis

14.3.1 Life Cycle, Vectors, and Morphology

14.3.1.1 *Loa loa*

This disease has various names including Calabar swelling, fugitive swelling, and filaria lacrimalis and was first described by the French surgeon Mongin in 1770, who isolated an adult worm from a child's eye (Mongin 1770). The life cycle was not elucidated until 1904 (Brumpt 1904; Kerr 1904), and it took almost another 100 years to discover that *L. loa* do not possess or require *Wolbachia* (Büttner et al. 2003). The genome of *L. loa* was published and confirmed the lack of *Wolbachia* endosymbionts (Desjardins et al. 2013).

Humans are the only known host for *L. loa* infestations although patent in vivo experiments are possible with *Mandrillus leucophaeus* (Duke 1957). Adult *L. loa* worms are fully developed after 12 months and throughout their life span (up to 17 years) continuously migrate through subcutaneous tissues (Eveland et al. 1975). Female adult worms are bigger than males (Table 14.1), and although fecundity usually begins after 6 months, it can take years (Klion and Nutman 2011). The sheathed Mf are found in peripheral blood and occasionally in spinal fluid, urine, or sputum. In comparison with *W. bancrofti* infection, *L. loa* Mf also display a periodicity but appear in peripheral blood during the day and reside in lung tissue overnight. The African-restricted deer fly species *Chrysops* (*C. silacea*, *C. dimidiate*, *C. langi*) serves as the vector and contain enough L3 larvae that a single bite may result in infection (Padgett and Jacobsen 2008; Wanji et al. 2002). Interestingly, although they reside in canopied rainforest areas, they bite in the open, attracted to smoke from wood fires and preferentially consume human blood.

14.3.1.2 *Onchocerca volvulus*

The first associations of Mf in the skin with papular dermatitis were made in Ghana in 1875 by the Irish naval surgeon O'Neill (Nelson 1991). Adult worms were isolated from subcutaneous nodules by missionaries in Ghana and described by the German zoologist Leuckart in 1893. A connection between symptoms and transmission by *Simulium* vectors was suggested by Robles in 1917 (Delaporte 2008), and in 1932 the Belgian ophthalmologist Hissette connected Mf with the development of ocular disease (Kluxen and Hoerauf 2008).

Transmission of infectious L3 larvae is via the female blackfly of the genus *Simulium*, and within the host they molt twice within the first year to become mature adults. Female adults reside in subcutaneous or intramuscular nodules, and males travel between nodules to inseminate females. Infections are chronic and the helminth's life span averages 9–10 years with a maximum of 15 years (Hoerauf 2011; Specht et al. 2009; Udall 2007). Again, adult females are longer than males, and

females can produce ~700 unsheathed Mf a day (Hoerauf 2011) (Table 14.1) which then reside within the skin for 6–30 months awaiting uptake by vectors. Mf can also be found in the lymphatics, sputum, urine, and blood, but it is the migration into the ocular regions that instigates damage, hence the colloquial term for the infection "river blindness." *O. volvulus* worms also harbor *Wolbachia* (Hoerauf et al. 2000).

14.3.1.3 *Mansonella perstans*, *M. ozzardi*, and *M. streptocerca*

The three *Mansonella* species infecting humans, *M. perstans* (synonyms: *Dipetalonema perstans*, *Tetrapetalonema perstans*, *Acanthocheilonema perstans*), *M. ozzardi*, and *M. streptocerca* (synonyms: *Dipetalonema streptocerca*, *Tetrapetalonema streptocerca*) vary in distribution, vector specificity, site of infection, and pathology. Due to their presence in the blood, infections of *M. perstans* and *M. ozzardi* were discovered earlier than *M. streptocerca*. Manson discovered both *M. perstans and M. ozzardi* Mf in 1890 and 1897, respectively (Manson 1891, 1897). In 1898 he further identified adult *M. perstans* worms in the mesentery (Manson 1899), while Daniels described adult *M. ozzardi* worms in mesentery and fat tissues around the pancreas and pericardium (Daniels 1898). As with *O. volvulus*, the Mf of *M. streptocerca* are located within the skin and were first identified by Macfie and Corson in Ghana in 1922. However, it took another 50 years before the corresponding adult worms were identified (Neafie et al. 1975).

Although humans are the primary host, infections have been found in other primates, and *M. ozzardi* has even been found in birds and amphibians (Habermann and Menges 1968; Peel 1946). Whereas *M. streptocerca* is specifically transmitted by *Culicoides grahamii* midges (Duke 1954), several species of *Culicoides* can transmit *M. perstans* and *M. ozzardi* larvae (Mediannikov and Ranque 2018; Ta-Tang et al. 2018). Indeed, it was observed in the southwest region of Cameroon that *M. perstans* can be transmitted by night-biting *C. milnei* (Wanji et al. 2019), whereas it appears that *Culicoides inornatipenni* maybe the potential vector in Ghana (Debrah et al. 2017). In addition, *M. ozzardi* can also be transmitted by the blackfly *S. amazonicum* (Cerqueira 1959). Development into adulthood occurs over several months even years with *M. perstans* and *M. ozzardi* worms residing in the cavities of peritoneum, pleura, and pericardium. Occasionally, *M. perstans* have been detected in the mesenterial, retroperitoneal, and perirenal tissues and *M. ozzardi* in the lymphatics. *M. streptocerca* adult worms reside in the dermal skin layer. In keeping with all other filarial nematodes, there is a length dimorphism (Table 14.1). All Mf species are unsheathed, and although *M. streptocerca* and *M. ozzardi* have no periodicity, those of *M. perstans* present weak diurnal activity (Asio et al. 2009a). With regard to *Wolbachia*, both *M. perstans* (confirmed for West and Central Africa) and *M. ozzardi* contain *Wolbachia* (Casiraghi et al. 2001; Keiser et al. 2008), which enables to eliminate infections using doxycycline therapy (Batsa Debrah et al. 2019; Coulibaly et al. 2009). Interestingly, a newly emerging *Mansonella* species termed "DEUX" in the Gabon also contains *Wolbachia* (Mourembou et al. 2015; Sandri

et al. 2021; Simonsen et al. 2011). It remains to be confirmed whether *M. streptocerca* worms also harbors *Wolbachia*.

14.3.2 Epidemiology of Infection

14.3.2.1 *Loa loa*

Loiasis occurs in the tropical forests of Central and Western Africa, between Benin and Uganda, southern Chad, and in the north and south of the Sudan and Zambia, respectively (Zoure et al. 2011). 14.4 million people live in the two main loci of high endemic loiasis (prevalence >40%). The Western locus includes Cameroon, Equatorial Guinea, Gabon, the Democratic Republic of Congo (DRC), Chad, and the Central African Republic, whereas the Eastern locus is mainly composed of the northeastern part of DRC, parts of the Sudan, and the Central African Republic (Zoure et al. 2011). Although large sections of these countries have low or no prevalence of loiasis, an additional 15.2 million individuals are thought to be at intermediate risk (prevalence 20–40%) (Zoure et al. 2011).

14.3.2.2 *Onchocerca volvulus*

Onchocerciasis is most prominent in sub-Saharan Africa where 99% of all onchocerciasis cases occur. However, alongside these 31 sub-Saharan countries, additional transmission sites remain around the border of Brazil and Venezuela and Western parts of Yemen (Basanez et al. 2006; MMWR 2013; WHO 2020c). Detailed maps can be obtained from the ESPEN (Expanded Special Project for Elimination of Neglected Tropical Diseases) homepage (https://espen.afro.who.int/diseases/onchocerciasis). Based on the Global Burden of Disease study 2017 (Global Burden of Disease Study 2017 2018), approximately 21 million people are infected with *O. volvulus*, with an estimated 218 million people living in risk of infection in endemic areas (Global Burden of Disease Study 2017 2018). Of those infected, 1,200,000 suffer varying forms of visual impairment, 270,000 individuals have become blind, and 15 million developed severe forms of dermatitis, which accounts to estimated 205 million DALYs (Basanez et al. 2006; Global Burden of Disease Study 2017 2018). River blindness or onchocerciasis occurs along fast-flowing rivers that serve as the *Simulium* vector's breeding sites. In hyperendemic areas (>60% microfilaridermia), 30–40% of patients have skin pathology, but palpable nodules are less frequent (>30% of patients) (Hoerauf 2011). In mesoendemic areas (30–60% microfilaridermia), nodules are detectable in ~20% of patients, whereas in hypoendemic areas less than 30% of patients have microfilaridermia (Hoerauf 2011). Disease pathology further differs between the New and Old World due to a tenfold lesser transmission rate in the former by *S. ochraceum* (Basanez et al. 2006) which has resulted in reduced parasitology and pathology. Moreover, there is only a patchy

distribution of the disease in Latin America, whereas in Africa it is common that in hyperendemic areas everyone is infected with *O. volvulus.*

14.3.2.3 *Mansonella perstans*, *M. ozzardi*, and *M. streptocerca*

An estimated 114 million people are infected with *M. perstans* in 33 sub-Saharan countries, and parts of the neotropical region of Central and South America and more than 600 million people in total live in risk of infection. Minor foci also include Algeria and Tunisia, and in highly endemic areas, almost every infection develops into a patent form. Recently, long-term studies over a 19 year period in Spain have shown an import of *M. perstans* infections by migrants from Africa (Puente et al. 2020). *M. ozzardi* is restricted to Latin America (Columbia, Venezuela, Guyana, Suriname, Brazil, Argentina, Bolivia, Puerto Rico, Antigua, Guadeloupe, Nevis), but there are also cases in the Caribbean (Dominican Republic, Haiti, Martinique, St. Kitts, St. Lucia, St. Vincent, and Trinidad). A recent study also showed a high prevalence ($\geq$20%) of *M. ozzardi* in Ecuador (Calvopina et al. 2019). Although the actual number of infected patients is not known, up to 70% of patients in endemic areas are Mf^+ (Marinkelle and German 1970). Endemic areas for *M. streptocerca* are the tropical rainforest regions of West, Central, and Eastern Africa (Angola, Cameroon, Central African Republic, Congo, Equatorial Guinea, Nigeria, Uganda, and DRC). Again, the actual number of infected individuals remains unknown, but in endemic areas of Uganda, infection rates range from 60 to 90% with the development of detectable Mf in 58% of individuals and skin disease in 24% of individuals (Fischer et al. 1997).

14.3.3 The Host Response to the Parasite

14.3.3.1 *Loa loa*

Infections with *L. loa* are often asymptomatic and remain undetected even in cases of high parasite burden. As with other helminth infections, hallmarks of *L. loa* infections include eosinophilia and high IgE levels in the sera, and, interestingly, these parameters are more pronounced in amicrofilaremic symptomatic patients than Mf^+ asymptomatic individuals (Nutman et al. 1986). The different outcomes are thought to be influenced by genetic predisposition, duration of parasite exposure, and prenatal contact to *L. loa* antigens (Akue and Devaney 2002; Akue et al. 2002; Garcia et al. 1999). Several studies have compared the immune responses of expatriates with EN, and it was shown that the former is usually Mf^- but present more Calabar swellings (Klion et al. 1991). Accordingly, expatriates develop stronger filarial-specific lymphoproliferation responses and have increased eosinophilia and higher levels of filarial-specific IgG and IgE (Klion et al. 1991; Nutman et al. 1988). When compared to Mf^+ individuals, filarial-specific T-cell proliferation and

cytokine responses were strongly elevated in PBMCs from endemic amicrofilaremic patients (Baize et al. 1997). In contrast, PBMCs from Mf^+ patients revealed increased frequencies of IL-4^+, IL-10^+, and IL-13^+ $CD4^+$ T-cell populations when compared to Mf^- groups, whereas no differences were observed in $CD4^+$ or $CD8^+$ T-cell subsets producing IFN-γ^+ (Winkler et al. 1999). Interestingly, *L. loa* Mf have been shown to modulate complement activation in vivo and cover their sheath surface with host complement regulatory factor H and C4b-binding protein, indicating that active complement modulation by *L. loa* may prevent the development of protective immune responses (Haapasalo et al. 2009). Current studies on immune responses by loiasis patients have revealed marked difference between Mf-positive and Mf-negative individuals. Indeed, all participants produced IL-10, IL-13, IL-5, IL-4, and IL-9 in response to filarial antigen, indicating a common infection-driven response. However, Mf-positive individuals had significantly increased filarial antigen-driven IL-24 and IL-19 responses when compared to Mf-negative subjects and revealed higher frequencies of T cells producing IL-19. T-cell expression of IL-19 and IL-24 was shown to be positively regulated by IL-10 and IL-1β (Ricciardi and Nutman 2021). More recently rodent animal models with *L. loa* were established (Chunda et al. 2020; Pionnier et al. 2019; Tendongfor et al. 2012), which could further enhance our understanding of immune responses to *L. loa* and the testing of new treatment candidates.

14.3.3.2 *Onchocerca volvulus*

O. volvulus-infected individuals present a spectrum of disease symptoms with two polar forms, generalized onchocerciasis (GEO), or Sowda. GEO or hyporesponsive individuals have over 10 Mf per mg skin and palpable nodules under their skin but no strong pathology (King and Nutman 1991), whereas hyperactive (Sowda) patients have no or few Mf but severe skin pathology (Adjobimey and Hoerauf 2010; Tamarozzi et al. 2011). Thus, despite being elicited by the same parasite, the range of clinical manifestations is quite broad, and such diversity is thought to reflect the intensity and type of host immune responses to the parasite, its products, the *Wolbachia*, and even anti-helminthic therapy (Hoerauf et al. 2009). With regard to the latter, in hyperendemic areas that have received multiple rounds of IVM, a further group of individuals have been reported. These patients have adult worms and nodules but are skin Mf-negative and display little pathology and it is now hypothesized that they stem from MDA and are thus "man-made." However, the immune responses in this latter population are not well defined.

In individuals with GEO, *O. volvulus* worms manipulate the host's immune system by inducing regulatory networks that reduce filarial-specific and bystander reactions (Doetze et al. 2000; Hoerauf and Brattig 2002; Korten et al. 2009; Satoguina et al. 2002). Such regulation was confirmed by blocking IL-10 or TGF-β signalling or microfilaricidal chemotherapy which also reversed immunosuppression (Doetze et al. 2000; Gallin et al. 1988; Hoerauf and Brattig 2002; Soboslay et al. 1992, 1999; Ward et al. 1988). IL-10 is a hallmark of onchocerciasis,

and the majority stems from $CD4^+$ T cells (Mitre et al. 2008) or ex vivo-derived Tr1 clones (Satoguina et al. 2002). Moreover, certain promoter haplotypes of IL-10 have been shown to influence filarial-specific proliferation (Timmann et al. 2004). GEO patients also have high *Onchocerca*-specific IgG4 when compared to hyperreactive/Sowda cases which present more IgE (Hoerauf and Brattig 2002; Soboslay et al. 1997). Since IgG4 binds to the same receptor as IgE and can be induced by Treg, it is hypothesized that elevations of this Ig prevent overt responses (Adjobimey et al. 2013; Ottesen et al. 1985; Satoguina et al. 2005, 2008). This theory is supported by the contents of nodules in GEO patients (IgG4, IL-10, TGF-β, and $Foxp3^+$Treg) highlighting the helminth's control over local immune responses (Brattig et al. 2009; Korten et al. 2010, 2011). With regard to bystander stimuli, onchocerciasis patients have impaired responses to BCG and tetanus vaccination (Cooper et al. 1998; Kilian and Nielsen 1989a,b) which are thought to be mediated by immunomodulatory products. For example, cystatin, derived from L3 larvae, modulates antigen presentation by inhibiting cysteine proteases which results in reduced T-cell priming (Manoury et al. 2001) or PBMC activation through the induction of IL-10 (Schonemeyer et al. 2001). Patients that present the Sowda form of onchocerciasis do not display regulatory profiles; those patients have a dominant Th2 milieu with high levels of total IgE (Hoerauf and Brattig 2002). This profile was expanded to include Th17 responses following studies from Ghana, which revealed that elevated frequencies of Th17 and Th2 cells form part of the immune network instigating the development of severe onchocerciasis (Katawa et al. 2015). This pro-inflammatory state is thought to activate effector cells which successfully eliminate Mf within the skin but simultaneously elicit severe dermatitis. Since Sowda cases are often clustered within families, it is further assumed that there is a genetic predisposition to the development of Sowda: associations have already been linked to the IL-13 gene (Hoerauf et al. 2002). As for most filariae that parasitize man, defined studies lack specific antigens for ex vivo stimulation. Recently, an in vitro system was developed in which *O. volvulus* L3 larvae can be maintained in culture leading to the development of (pre-)adult stages (Gandjui et al. 2021; Voronin et al. 2019). Furthermore, using humanized mice, a mouse model for *O. volvulus* was recently developed (Patton et al. 2018). These new assays may provide a platform to investigate specific immune responses and macrofilaricidal drug screening.

14.3.3.3 *Mansonella perstans*, *M. ozzardi*, and *M. streptocerca*

The *Mansonella* species are well adapted to the human immune system and do not normally induce strong inflammatory immune responses. Since there is also no overt pathology, this patient group has gone undetected for many years, and therefore there is surprisingly little research on the immune responses of these patients. As with most filarial infections, *M. streptocerca*, *M. perstans*, and *M. ozzardi* infections induce blood eosinophilia and increased IgE levels (Almaviva et al. 1984; Baird et al. 1988; McNeeley et al. 1989; Meyers et al. 1972; Nutman et al. 1987b; Wiseman 1967). A major drawback to decipher *Mansonella*-specific immune

responses was the lack of specific antigen. However, due to the establishment of an in vitro culture-based system which allows the development of viable worms (Njouendou et al. 2017, 2019), initial filarial-specific immune responses could be determined in *M. perstans*-infected individuals. In short, upon restimulation with worm antigen extract, IFN-γ, IL-13, IL-10, and IL-17A secretion was enhanced in cell cultures from *M. perstans* Mf+ individuals when compared to those from cultures of healthy European individuals. Moreover, these individuals had increased type 2 helper T (Th2), natural killer (NK), and regulatory B- and T-cell subsets but decreased type 1 regulatory T (Tr1) cells (Ritter et al. 2018). Other studies also showed that PBMC from *M. perstans*-infected individuals produced without any stimulation significantly higher levels of eotaxin-2, IL-27, IL-8, MCP-4, and MDC than cells from noninfected individuals, while IFN-γ and IP-10 levels were lower (Wangala et al. 2019). Interestingly, immune studies on *M. ozzardi* revealed that the complex network of cytokines (IL-6/IL-10 axis) during infection depends on a fine balance to elicit either host protective or filarial persistent responses (Costa et al. 2018).

14.3.4 Immunopathological Processes and Disease

14.3.4.1 *Loa loa*

Previously, due to the lack of severe pathology, this infection was considered a nuisance. Indeed, clinical symptoms may take years to develop and are more common in non-endemic subjects (Boussinesq 2006; Buell et al. 2019; Churchill et al. 1996; Klion et al. 1991; Nutman et al. 1986). Accordingly, 16% of endemic loiasis patients but 95% of expatriates develop a localized angioedema which is colloquially named Calabar swelling (Klion et al. 1991). These develop in subcutaneous tissues, often located on the face, limbs, or near joints (Klion et al. 1991; Nutman et al. 1986), and it is hypothesized that they stem from allergic responses to worms or Mf (Nutman et al. 1986). They are associated with local or disseminated pruritus, urticaria, and maybe painful and restrict movement. Although they normally resolve after 2–4 days, they can persist and may even reoccur (Nutman et al. 1986). The other prominent consequence of *L. loa* is "African eye worm," caused by the migration of worms across the eye. 10–20% of infected individuals, both endemics and expatriates (Churchill et al. 1996; Nutman et al. 1986), suffer from this symptom which can last up to several days causing inflammation, itching, light sensitivity, congestion, and severe pain. The ensuing damage is generally minimal and not permanent. 30% of patients also present proteinuria and/or hematuria due to removal of high Mf loads and immune complex glomerulonephritis. Sometimes these symptoms are accentuated following chemotherapy but do not normally lead to renal failure (Klion et al. 1991; Nutman et al. 1986; Zuidema 1971). Other more seldom pathologies include inflammation of the lymph glands (Paleologo et al. 1984), arthritis (Bouvet et al. 1977), scrotal swellings (https://www.cdc.gov/

parasites/loiasis/diseases.html), eosinophilic lung infiltrates (Klion et al. 1992), and endomyocardial fibrosis (Brockington et al. 1967; Nutman et al. 1986). A recent systemic review indicated that such atypical symptoms including respiratory, cardiac, neurological gastrointestinal, renal, and ophthalmological pathologies were reported in almost half of the case reports on loiasis, and thus loiasis should be considered as a significant public health problem (Buell et al. 2019).

14.3.4.2 *Onchocerca volvulus*

Classical symptoms of onchocerciasis are dermatitis, keratitis, and chorioretinitis. Adult worm-harboring nodules do not elicit overt responses and are associated with mild clinical symptoms. They lie in subcutaneous or deeper intramuscular tissues surrounded by a fibrous capsule which contains blood, lymphatic vessels (Attout et al. 2009), and cellular infiltrates which are mainly composed of macrophages (Brattig et al. 2001; Parkhouse et al. 1985; Wildenburg et al. 1998). Severe disease manifestations have been linked to dead or dying Mf that are responsible for severe pruritus in heavily infected patients, occasional rashes, erythema, and angioedema. The severity, activity, and distribution of dermatitis are graded as follows: (1) acute papular onchodermatitis, (2) chronic papular onchodermatitis, (3) lichenified onchodermatitis, (4) atrophy, and (5) depigmentation (Kipp and Bamhuhiiga 2002; Murdoch et al. 1993). Chronic skin inflammation may induce dermatological changes including depigmentation of the skin (Leopard skin, Fig. 14.3b) and loss of elasticity. Sowda patients classically present hyperpigmented papules and plaques and can suffer from severe itching and edema that are often restricted to one limb, usually a leg (Fig. 14.3a). Bacterial superinfections may also occur due to scratching and disruption of the skin barrier. Sowda is usually accompanied by enlarged regional lymph nodes which present prominent follicular hyperplasia indicating aberrant humoral hyper-responsiveness (Hoerauf 2011). Those skin pathologies account for 50% of onchocerciasis-associated disability adjusted life years (DALYs) (Murdoch et al. 1993, 2002). Occasionally, patients develop lymphadenopathy or "hanging groin" which results from atrophic skin slings that contain accumulating inguinofemoral lymph node conglomerates. Naturally, *O. volvulus* infections are more commonly known through their layman's term "river blindness" which remains the second most prominent cause of blindness in the tropics. The disease involves all eye-related tissues, but the initial temporary keratitis is initiated by pro-inflammatory responses to lodged Mf in the conjunctival and intraocular tissues. Characteristically, opacity develops from the corners of the cornea to the center, and it is common to have varying degrees of visual impairment such as punctuate keratitis or iridocyclitis. Permanent exposure, however, can lead to irreversible sclerosing keratitis which may develop into blindness. Interestingly, through neutrophil and macrophage recruitment, *Wolbachia* also seem to play a role in these developing pathologies (Abiose 1998; Pearlman and Gillette-Ferguson 2007; Saint Andre et al. 2002; Tamarozzi et al. 2011).

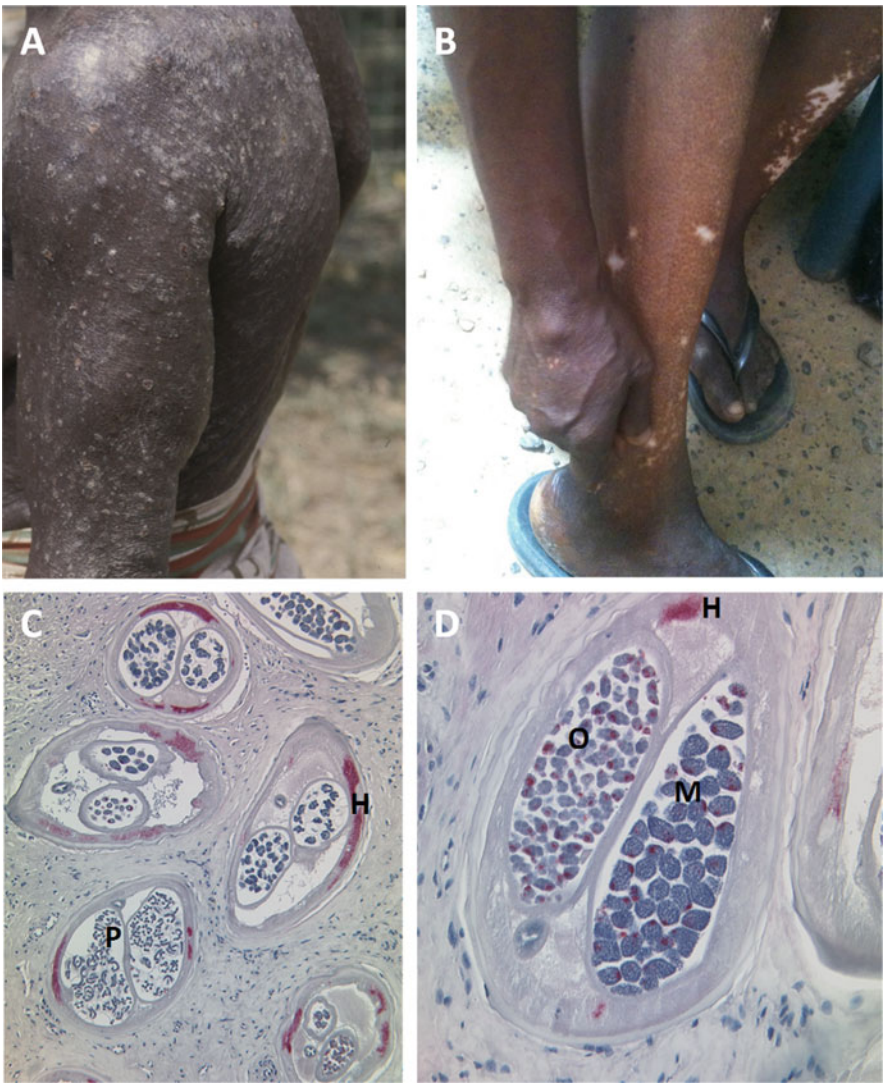

Fig. 14.3 *O. volvulus*. (**a**) Chronic onchodermatitis at shoulder and upper arm, (**b**) depigmentation (leopard skin) at legs and hand. (**c, d**) Histological sections of one female adult *O. volvulus* worm with embryonic stages in the uterus. Presence of *Wolbachia* (red dots) in hypoepidermis (H) and embryonic stages (O, oocytes; M, morulae; P, pretzel stage) within the uterus (*Wolbachia* surface protein staining)

14.3.4.3 *Mansonella perstans*, *M. ozzardi*, and *M. streptocerca*

The dermatological pathology induced by *M. streptocerca* is similar to that observed in *O. volvulus*-infected individuals (Meyers et al. 1972). Spotty depigmentation is

usually located around the thorax and shoulders, an area where Mf are often detected (Fischer et al. 1997). Mf are also found in the buttock area and may be related to inguinal lymph node swellings and thickening of the dermis. Development of severe lymphedema has been suggested, but to date there are no definite case studies (Klion and Nutman 2011). Infections with both *M. perstans* and *M. ozzardi* are generally asymptomatic presenting transient itching, swellings (e.g., of the skin) and rashes. Some case studies have also reported fever, headache, tiredness, pulmonary symptoms, joint pain, and lymph node enlargement (Adolph et al. 1962; Holmes et al. 1969; Sondergaard 1972). In exceptional cases *M. perstans* infections have been linked to pericarditis (Foster 1956), hepatitis (Dukes et al. 1968; Gelfand and Wessels 1964), meningoencephalitis, neurological disorders (Adolph et al. 1962; Dukes et al. 1968), and ocular pathology (Baird et al. 1988; Bregani et al. 2002).

14.3.5 Diagnosis (Inclusive of Histopathology)

14.3.5.1 *Loa loa*

Diagnosis in endemic areas relies mainly on the detection and identification of the sheathed Mf which are diurnal in nature (Table 14.1). Therefore, sampling should be coordinated with their highest activity (10am–2pm) but should also be based on information from the endemic regions. However, amicrofilaremic infections are common, especially in patients with pathology (Dupont et al. 1988). Adult worms can be surgically removed from either subcutaneous tissue or the eye and can be differentiated by their size and characteristics (Table 14.1). Laboratory tests include novel serological assays that have an improved sensitivity and specificity compared to previous serological tests (Ambroise-Thomas 1974; Gobbi et al. 2020; Ottesen et al. 1982; Pedram et al. 2017; Klion et al. 2003), but these are not able to differentiate between active infections and previous exposures or cured infections and are therefore only recommended for repatriates. Recently, molecular PCR and LAMP assays were developed that can identify *L. loa* in human blood or the transmitting vector and allow the differentiation between active and prior infections (Amambo et al. 2021; Drame et al. 2014; Fink et al. 2011). To identify *L. loa* patients with high Mf loads, which are at risk to develop SAEs following ivermectin or DEC treatment, the LoaScope was developed, which detects the movement of Mf in fresh blood smears using a cell phone (Emukah et al. 2018).

14.3.5.2 *Onchocerca volvulus*

The traditional way to diagnose infection is a clinical examination of the entire body to detect any signs of dermatitis and subcutaneous onchocercoma which occur in areas that correspond to the biting preferences of vectors. In Africa nodules are mainly located on the hips, sacral bones, lower limbs, thorax, and near the knee,

whereas in Latin America they are found on the upper body and head (Hoerauf 2011). Histological assessment of nodules and ultrasound (filarial dance sign; infrequently found) are also diagnostic tools but require expertise (Leichsenring et al. 1990; Mand et al. 2005). Therefore, confirmation usually requires the identification of Mf from two to four skin snip biopsies taken with a corneoscleral punch or razor and is restricted to the upper dermis since deeper punctures do not increase diagnostic sensitivity and increase the risk of bleeding and contamination with Mf of other species. In general, skin snips from African individuals are from the iliac crest and may be supplemented by biopsies from calf and scapulae. Following incubation, 6–20 hours, motile Mf can be assessed using a dissecting microscope (Mand et al. 2005; Nutman et al. 1996). Mf burdens can exceed 100 Mf/mg tissue in endemic patients, and, interestingly, the highest levels of Mf in Latin American-infected individuals are found in scapulae skin snips, whereas in the Old World, those from the iliac crest harbor the most Mf. Finally, examination of the eye using a split lamp may also reveal Mf. Keeping the head bent forward for 10 minutes will allow the Mf to migrate to the visible part of the eye (Hoerauf 2011). However, not all *O. volvulus* infections lead to microfilaridermia, as nonresident travellers and endemics receiving ivermectin MDAs are often amicrofilaridermic.

Serological tests using highly sensitive (100%) filarial-specific Igs lack specificity due to cross-reactivity and are therefore only useful for non-endemic patients that were likely to be initially seronegative or to detect alterations in IgG levels after chemotherapeutic interventions. A rapid-format antibody card test that detects IgG4 antibodies against *O. volvulus* antigen Ov-16 is also available for onchocerciasis and invaluable for screening in the field since it is fast and inexpensive (Lipner et al. 2006a, b; Weil et al. 2000). Patients that are suspected of onchocerciasis but lack detectable Mf may be identified using the Mazzotti test, developed in 1947 (Taylor 1992). This test is based on the strong immune reaction that develops following systemic DEC treatment and correlates with the intensity of infection. However, it can be life-threatening, so current applications use topical administrations of DEC that result in locally contained acute dermatitis (Kilian 1988). In a similar sensitivity to the Mazzotti test, several molecular tests (PCR and field-applicable LAMP assays) have been developed to detect *O. volvulus* DNA from skin snips, scrapings, or the transmitting vector (Abong et al. 2020, 2021; Boatin et al. 2002; Lloyd et al. 2015; Poole et al. 2017; Prince-Guerra et al. 2018).

14.3.5.3 *Mansonella perstans*, *M. ozzardi*, and *M. streptocerca*

Mansonella infections are suspected on the development of pruritus, rash, and dermatitis, and since adult worms are rarely detected due to their location in serous cavities (*M. perstans* and *M. ozzardi*) or dermis (*M. streptocerca*), unequivocal diagnosis can only be ascertained by identifying the unsheathed Mf in skin biopsies (*M. streptocerca* and *M. ozzardi*) or blood (*M. perstans* and *M. ozzardi*) (Table 14.1). As all three *Mansonella* species have no periodicity, samples can be collected at any time for Mf diagnosis. *M. streptocerca* Mf are easy to distinguish since they have a

unique hook-structured tail called the "shepherd's crook" (Eberhard and Lammie 1991; Orihel 1984). Detection of blood dwelling Mf may require concentration techniques similar to those described for *W. bancrofti* (Knott's or filtration technique). Again, diagnostic PCRs and LAMP assays on skin snip or blood samples as well as the transmitting vectors can specifically identify each *Mansonella* species (Doret et al. 2021; Drame et al. 2016; Fischer et al. 1998; Formenti et al. 2021; Kwarteng et al. 2021; Morales-Hojas et al. 2001; Poole et al. 2017). Serological tests to diagnose *Mansonella* infections are not yet available.

14.4 Alterations in Clinical Manifestations of Immunocompromised Filarial-Infected Individuals

In all regions where filarial infections are endemic, they overlap with diseases such as malaria, tuberculosis, and viral infections (HIV, HCV). Since each infection has its own peculiarities, it stands to reason that coinfections will have both direct and indirect influences on immunological and pathological responses. In addition, the sequence of infections will also play a significant role; for example, filarial infections are likely to precede tuberculosis, whereas maternally transferred HIV or malaria are likely to occur before filariasis. All filarial infections modulate $CD4^+$ T-cell responses, and the chronic regulatory phases of infection are thought to influence responses to bystander antigens. As seen with experimental and human filarial infections, these can be beneficial like the suppression of autoimmunity (Hübner et al. 2009, 2012) or can potentially hinder routine vaccinations (Cooper et al. 1998; Elias et al. 2007, 2008; Wammes et al. 2010). Interestingly, it is still a matter of debate whether the strong Th1 responses upon Calmette-Guérin (BCG) vaccination at birth reduces the susceptibility to filarial infection or vice versa whether the success of BCG vaccination depends in part on (intrauterine) exposure to filarial antigens. Several studies have documented that onchocerciasis patients have poor in vitro responses to PPD (Soboslay et al. 1992) and, interestingly, the incidence of nontuberculous *Mycobacterium leprae* infections are twice as high in areas of onchocerciasis (Prost et al. 1979). Moreover, *O. volvulus* but not LF patients were shown to modulate delayed-type hypersensitivity reactions to tuberculin skin tests (Lipner et al. 2006a; b; Rougemont et al. 1977). Advanced flow cytometry further revealed that filarial infection induces Ag-specific, exaggerated IL-4 responses in distinct T-cell memory compartments to *M. tuberculosis*-specific Ags that were dampened in individuals able to mount a delayed-type hypersensitivity reaction to *M. tuberculosis* (Chatterjee et al. 2015). With regard to malaria, both vector-borne diseases thrive together in varying prevalence and with regard to LF are even transmitted by the same vector in areas of West African and Papua New Guinea (Chadee et al. 2003). In the animal studies of filaria and malaria coinfections, some have shown dampened malaria responses (Fernandez Ruiz et al. 2008; Specht et al. 2010), whereas other showed exacerbated malaria severity (Graham et al. 2005).

Indeed, a severe drawback at the moment in studying the effects of filaria-induced pathology with concomitant coinfections or an immunocompromised state is the lack of suitable animal models (rodents can become patent but do not develop pathology) or very large cross-sectional human studies.

In contrast to tuberculosis or leishmaniasis, filariae do not seem to be opportunistic infections during HIV. However, one has to keep in mind that present studies investigated HIV patients that were mainly non-immunocompromised, and there is a lack of studies that investigated immunocompromised AIDS patients. In many endemic lands, the rates of HIV infection remain stable, and no differences in filarial-specific antigens have been detected in coinfected groups (Talaat et al. 2008). There is a high coexistence of loiasis and HIV, but very few studies have investigated the influence of HIV although a recent case study reported that an HIV-positive individual presented a rare case of pulmonary *L. loa* Mf (Cambanis 2010) which may be due to reduced protective immune response to *L. loa* or simply a coincidence. In Western Uganda, it has been suggested that *O. volvulus*-induced skin disease is exacerbated in HIV-coinfected patients (Kipp et al. 2003) and HIV-coinfected individuals were shown to have reduced *O. volvulus*-specific Ig levels when compared to non-HIV-infected persons (Tawill et al. 1996). Reduced filarial-specific cytokine profiles have also been reported in HIV-coinfected *O. volvulus* patients (Sentongo et al. 1998). However, HIV infection does not seem to impact the protective immune response against *O. volvulus*, since coinfected patients have similar Mf levels and respond equally to IVM (Fischer et al. 1995). In LF patients, coinfection with HIV showed significantly higher levels of filarial-specific IgG3 before DEC treatment but reduced levels of IgG4 following treatment indicating that therapy had a stronger antifilarial effect in these individuals (Petersen et al. 2009). In correlation, DEC therapy in coinfected patients in Tanzania showed a significant drop in viral titer and a slight increase in $CD4^+$ T cells indicating that such MDA programs might have beneficial effects in coinfected patients (Nielsen et al. 2007), although it cannot rule out that this was due to an unspecific drug effect. In a population-based cohort study in Tanzania, HIV and filarial infection were documented in 18,000 individuals. The study revealed that HIV incidence in lymphatic filariasis-positive participants was significantly higher than the incidence in lymphatic filariasis-negative participants, thus demonstrating that there was an increased risk of acquiring HIV for *W. bancrofti*-infected individuals (Kroidl et al. 2016). Coinfections of *M. perstans* and HIV have not been associated with reduced $CD4^+$ T-cell counts, higher viral loads, or a faster progression of HIV disease (Brown et al. 2004), suggesting that *Mansonella* infections may not negatively influence HIV disease. Furthermore, it seems that immunosuppression due to HIV infection does not exacerbate *M. perstans*-induced pathology (Molina et al. 1999), but whether these effects are also observed in *M. streptocerca*- or *M. ozzardi*-infected individuals has still to be investigated. Although those reported studies lacked defined immunocompromised patient cohorts, current and future MDA programs will further raise the awareness of HIV coinfections. However, there remains the main problem that behavioral and environmental factors may contribute to the

observed differences which may impair future studies about the impact of HIV or AIDS on filarial infections.

Another area which has been studied over the last years is the impact of filarial infections during pregnancy. A birth cohort study showed a causal association between maternal filarial infection and impaired or altered immune responses in children. Moreover children were potentially more susceptible to filarial infection during early childhood (Bal et al. 2018). Children from filaria-infected mothers are also less responsive to vaccination perhaps due to skewing of immune responses in utero. Indeed, in cord blood a negative association between $CD4^+$ $CD25^{hi}$ $FOXP3^+$ T cells and $CD4^+$ $Tbet^+$ as well as $CD4^+$ $ROR\gamma t^+$ T cells was observed in the infected group (Ateba-Ngoa et al. 2014). This may have effects on their entire immunological lives as observed in studies on allergy (Aguiar-Santos et al. 2018).

14.5 Treatment and Prognosis

Filariasis is a serious public health issue and has driven the development of control and elimination programs on a global level. The aim of these MDA programs was to interrupt disease transmission by systematically administrating microfilaricidal drugs to affected communities until the desired block of transmission was reached (Amazigo 2008; Ottesen et al. 2008; Sauerbrey 2008). These programs have used diethylcarbamazine/albendazole (DEC/ALB) for LF (5–8 years) or a combination of ivermectin and albendazole (IVM/ALB) for either LF in Africa (where onchocerciasis is often co-endemic and DEC is therefore contraindicated) or onchocerciasis (10–14 years). More recently the WHO recommends a triple therapy of IVM/DEC/ALB for LF in areas not co-endemic for onchocerciasis or loiasis (WHO 2017). Although the impact of these MDA programs has been extremely impressive, there remain several hurdles before elimination is achieved (Bockarie and Deb 2010; Chu et al. 2010; Coffeng et al. 2013; Mackenzie et al. 2012). For example, although originally included in the Global Programme to Eliminate Lymphatic Filariasis (GPELF), ten countries have not yet begun treatment due to internal conflicts or the endemic regions being hard to access. Moreover, an emerging future goal of such programs is the development of safe macrofilaricidal agents that allow treatments of two weeks or less since extra safety measures have had to be implemented in areas co-endemic for *L. loa* and there is growing evidence of IVM suboptimal performance (Osei-Atweneboana et al. 2011; Taylor et al. 2009). Indeed, suboptimal IVM responses have already been observed for onchocerciasis in northern Ghana, an area that obtained more than 15 rounds of IVM (Awadzi et al. 2004a,b).

Although adult *L. loa* worms can be surgically removed, such approaches are not a cure since not all adult worms are detected. This is also true for onchocerciasis although mass nodulectomies proved quite successful in endemic areas of Latin America (Guderian et al. 1987). With regard to other filarial infections, surgical procedures are not feasible, and treatment relies solely on drugs. Chemotherapeutically, loiasis is primarily treated using a 2- to 4-week daily treatment with 5–10

mg/kg DEC since it has both micro- and macrofilaricidal effects. However, this agent has been shown to elicit serious adverse effects (SAE) in patients, and therefore, professionals must evaluate the risk factors in each individual before administration. These include medical conditions, additional infections, or high Mf burden (Boussinesq 2012), since the rapid death of numerous Mf may lead to renal failure, shock, coma, and encephalitis (Gardon et al. 1997; Gentilini and Carme 1981; Gobbi et al. 2018). Therapy should be started under hospital surveillance so that corticosteroids or antihistamines are readily available to lessen rashes, joint pain, and fever (Boussinesq 2012; Nutman et al. 1986). Within the first 48h after DEC treatment, adult worms are sometimes found as subcutaneous eruptions and can be surgically removed (Nutman and Kradin 2002).

In combination with ALB (400 mg) and IVM (200μg/kg), DEC is also used as an MDA agent in endemic areas of LF and is administered at a dose of 6 mg/kg either semiannually or annually (WHO 2017). However, SAE can occur in LF patients treated with the IVM/DEC/ALB triple therapy or the previously used DEC/ALB combination, and the development and severity of such responses are correlated to Mf load, as well as locations of adult worms (e.g., scrotal pain is related to death of adult worms residing at this location and has led to reluctance of men taking MDA). This treatment is actually contraindicated for onchocerciasis patients because the DEC-mediated rapid killing of Mf has been shown to lead to strong inflammatory immune responses within the skin and eye and may lead to urticaria, angioedema, irreversible eye damage, hypotension, and even death (Greene et al. 1985) and is therefore limited to areas that are not co-endemic for onchocerciasis (WHO 2017). Except for the risk of vision impairment, similar consequences of DEC therapy are also seen when treating *M. streptocerca*-infected individuals with 6 mg/kg daily for 14–21 days. Side effects include a transient worsening of pruritus, urticaria, and popular eruptions and may further include headache, fever, nausea, joint and muscle pain, which generally begin 1–2 days after treatment (Meyers et al. 1978, 1972). DEC treatment for 21 days with 2–6 mg/kg eliminates both *M. streptocerca* microfilariae as well as adult worms.

Similar to onchocerciasis and LF, *M. streptocerca* and loiasis can be treated with IVM (Meyers et al. 1978, 1972). The macrocyclic lactone IVM succeeded suramin, a macrofilaricidal drug that is no longer used due to SAE. IVM is specific for a glutamate-gated chloride channel in nematodes which results in cellular hyperpolarization (Cully et al. 1994), and it was shown to block excretory/secretory vesicles (Moreno et al. 2010). Despite having a serum half-life of only 12 hours, this agent is very effective, depleting Mf within a few days (Boussinesq 2012; Martin-Prevel et al. 1993). In general, the effects are long-lasting, and Mf loads slowly begin to reappear after 3–4 months (Basanez et al. 2008; Duke et al. 1991) meaning that transmission is also interrupted during that time. Although it is extremely rapid, IVM works a little slower than DEC, but this allows effector cells of the immune system to clear away Mf debris. Moreover, since this occurs at a safe distance from the eye, it also prevents severe ocular adverse reactions and has therefore made IVM therapy the drug of choice for MDA against onchocerciasis (150–200 μg/kg every 6–12 months). These treatments can transiently improve cellular responsiveness

(Soboslay et al. 1992; Steel et al. 1991, 1994) and prevent or delay the development of ocular damage and skin disease but do not restore or improve visual impairment (Molyneux et al. 2003; Tielsch and Beeche 2004). Although one trial study revealed that multiple doses (x4/year) of IVM had some macrofilaricidal effects in onchocerciasis patients (Gardon et al. 2002), others debate that increasing the number of doses enhances the effect. Indeed, in *O. volvulus* patients, if the drug is given too often, it can even lead to SAE such as skin edema or ocular inflammation (Gardon et al. 2002; Kamgno et al. 2004).

With regard to LF, a meta-analysis on 15 individual IVM studies noted that a single dose of IVM resulted in almost complete clearance of Mf within 30 days with a gradual recurrence, and these effects improved at higher doses (Cao et al. 1997). Unlike IVM/ALB combinations, IVM/DEC further improved the microfilaricidal effect for LF and extended the amicrofilaremic period up to 1 year (Shenoy et al. 1998). GPELF supported the annual administration of IVM/ALB in Africa, but the weak, if any, adulticidal capacity of IVM/ALB and DEC/ALB required several rounds of MDA (Addiss 2010; Hoerauf et al. 2011). Today, the WHO recommends a triple therapy consisting of a single treatment with IVM (200 μg/kg)/DEC 6 mg/kg)/ALB (400 mg) for LF, as it leads to a clearance of the microfilaremia for more than 2 years and may also mediate some macrofilaricidal efficacy (King et al. 2018; Thomsen et al. 2016; Weil et al. 2019). Thus, with the implementation of the triple therapy, it is thought that the WHO road map for neglected tropical diseases 2021–2030 goal to achieve the elimination of LF as public health problem in 80% of the endemic countries can be met. However, areas co-endemic for *O. volvulus* or *L. loa* are excluded from this triple therapy (as well as treatment with DEC/ALB), as DEC treatment may elicit the abovementioned side effects in onchocerciasis and loiasis patients (WHO 2017).

First time IVM therapy in more heavily infected *O. volvulus* patients (20–50 Mf/mg skin and above) has to be administered with care since a substantial number of patients develop SAE including exacerbated pruritus, acute dermatitis, fever, rash, hypotension, and swellings of the limbs, face, and lymph nodes (Awadzi 2003; Chijioke and Okonkwo 1992; Pacque et al. 1991). These effects can be alleviated with antihistamines and analgesics, and further treatments are generally better tolerated. This also applies to *L. loa* patients in which IVM therapy is only recommended for patients with less than 20,000 Mf/ml blood (Boussinesq 2012; Ducorps et al. 1995) due to the risk of life-threatening neurological symptoms such as encephalopathy and coma. To identify loiasis patients with such high Mf counts, the cell phone-based method of the LoaScope was developed (Pion et al. 2020). Loiasis patients with high Mf loads can be initially treated with ALB (200 mg, twice daily, for 21d) before continuing with IVM since the drug works more slowly and causes no severe clinical adverse effects (Boussinesq 2012; Klion et al. 1993). Moreover, all loiasis patients still require therapy with DEC (5–10 mg/kg for 2–4 weeks), as this is the only treatment that provides some macrofilaricidal efficacy (Boussinesq 2012). Since *L. loa* does not harbor *Wolbachia* (Büttner et al. 2003; Desjardins et al. 2013) and is therefore immune to such therapies, there is a substantial need for new drugs that slowly reduce Mf loads in *L. loa* patients. One

possible clinical candidate is oxfendazole, which was shown in preclinical rodent studies to be active against adult filariae, but not microfilariae (Hübner et al. 2020; Pionnier et al. 2019).

Successful field studies in endemic regions of *Onchocerca* and LF have proven that anti-*Wolbachia* therapy has long-term sterilizing effects, is a safe macrofilaricidal treatment, and provides not only a superior therapeutic prognosis but improves clinical pathology in LF (reviewed in (Hoerauf 2008; Mand et al. 2012; Taylor et al. 2013)). Doxycycline is the first and, so far, only macrofilaricidal drug against onchocerciasis. In addition, doxycycline was found to be the only effective macrofilaricidal agent against *M. perstans* (Batsa Debrah et al. 2019; Coulibaly et al. 2009; Hoerauf 2009), and the presence of *Wolbachia* in *M. ozzardi* (Casiraghi et al. 2001) suggests a similar susceptibility. This is of importance, as the current MDA treatment of a single-dose IVM/ALB has no or only a minor effect on *M. perstans* microfilaremia (Asio et al. 2009b; Wanji et al. 2016). Only extended treatments with DEC (200 mg twice daily) for 21 days or ALB treatments (100 mg twice daily) for 28 days, and especially their combination, led to the reduction of *M. perstans* microfilaremia (Bregani et al. 2006). On the other hand, *M. ozzardi* microfilaremia is reduced long term following a single treatment with 150 mg/kg IVM, but not affected by DEC treatment (Ferreira et al. 2021; Bartholomew et al. 1978).

Currently, safe, affordable, and readily available antibiotics such as doxycycline are used in endemic communities. Field trials have demonstrated that a daily administration of doxycycline (100–200 mg) over a 4- to 6-week period reduced *Wolbachia* levels by 95%, had a 50–70% macrofilaricidal effect, and sterilized the remaining female adult worms (Hoerauf et al. 2001, 2000, 2009, 2008; Walker et al. 2015). At that time IVM therapy was also given to eliminate Mf (Hoerauf 2008). Today, a 4-week treatment with 200 mg/day doxycycline is recommended for the treatment of LF, as this treatment permanently depletes *Wolbachia* endosymbionts and therefore leads to a permanent sterilization of the adult female filariae and the death of the adult filariae by 18 months after treatment (Debrah et al. 2011b). Similarly, for onchocerciasis, a doxycycline treatment with 200 mg/day for 6 weeks is recommended to achieve adult worm death within 18–24 months (Hoerauf et al. 2008; Turner et al. 2010; Walker et al. 2015). Permanent filarial sterility and amicrofilaridermia can be achieved following 4-week doxycycline treatment with 200 mg/day or 5-week treatment with 100 mg/day (Hoerauf et al. 2009, 2008; Walker et al. 2015). Importantly, as anti-wolbachials have no direct effect on the microfilaremia, doxycycline treatment induces a slow clearance of the Mf, which is due to the natural removal of the Mf and inhibited filarial embryogenesis, and prevents the pathology observed following treatment with Mf-targeting drugs such as DEC or IVM. If a faster removal of the Mf is required, doxycycline treatment can be combined with a single treatment with IVM (Hoerauf et al. 2001; Turner et al. 2010). Doxycycline therapy is now recommended by the WHO as individual therapy for onchocerciasis and for clearing remaining onchocerciasis spots in the Americas (WHO 2019). Further, doxycycline therapy is an option in individuals with persisting severe dermatitis despite several rounds of IVM and eventually patients that developed suboptimal IVM responses. It is also a safer way

to treat co-endemic areas of *L. loa* since as mentioned above, this nematode lacks *Wolbachia* (Taylor et al. 2005). The only drawback is that daily administration of doxycycline for multiple weeks is not feasible for MDA and cannot be given during pregnancy or to children under the age of 8. Clinical trials that aimed to reduce the required doxycycline treatment time showed in LF patients that a 3-week course of doxycycline with a single dose of IVM/ALB resulted in reduced fertility, but did not provide macrofilaricidal effects (Turner et al. 2006). However, 3-week doxycycline administered in combination with a single dose of DEC had a macrofilaricidal efficacy in *W. bancrofti* patients, demonstrating that different combinations with doxycycline do allow shorter regimens (Mand et al. 2009).

In order to find alternative tetracycline candidates that could be used in shorter regimens, the Anti-*Wolbachia* (A-WOL) consortium, funded by the Bill & Melinda Gates Foundation, was formed in 2007. In addition, the Bill & Melinda Gates Foundation established in 2014 the Macrofilaricidal Drug Accelerator Program, to identify novel macrofilaricidal drug candidates via collaboration of partners from industry, academia, and nonprofit organizations. Alternative strategies include a 2- to 4-week course of rifampicin (10mg/kg/d) which showed anti-*Wolbachia* activity and reduced worm fertility in *O. volvulus* patients (Specht et al. 2008), and a pilot study of LF infected males presented promising effects by combining doxycycline and rifampicin for just 3 weeks (Debrah et al. 2011b). Recent preclinical data indicated that the 10 mg/kg/day rifampicin treatments were suboptimal and a high-dose (30–35 mg/kg/day) rifampicin treatment is superior to doxycycline (Aljayyoussi et al. 2017). Such high-dose rifampicin treatments are currently performed in clinical studies in onchocerciasis patients. Furthermore, the tylosin analogue ABBV-4083 presents a novel anti-wolbachial candidate (Taylor et al. 2019) that is currently tested in phase 2 clinical studies in onchocerciasis patients. Additional novel candidates for the treatment of onchocerciasis that passed phase 1 clinical studies are the two direct-acting compounds emodepside (Krücken et al. 2021) and oxfendazole (Hübner et al. 2020). Anti-wolbachial candidates that are currently under preparation for or just entered first-in-men studies are corallopyronin A (Schiefer et al. 2020) and AWZ-1066S (Hong et al. 2019), respectively.

Another novel drug that is now available for onchocerciasis treatment is moxidectin. Moxidectin is—as IVM—a macrocyclic lactone, which is microfilaricidal and leads to an amicrofilaridermia that exceeds 1 year and is therefore superior to IVM (Opoku et al. 2018). Thus, single-dose annual moxidectin treatments could replace semiannual IVM treatments for onchocerciasis MDA. However, so far IVM is used for MDA, as it has the advantage that it is donated, registered for children below the age of 12, and has been used on a large scale since more than three decades. Furthermore, moxidectin treatment may lead to similar SAEs in loiasis patients with high Mf loads, as was observed with IVM.

14.6 Prevention and Control

On an individual's level, the best prevention against filariasis is to limit contact with the responsible vectors. Against nocturnal biting mosquitoes, this can be achieved by using insecticide-soaked bednets. Interestingly, vector control programs for malaria have proven beneficial for hindering the transmission of LF by anophelines in Africa (WHO 2012a). For tissue filarial agents, the CDC recommends using insect repellents and long clothing and sleeping in air-conditioned rooms under a mosquito net. One should also avoid potential habitats since as mentioned above deer flies are attracted to camp fire smoke and travel fair distances to do so. Although there are no vaccines and no specific prophylactic therapies, the CDC does suggest that long-term travellers in endemic areas of *L. loa* take 300 mg DEC per week (https://www.cdc.gov/parasites/loiasis/prevent.html). Although off-label, travellers to endemic regions of *Wolbachia*-containing nematodes could take doxycycline since this was shown in several animal models to prevent the molting from infectious L3 larvae to adult worms (Hoerauf et al. 1999; Rao et al. 2002; Smith and Rajan 2000; Specht et al. 2018). Accordingly, travellers that are visiting areas that are co-endemic for malaria and onchocerciasis may consider using doxycycline as malaria prophylaxis (Tan et al. 2011) thus reducing the risk to obtain onchocerciasis.

No vector control programs have been implemented for *Mansonella* species and strategies for *L. loa* vectors have had only limited success due to the remote location of *Chrysops* breeding sites (Fain 1978). From 1974 to 2002, the WHO implemented the onchocerciasis Control Program (OCP) in 11 West Africa countries (Boatin and Richards 2006; Molyneux et al. 2003), distributing larvicides (e.g., Abate, *Bacillus thuringiensis*) to eliminate *Simulium* vectors along 50,000 km of rivers. Since 1988, this was carried out in combination with annual IVM treatment, which significantly reduced transmission and the development of onchocerciasis (Borsboom et al. 2003). The program was stopped in 2002, and current programs now rely solely on IVM distributions, but there remains the issue that individuals may migrate and transmit the disease to areas that have already been cleared.

With regard to immunity and vaccine development, EN residing in regions of *L. loa* do often not develop symptoms or Mf suggesting a certain level of immunity to loiasis (Buell et al. 2019; Noireau et al. 1990). For *L. loa* and *Mansonella* species, there are no available vaccines or even studies trying to develop them. One major hurdle was the lack of suitable small animal models that allow immunological studies and testing of experimental vaccines. However, with the recent development of *L. loa* mouse models (Chunda et al. 2020; Pionnier et al. 2019; Tendongfor et al. 2012), new investigations may open up in to this field. Material from LF and onchocerciasis is also limited and vaccine studies have to be performed in related animal models. In *O. volvulus* patients, protective immune responses that reduce infection burden seem to develop over time. Accordingly, repeated injections with irradiated L3 larvae in animal models and repeated exposure of *O. ochengi* in cattle induced protective immune responses and partial immunity when challenged with further infections (Abraham et al. 2002; Allen et al. 2008; Hübner et al. 2010; Lange

et al. 1994; Tchakoute et al. 2006). Recently, two recombinant antigens, Ov-103 and RAL-2, were identified that are candidates for a potential onchocerciasis vaccine (George et al. 2019; Lustigman et al. 2002). Development of such vaccines is essential, since the overall effectiveness of MDA in several regions is worrying due to noncompliance or potential drug resistance.

By 2020, the Global Programme to Eliminate Lymphatic Filariasis (GPELF) had provided combinations of DEC/ALB or ALB/IVM and more recently IVM/DEC/ALB to 923 million individuals in 63 of 73 endemic countries. Annual therapy is needed for at least 5 years and should reach 65% of the population to reduce the prevalence of infection which interrupts transmission. The strategy has managed to remove LF in 18 of the originally 73 endemic countries, where validation of elimination of LF transmission is now ongoing, and 22 additional countries have provided MDA in all endemic areas and are on track for elimination, but 33 countries still have to reach a geographical MDA coverage of 100% of the endemic areas and 10 of these countries did not start with the MDA treatments yet or did not confirm that they are not LF endemic countries. Although GPELF ended in 2020, MDAs for LF are ongoing in 45 endemic countries, and despite the fact that GPELF missed its goal to eliminate LF globally by 2020, it created a platform that endemic countries can use to achieve the elimination of LF as public health problem in 80% of the endemic countries by 2030, as stated by the WHO NTD roadmap 2021–2030 (Hooper et al. 2014; Ichimori et al. 2014; Ottesen and Horton 2020; Ramaiah and Ottesen 2014).

In 1995, the African Program for Onchocerciasis Control (APOC) started with the aim to establish a platform by 2015 that allows elimination of onchocerciasis as a public health problem in African countries (WHO 2011). Until 2014, more than 180,000 endemic communities in 20 African countries participated in the APOC program (Tekle et al. 2016). Ivermectin was donated by Merck and distributed on a community-directed annual treatment to 112 million people in 2014 (Tekle et al. 2016). APOC was estimated to prevent 8.2 million onchocerciasis-associated DALYs between 1995 and 2010 another 9.2 million DALYs by 2015 (Coffeng et al. 2013). While OCP (1974–2002) significantly reduced transmission of onchocerciasis, APOC focused on hyperendemic areas and aimed to prevent onchocerciasis as a public health problem. Expansion of this goal to eliminate onchocerciasis was challenged in several countries due to low coverage rates, political unrest, and missing funds for MDA distribution. Nevertheless, foci in Senegal and Mali achieved the interruption of onchocerciasis transmission (Diawara et al. 2009). Areas that are co-endemic for *L. loa* further complicate MDA of IVM due to the risk of encephalopathy. The Rapid Assessment Procedure for Loiasis (RAPLOA) estimates the risk to develop "ivermectin caused adverse reactions" by the prevalence of reported "eye worm" passings within an area. Prevalence rates >40% correlated with either Mf loads >30.000 per ml in 2% of individuals or microfilaremia in >20% of individuals and presented a risk for adverse reactions that is too high to perform MDA (Takougang et al. 2002; Zoure et al. 2011). Furthermore, the LoaScope was recently developed to identify loiasis patients with high *L. loa* Mf counts > 20.000 per ml (Pion et al. 2020) to implement a test and not

treat strategy (Kamgno et al. 2017). *Wolbachia*-targeting drugs such as doxycycline may be an alternative in those co-endemic areas, as they have no impact on the *Wolbachia*-free *L. loa* worms. Similarly, novel macrofilaricidal candidates with no or only minor microfilaricidal efficacy would support the elimination of onchocerciasis in those co-endemic areas and may additionally clear loiasis infections. Preclinical data indicate that oxfendazole may be such a predominant macrofilaricidal candidate (Hübner et al. 2020; Pionnier et al. 2019). Based on the success of APOC and the following ESPEN (the Expanded Special Program to Eliminate Neglected Tropical Diseases) program, the WHO Neglected Tropical Disease roadmap 2021–2030 now pronounced to target the elimination of the transmission of onchocerciasis by 2030 (WHO 2020b,c).

Strategies in South America were managed through the Onchocerciasis Elimination Program in the Americas (OEPA), which was launched in 1991 with the goal to eliminate transmission until 2012. For this purpose biannual treatments with IVM were given with an 85% coverage in 13 endemic foci. By the end of 2012, four out of six endemic countries had interrupted or eliminated onchocerciasis according to the WHO criteria (<1% of reversible (new) ocular disease and <0.1% of *O. volvulus*-specific antibodies in exposed school children and no recovery of transmission 3 years after treatment stopped). Active transmission receded from an original at risk population of 561,000 people to 26,000 in 2 foci that are located at the border areas of Venezuela and Brazil (MMWR 2013), and there are no new cases of blindness in the Americas. To eliminate onchocerciasis from the Americas, the OEPA now uses doxycycline therapy in the remaining areas (WHO 2019). In summary, it is obvious that despite all progress that has been made, filarial infections still present a major public health problem that has to be addressed. Current treatment options are often insufficient and may lead to severe adverse reactions. Thus, future challenges include the implementation of MDAs and the development and improvement of new drugs.

Acknowledgments We thank PD Dr. Sabine Specht for providing the two images of histological sections that are displayed in Fig. 14.3c, d. Further, we'd like to thank Constanze Kühn for her support with the life cycle figure. AH is a member of the Excellence ImmunoSensation2—the immune sensory system (DFG, EXS 2151). AH and MPH are members of the German Center for Infection Research (DZIF), Partner Site Bonn-Cologne, Bonn, Germany.

References

Abiose A (1998) Onchocercal eye disease and the impact of Mectizan treatment. Ann Trop Med Parasitol 92(Suppl 1):S11–S22

Abong RA, Amambo GN, Chounna Ndongmo PW, Njouendou AJ, Ritter M, Beng AA, Esum ME, Deribe K, Fru-Cho J, Fombad FF, Nji TM, Enyong PI, Poole CB, Pfarr K, Hoerauf A, Carlow CKS, Wanji S (2020) Differential susceptibility of *Onchocerca volvulus* microfilaria to ivermectin in two areas of contrasting history of mass drug administration in Cameroon: relevance of microscopy and molecular techniques for the monitoring of skin microfilarial repopulation within six months of direct observed treatment. BMC Infect Dis 20:726

Abong RA, Amambo GN, Hamid AA, Enow BA, Beng AA, Nietcho FN, Nji TM, Njouendou AJ, Ritter M, Esum ME, Deribe K, Cho JF, Fombad FF, Enyong PI, Poole C, Pfarr K, Hoerauf A, Carlow C, Wanji S (2021) The Mbam drainage system and onchocerciasis transmission post ivermectin mass drug administration (MDA) campaign. Cameroon. PLoS Negl Trop Dis 15: e0008926

Abraham D, Lucius R, Trees AJ (2002) Immunity to *Onchocerca* spp. in animal hosts. Trends Parasitol 18:164–171

Addiss D (2010) The 6th Meeting of the Global Alliance to Eliminate Lymphatic Filariasis: A half-time review of lymphatic filariasis elimination and its integration with the control of other neglected tropical diseases. Parasit Vectors 3:100

Adjobimey T, Hoerauf A (2010) Induction of immunoglobulin G4 in human filariasis: an indicator of immunoregulation. Ann Trop Med Parasitol 104:455–464

Adjobimey T, Satoguina J, Oldenburg J, Hoerauf A, Layland LE (2013) Co-activation through TLR4 and TLR9 but not TLR2 skews Treg-mediated modulation of Igs and induces IL-17 secretion in Treg: B cell co-cultures. Innate Immun 20:12–23

Adolph PE, Kagan IG, Mc QR (1962) Diagnosis and treatment of Acanthocheilonema perstans filariasis. Am J Trop Med Hyg 11:76–88

Aguiar-Santos AM, Montenegro S, Medeiros Z, Rocha A, Morais CNL, Silva AR, Bonfim C, Costa TR, Sarinho ESC (2018) Immunomodulation of allergic response in children and adolescents: What we can learn from lymphatic filarial infection. Allergologia et immunopathologia 46:167–174

Ajendra J, Specht S, Ziewer S, Schiefer A, Pfarr K, Parcina M, Kufer TA, Hoerauf A, Hübner MP (2016) NOD2 dependent neutrophil recruitment is required for early protective immune responses against infectious *Litomosoides sigmodontis* L3 larvae. Scientific reports 6:39648

Akue JP, Devaney E (2002) Transmission intensity affects both antigen-specific and nonspecific T-cell proliferative responses in *Loa loa* infection. Infect Immun 70:1475–1480

Akue JP, Devaney E, Wahl G, Moukana H (2002) Expression of filarial-specific IgG subclasses under different transmission intensities in a region endemic for loiasis. Am J Trop Med Hyg 66: 245–250

Aljayyoussi G, Tyrer HE, Ford L, Sjoberg H, Pionnier N, Waterhouse D, Davies J, Gamble J, Metugene H, Cook DA, Steven A, Sharma R, Guimaraes AF, Clare RH, Cassidy A, Johnston KL, Myhill L, Hayward L, Wanji S, Turner JD, Taylor MJ, Ward SA (2017) Short-course, high-dose rifampicin achieves *Wolbachia* depletion predictive of curative outcomes in preclinical models of lymphatic filariasis and onchocerciasis. Sci Rep 7:210

Allen JE, Adjei O, Bain O, Hoerauf A, Hoffmann WH, Makepeace BL, Schulz-Key H, Tanya VN, Trees AJ, Wanji S, Taylor DW (2008) Of mice, cattle, and humans: the immunology and treatment of river blindness. PLoS Negl Trop Dis 2:e217

Almaviva M, Galli M, Rizzi M, Simonelli E, Negri C, Vigevani GM (1984) Immune response in a symptomatic case of *Tetrapetalonema perstans* infection. Trans R Soc Trop Med Hyg 78:489–491

Al-Qaoud KM, Taubert A, Zahner H, Fleischer B, Hoerauf A (1997) Infection of BALB/c mice with the filarial nematode *Litomosoides sigmodontis*: role of CD4+ T cells in controlling larval development. Infect Immun 65:2457–2461

Al-Qaoud KM, Fleischer B, Hoerauf A (1998) The Xid defect imparts susceptibility to experimental murine filariasis--association with a lack of antibody and IL-10 production by B cells in response to phosphorylcholine. Int Immunol 10:17–25

Al-Qaoud KM, Pearlman E, Hartung T, Klukowski J, Fleischer B, Hoerauf A (2000) A new mechanism for IL-5-dependent helminth control: neutrophil accumulation and neutrophil-mediated worm encapsulation in murine filariasis are abolished in the absence of IL-5. Int Immunol 12:899–908

Amambo GN, Abong RA, Fombad FF, Njouendou AJ, Nietcho F, Beng AA, Manuel R, Esum ME, Deribe K, Cho JF, Enyong PI, Poole C, Hoerauf A, Carlow C, Wanji S (2021) Validation of

loop-mediated isothermal amplification for the detection of *Loa loa* infection in *Chrysops* spp in experimental and natural field conditions. Parasit Vectors 14:19

Amazigo U (2008) The African Programme for Onchocerciasis Control (APOC). Ann Trop Med Parasitol 102(Suppl 1):19–22

Ambroise-Thomas P (1974) Immunological diagnosis of human filariases: present possibilities, difficulties and limitations. Acta Trop 31:108–128

Anuradha R, George PJ, Hanna LE, Chandrasekaran V, Kumaran PP, Nutman TB, Babu S (2014a) Parasite-antigen driven expansion of IL-5(-) and IL-5(+) Th2 human subpopulations in lymphatic filariasis and their differential dependence on IL-10 and TGFβ. PLoS Negl Trop Dis 8 (e):2658

Anuradha R, George PJ, Hanna LE, Kumaran P, Chandrasekaran V, Nutman TB, Babu S (2014b) Expansion of parasite-specific CD4+ and CD8+ T cells expressing IL-10 superfamily cytokine members and their regulation in human lymphatic filariasis. PLoS Negl Trop Dis 8:e2762

Arndts K, Deininger S, Specht S, Klarmann U, Mand S, Adjobimey T, Debrah AY, Batsa L, Kwarteng A, Epp C, Taylor M, Adjei O, Layland LE, Hoerauf A (2012) Elevated adaptive immune responses are associated with latent infections of *Wuchereria bancrofti*. PLoS Negl Trop Dis 6:e1611

Arunkumar C, Pandiaraja P, Prince PR, Kaliraj P (2014) Immunological characterization of recombinant *Wuchereria bancrofti* cuticular collagen (COL-4) as putative vaccine candidate for human lymphatic filariasis. Asian Pac J Trop Med 7:505–512

Asio SM, Simonsen PE, Onapa AW (2009a) Analysis of the 24-h microfilarial periodicity of *Mansonella perstans*. Parasitol Res 104:945–948

Asio SM, Simonsen PE, Onapa AW (2009b) *Mansonella perstans*: safety and efficacy of ivermectin alone, albendazole alone and the two drugs in combination. Ann Trop Med Parasitol 103:31–37

Ateba-Ngoa U, Mombo-Ngoma G, Zettlmeissl E, van der Vlugt LE, de Jong SE, Matsiegui PB, Ramharter M, Kremsner PG, Yazdanbakhsh M, Adegnika AA (2014) CD4+CD25hiFOXP3+ cells in cord blood of neonates born from filaria infected mother are negatively associated with CD4+Tbet+ and CD4+RORγt+ T cells. PLoS One 9:e114630

Atmosoedjono S, Partono F, Dennis DT, Purnomo (1977) *Anopheles barbirostris* (Diptera: Culicidae) as a vector of the timor filaria on Flores Island: preliminary observations. J Med Entomol 13:611–613

Attout T, Hoerauf A, Denece G, Debrah AY, Marfo-Debrekyei Y, Boussinesq M, Wanji S, Martinez V, Mand S, Adjei O, Bain O, Specht S, Martin C (2009) Lymphatic vascularisation and involvement of Lyve-1+ macrophages in the human *Onchocerca* nodule. PLoS One 4: e8234

Awadzi K (2003) Clinical picture and outcome of Serious Adverse Events in the treatment of Onchocerciasis. Filaria J 2(Suppl 1):S6

Awadzi K, Attah SK, Addy ET, Opoku NO, Quartey BT, Lazdins-Helds JK, Ahmed K, Boatin BA, Boakye DA, Edwards G (2004a) Thirty-month follow-up of sub-optimal responders to multiple treatments with ivermectin, in two onchocerciasis-endemic foci in Ghana. Ann Trop Med Parasitol 98:359–370

Awadzi K, Boakye DA, Edwards G, Opoku NO, Attah SK, Osei-Atweneboana MY, Lazdins-Helds JK, Ardrey AE, Addy ET, Quartey BT, Ahmed K, Boatin BA, Soumbey-Alley EW (2004b) An investigation of persistent microfilaridermias despite multiple treatments with ivermectin, in two onchocerciasis-endemic foci in Ghana. Ann Trop Med Parasitol 98:231–249

Babu S, Nutman TB (2003) Proinflammatory cytokines dominate the early immune response to filarial parasites. J Immunol 171:6723–6732

Babu S, Nutman TB (2012) Immunopathogenesis of lymphatic filarial disease. Semin Immunopathol 34:847–861

Babu S, Nutman TB (2014) Immunology of lymphatic filariasis. Parasite Immunol 36:338–346

Babu S, Ganley LM, Klei TR, Shultz LD, Rajan TV (2000) Role of gamma interferon and interleukin-4 in host defense against the human filarial parasite *Brugia malayi*. Infect Immun 68:3034–3035

Babu S, Blauvelt CP, Kumaraswami V, Nutman TB (2006) Regulatory networks induced by live parasites impair both Th1 and Th2 pathways in patent lymphatic filariasis: implications for parasite persistence. J Immunol 176:3248–3256

Babu S, Bhat SQ, Pavan Kumar N, Lipira AB, Kumar S, Karthik C, Kumaraswami V, Nutman TB (2009) Filarial lymphedema is characterized by antigen-specific Th1 and th17 proinflammatory responses and a lack of regulatory T cells. PLoS Negl Trop Dis 3:1–9

Baird JK, Neafie RC, Connor DH (1988) Nodules in the conjunctiva, bung-eye, and bulge-eye in Africa caused by *Mansonella perstans*. Am J Trop Med Hyg 38:553–557

Baize S, Wahl G, Soboslay PT, Egwang TG, Georges AJ (1997) T helper responsiveness in human Loa loa infection; defective specific proliferation and cytokine production by CD4+ T cells from microfilaraemic subjects compared with amicrofilaraemics. Clin Exp Immunol 108:272–278

Bal M, Ranjit M, Satapathy AK, Khuntia HK, Pati S (2018) Filarial infection during pregnancy has profound consequences on immune response and disease outcome in children: A birth cohort study. PLoS Negl Trop Dis 12:e0006824

Bartholomew CF, Nathan MB, Tikasingh ES (1978) The failure of diethylcarbamazine in the treatment of *Mansonella ozzardi* infections. Trans R Soc Trop Med Hyg 72:423–424

Basanez MG, Pion SD, Churcher TS, Breitling LP, Little MP, Boussinesq M (2006) River blindness: a success story under threat? PLoS Med 3:e371

Basanez MG, Pion SD, Boakes E, Filipe JA, Churcher TS, Boussinesq M (2008) Effect of single-dose ivermectin on Onchocerca volvulus: a systematic review and meta-analysis. Lancet Infect Dis 8:310–322

Batsa Debrah L, Phillips RO, Pfarr K, Klarmann-Schulz U, Opoku VS, Nausch N, Owusu W, Mubarik Y, Sander AL, Lammer C, Ritter M, Layland LE, Jacobsen M, Debrah AY, Hoerauf A (2019) The Efficacy of Doxycycline Treatment on *Mansonella perstans* Infection: An Open-Label, Randomized Trial in Ghana. Am J Trop Med Hyg 101:84–92

Boatin BA, Richards FO Jr (2006) Control of onchocerciasis. Adv Parasitol 61:349–394

Boatin BA, Toe L, Alley ES, Nagelkerke NJ, Borsboom G, Habbema JD (2002) Detection of *Onchocerca volvulus* infection in low prevalence areas: a comparison of three diagnostic methods. Parasitology 125:545–552

Bockarie MJ, Deb RM (2010) Elimination of lymphatic filariasis: do we have the drugs to complete the job? Curr Opin Infect Dis 23:617–620

Borsboom GJ, Boatin BA, Nagelkerke NJ, Agoua H, Akpoboua KL, Alley EW, Bissan Y, Renz A, Yameogo L, Remme JH, Habbema JD (2003) Impact of ivermectin on onchocerciasis transmission: assessing the empirical evidence that repeated ivermectin mass treatments may lead to elimination/eradication in West-Africa. Filaria J 2:8

Bouchery T, Denece G, Attout T, Ehrhardt K, Lhermitte-Vallarino N, Hachet-Haas M, Galzi JL, Brotin E, Bachelerie F, Gavotte L, Moulia C, Bain O, Martin C (2012) The Chemokine CXCL12 Is Essential for the Clearance of the Filaria *Litomosoides sigmodontis* in Resistant Mice. PLoS One 7:e34971

Boussinesq M (2006) Loiasis. Ann Trop Med Parasitol 100:715–731

Boussinesq M (2012) Loiasis: new epidemiologic insights and proposed treatment strategy. J Travel Med 19:140–143

Bouvet JP, Therizol M, Auquier L (1977) Microfilarial polyarthritis in a massive *Loa loa* infestation: a case report. Acta Trop 34:281–284

Brattig NW, Büttner DW, Hoerauf A (2001) Neutrophil accumulation around *Onchocerca* worms and chemotaxis of neutrophils are dependent on *Wolbachia* endobacteria. Microbes Infect 3: 439–446

Brattig NW, Schwohl A, Hoerauf A, Büttner DW (2009) Identification of the lipid mediator prostaglandin E2 in tissue immune cells of humans infected with the filaria *Onchocerca volvulus*. Acta Trop 112:231–235

Bregani ER, Ceraldi T, Rovellini A, Ghiringhelli C (2002) Case report: intraocular localization of *Mansonella perstans* in a patient from south Chad. Trans R Soc Trop Med Hyg 96:654

Bregani ER, Rovellini A, Mbaidoum N, Magnini MG (2006) Comparison of different anthelminthic drug regimens against *Mansonella perstans* filariasis. Trans R Soc Trop Med Hyg 100:458–463

Brockington IF, Olsen EG, Goodwin JF (1967) Endomyocardial fibrosis in Europeans resident in tropical Africa. Lancet 1:583–588

Brown M, Kizza M, Watera C, Quigley MA, Rowland S, Hughes P, Whitworth JA, Elliott AM (2004) Helminth infection is not associated with faster progression of HIV disease in coinfected adults in Uganda. J Infect Dis 190:1869–1879

Brumpt EJA (1904) La Filaria loa, Guyot, est la forme adulte de la microfilaire désignée sous le nom de Filaria diurna Manson. Comp Rend Soc Biol Paris 56:630

Buell KG, Whittaker C, Chesnais CB, Jewell PD, Pion SDS, Walker M, Basáñez MG, Boussinesq M (2019) Atypical clinical manifestations of Loiasis and their relevance for endemic populations. Open Forum Infect Dis 6:ofz417

Burri H, Loutan L, Kumaraswami V, Vijayasekaran V (1996) Skin changes in chronic lymphatic filariasis. Trans R Soc Trop Med Hyg 90:671–674

Büttner DW, Wanji S, Bazzocchi C, Bain O, Fischer P (2003) Obligatory symbiotic Wolbachia endobacteria are absent from Loa loa. Filaria J 2:10

Calvopina M, Chiluisa-Guacho C, Toapanta A, Fonseca D, Villacres I (2019) High prevalence of *Mansonella ozzardi* infection in the Amazon Region, Ecuador. Emerg Infect Dis 25:2081–2083

Cambanis A (2010) Pulmonary loiasis and HIV coinfection in rural Cameroon. PLoS Negl Trop Dis 4:e572

Cao WC, Van der Ploeg CP, Plaisier AP, van der Sluijs IJ, Habbema JD (1997) Ivermectin for the chemotherapy of bancroftian filariasis: a meta-analysis of the effect of single treatment. Trop Med Int Health 2:393–403

Casiraghi M, Favia G, Cancrini G, Bartoloni A, Bandi C (2001) Molecular identification of *Wolbachia* from the filarial nematode *Mansonella ozzardi*. Parasitol Res 87:417–420

Cerqueira N (1959) Sobbre a transmissao da *Mansonella ozzardi*. J Bras Med (Rio) 1:885–914

Chadee DD, Rawlins SC, Tiwari TS (2003) Short communication: concomitant malaria and filariasis infections in Georgetown, Guyana. Trop Med Int Health 8:140–143

Chatterjee S, Clark CE, Lugli E, Roederer M, Nutman TB (2015) Filarial infection modulates the immune response to *Mycobacterium tuberculosis* through expansion of CD4+ IL-4 memory T cells. J Immunol 194:2706–2714

Chauhan N, Sharma R, Hoti SL (2015) Identification and biochemical characterization of macrophage migration inhibitory factor-2 (MIF-2) homologue of human lymphatic filarial parasite, *Wuchereria bancrofti*. Acta Trop 142:71–78

Chijioke CP, Okonkwo PO (1992) Adverse events following mass ivermectin therapy for onchocerciasis. Trans R Soc Trop Med Hyg 86:284–286

Chitkara RK, Krishna G (2006) Parasitic pulmonary eosinophilia. Semin Respir Crit Care Med 27: 171–184

Chu BK, Hooper PJ, Bradley MH, McFarland DA, Ottesen EA (2010) The economic benefits resulting from the first 8 years of the Global Programme to Eliminate Lymphatic Filariasis (2000-2007). PLoS Negl Trop Dis 4:e708

Chunda VC, Ritter M, Bate A, Gandjui NVT, Esum ME, Fombad FF, Njouendou AJ, Ndongmo PWC, Taylor MJ, Hoerauf A, Layland LE, Turner JD, Wanji S (2020) Comparison of immune responses to Loa loa stage-specific antigen extracts in *Loa loa*-exposed BALB/c mice upon clearance of infection. Parasit Vectors 13:51

Churchill DR, Morris C, Fakoya A, Wright SG, Davidson RN (1996) Clinical and laboratory features of patients with loiasis *(Loa loa* filariasis) in the U.K. J Infect 33:103–109

Coffeng LE, Stolk WA, Zoure HG, Veerman JL, Agblewonu KB, Murdoch ME, Noma M, Fobi G, Richardus JH, Bundy DA, Habbema D, de Vlas SJ, Amazigo UV (2013) African Programme

For Onchocerciasis Control 1995-2015: model-estimated health impact and cost. PLoS Negl Trop Dis 7(e):2032

Cooper PJ, Espinel I, Paredes W, Guderian RH, Nutman TB (1998) Impaired tetanus-specific cellular and humoral responses following tetanus vaccination in human onchocerciasis: a possible role for interleukin-10. J Infect Dis 178:1133–1138

Costa AG, Sadahiro A, Monteiro Tarragô A, Pessoa FAC, Pires Loiola B, Malheiro A, Medeiros JF (2018) Immune response in *Mansonella ozzardi* infection modulated by IL-6/IL-10 axis in Amazon region of Brazil. Cytokine 104:98–103

Coulibaly YI, Dembele B, Diallo AA, Lipner EM, Doumbia SS, Coulibaly SY, Konate S, Diallo DA, Yalcouye D, Kubofcik J, Doumbo OK, Traore AK, Keita AD, Fay MP, Traore SF, Nutman TB, Klion AD (2009) A randomized trial of doxycycline for *Mansonella perstans* infection. N Engl J Med 361:1448–1458

Cully DF, Vassilatis DK, Liu KK, Paress PS, Van der Ploeg LH, Schaeffer JM, Arena JP (1994) Cloning of an avermectin-sensitive glutamate-gated chloride channel from *Caenorhabditis elegans*. Nature 371:707–711

Dakshinamoorthy G, Munirathinam G, Stoicescu K, Reddy MV, Kalyanasundaram R (2013) Large extracellular loop of tetraspanin as a potential vaccine candidate for filariasis. PLoS One 8: e77394

Daniels CW (1898) Discovery of the parenteral form of a British guiana blood worm. Br Med J 1: 1011–1012

Darwiche R, Lugo F, Drurey C, Varossieau K, Smant G, Wilbers RHP, Maizels RM, Schneiter R, Asojo OA (2018) Crystal structure of *Brugia malayi* venom allergen-like protein-1 (BmVAL-1), a vaccine candidate for lymphatic filariasis. Int J Parasitol 48:371–378

Das BK, Sahoo PK, Ravindran B (1996) A role for tumour necrosis factor-alpha in acute lymphatic filariasis. Parasite Immunol 18:421–424

Das NC, Sen Gupta PS, Biswal S, Patra R, Rana MK, Mukherjee S (2021) In-silico evidences on filarial cystatin as a putative ligand of human TLR4. J Biomol Struct Dyn 2021:1–17

Debrah AY, Mand S, Specht S, Marfo-Debrekyei Y, Batsa L, Pfarr K, Larbi J, Lawson B, Taylor M, Adjei O, Hoerauf A (2006) Doxycycline reduces plasma VEGF-C/sVEGFR-3 and improves pathology in lymphatic filariasis. PLoS Pathog 2:e92

Debrah AY, Mand S, Toliat MR, Marfo-Debrekyei Y, Batsa L, Nurnberg P, Lawson B, Adjei O, Hoerauf A, Pfarr K (2007) Plasma vascular endothelial growth Factor-A (VEGF-A) and VEGF-A gene polymorphism are associated with hydrocele development in lymphatic filariasis. Am J Trop Med Hyg 77:601–608

Debrah AY, Batsa L, Albers A, Mand S, Toliat MR, Nurnberg P, Adjei O, Hoerauf A, Pfarr K (2011a) Transforming growth factor-beta1 variant Leu10Pro is associated with both lack of microfilariae and differential microfilarial loads in the blood of persons infected with lymphatic filariasis. Hum Immunol 72:1143–1148

Debrah AY, Mand S, Marfo-Debrekyei Y, Batsa L, Albers A, Specht S, Klarmann U, Pfarr K, Adjei O, Hoerauf A (2011b) Macrofilaricidal Activity in *Wuchereria bancrofti* after 2 Weeks Treatment with a Combination of Rifampicin plus Doxycycline. J Parasitol Res 2011:201617

Debrah LB, Nausch N, Opoku VS, Owusu W, Mubarik Y, Berko DA, Wanji S, Layland LE, Hoerauf A, Jacobsen M, Debrah AY, Phillips RO (2017) Epidemiology of *Mansonella perstans* in the middle belt of Ghana. Parasit Vectors 10:15

Delaporte F (2008) The discovery of the vector of Robles disease. Parasitologia 50:227–231

Desjardins CA, Cerqueira GC, Goldberg JM, Hotopp JC, Haas BJ, Zucker J, Ribeiro JM, Saif S, Levin JZ, Fan L, Zeng Q, Russ C, Wortman JR, Fink DL, Birren BW, Nutman TB (2013) Genomics of *Loa loa*, a *Wolbachia*-free filarial parasite of humans. Nat Genet 45:495–500

Diawara L, Traore MO, Badji A, Bissan Y, Doumbia K, Goita SF, Konate L, Mounkoro K, Sarr MD, Seck AF, Toe L, Touree S, Remme JH (2009) Feasibility of onchocerciasis elimination with ivermectin treatment in endemic foci in Africa: first evidence from studies in Mali and Senegal. PLoS Negl Trop Dis 3:e497

Dimock KA, Eberhard ML, Lammie PJ (1996) Th1-like antifilarial immune responses predominate in antigen-negative persons. Infect Immun 64:2962–2967

Doetze A, Satoguina J, Burchard G, Rau T, Loliger C, Fleischer B, Hoerauf A (2000) Antigen-specific cellular hyporesponsiveness in a chronic human helminth infection is mediated by T(h) 3/T(r)1-type cytokines IL-10 and transforming growth factor-beta but not by a T(h)1 to T(h)2 shift. Int Immunol 12:623–630

Doret MPM, Nana-Djeunga HC, Nzune-Toche N, Pion SDS, Chesnais CB, Boussinesq M, Kamgno J, Varlet-Marie E, Locatelli S (2021) Limitations of PCR detection of filarial DNA in human stools from subjects non-infected with soil-transmitted helminths. Parasite 28:47

Drame PM, Fink DL, Kamgno J, Herrick JA, Nutman TB (2014) Loop-mediated isothermal amplification for rapid and semiquantitative detection of *Loa loa* infection. J Clin Microbiol 52:2071–2077

Drame PM, Montavon C, Pion SD, Kubofcik J, Fay MP, Nutman TB (2016) Molecular Epidemiology of Blood-Borne Human Parasites in a *Loa loa*-, *Mansonella perstans*-, and *Plasmodium falciparum*-Endemic Region of Cameroon. Am J Trop Med Hyg 94:1301–1308

Dreyer G, Amaral F, Noroes J, Medeiros Z (1994) Ultrasonographic evidence for stability of adult worm location in bancroftian filariasis. Trans R Soc Trop Med Hyg 88:558

Dreyer G, Medeiros Z, Netto MJ, Leal NC, de Castro LG, Piessens WF (1999) Acute attacks in the extremities of persons living in an area endemic for bancroftian filariasis: differentiation of two syndromes. Trans R Soc Trop Med Hyg 93:413–417

Dreyer G, Noroes J, Figueredo-Silva J, Piessens WF (2000) Pathogenesis of lymphatic disease in bancroftian filariasis: a clinical perspective. Parasitol Today 16:544–548

Ducorps M, Gardon-Wendel N, Ranque S, Ndong W, Boussinesq M, Gardon J, Schneider D, Chippaux JP (1995) Secondary effects of the treatment of hypermicrofilaremic loiasis using ivermectin. Bull Soc Pathol Exot 88:105–112

Duke BO (1954) The uptake of the microfilariae of *Acanthocheilonema streptocerca* by *Culicoides grahamii*, and their subsequent development. Ann Trop Med Parasitol 48:416–420

Duke BO (1957) Experimental transmission of *Loa loa* from man to monkey. Nature 179:1357–1358

Duke BO, Zea-Flores G, Munoz B (1991) The embryogenesis of *Onchocerca volvulus* over the first year after a single dose of ivermectin. Trop Med Parasitol 42:175–180

Dukes DC, Gelfand M, Gadd KG, Clarke VD, Goldsmid JM (1968) Cerebral filariasis caused by *Acanthocheilonema perstans*. Cent Afr J Med 14:21–27

Dupont A, Zue-N'dong J, Pinder M (1988) Common occurrence of amicrofilaraemic *Loa loa* filariasis within the endemic region. Trans R Soc Trop Med Hyg 82:730

Eberhard ML, Lammie PJ (1991) Laboratory diagnosis of filariasis. Clin Lab Med 11:977–1010

Ehrens A, Lenz B, Neumann AL, Giarrizzo S, Reichwald JJ, Frohberger SJ, Stamminger W, Buerfent BC, Fercoq F, Martin C, Kulke D, Hoerauf A, Hübner MP (2021) Microfilariae trigger eosinophil extracellular DNA traps in a dectin-1-dependent manner. Cell Rep 34:108621

Elias D, Britton S, Kassu A, Akuffo H (2007) Chronic helminth infections may negatively influence immunity against tuberculosis and other diseases of public health importance. Expert Rev Anti Infect Ther 5:475–484

Elias D, Britton S, Aseffa A, Engers H, Akuffo H (2008) Poor immunogenicity of BCG in helminth infected population is associated with increased in vitro TGF-beta production. Vaccine 26: 3897–3902

Emukah E, Rakers LJ, Kahansim B, Miri ES, Nwoke BEB, Griswold E, Saka Y, Anagbogu I, Davies E, Ityonzughul C, D'Ambrosio M, Bakalar M, Fletcher DA, Nutman T, Kamgno J, Richards FO (2018) In Southern Nigeria *Loa loa* blood microfilaria density is very low even in areas with high prevalence of loiasis: results of a survey using the new loa scope technology. Am J Trop Med Hyg 99:116–123

Eveland LK, Yermakov V, Kenney M (1975) *Loa loa* infection without microfilaraemia. Trans R Soc Trop Med Hyg 69:354–355

Fain A (1978) Current problems of loaiasis. Bull World Health Organ 56:155–167

Fernandez Ruiz D, Dubben B, Saeftel M, Endl E, Deininger S, Hoerauf A, Specht S (2008) Filarial infection induces protection against P. berghei liver stages in mice. Microbes Infect 11:172–180
Ferreira MU, Crainey JL, Luz SLB (2021) Mansonella ozzardi. Trends Parasitol 37:90–91
Fink DL, Kamgno J, Nutman TB (2011) Rapid molecular assays for specific detection and quantitation of *Loa loa* microfilaremia. PLoS Negl Trop Dis 5:e1299
Fischer P, Kipp W, Kabwa P, Büttner DW (1995) Onchocerciasis and human immunodeficiency virus in western Uganda: prevalences and treatment with ivermectin. Am J Trop Med Hyg 53: 171–178
Fischer P, Bamuhiiga J, Büttner DW (1997) Occurrence and diagnosis of *Mansonella streptocerca* in Uganda. Acta Trop 63:43–55
Fischer P, Büttner DW, Bamuhiiga J, Williams SA (1998) Detection of the filarial parasite *Mansonella streptocerca* in skin biopsies by a nested polymerase chain reaction-based assay. Am J Trop Med Hyg 58:816–820
Formenti F, Tang TT, Tamarozzi F, Silva R, La Marca G, Pajola B, Piubelli C, Perandin F, Rubio JM, Escolar EM, Bisoffi Z, Gobbi F (2021) Preliminary comparison between an in-house real-time PCR vs microscopy for the diagnosis of *Loa loa* and *Mansonella perstans*. Acta Trop 216: 105838
Foster DG (1956) Filariasis, a rare cause of pericarditis. J Trop Med Hyg 59:212–214
Freedman DO, de Almeida Filho PJ, Besh S, Maia e Silva MC, Braga C, Maciel A (1994) Lymphoscintigraphic analysis of lymphatic abnormalities in symptomatic and asymptomatic human filariasis. J Infect Dis 170:927–933
Freedman DO, Bui T, De Almeida Filho PJ, Braga C, Maia e Silva MC, Maciel A, Furtado AF (1995) Lymphoscintigraphic assessment of the effect of diethylcarbamazine treatment on lymphatic damage in human bancroftian filariasis. Am J Trop Med Hyg 52:258–261
Frohberger SJ, Ajendra J, Surendar J, Stamminger W, Ehrens A, Buerfent BC, Gentil K, Hoerauf A, Hübner MP (2019) Susceptibility to *L. sigmodontis* infection is highest in animals lacking IL-4R/IL-5 compared to single knockouts of IL-4R, IL-5 or eosinophils. Parasit Vectors 12:248
Frohberger SJ, Fercoq F, Neumann AL, Surendar J, Stamminger W, Ehrens A, Karunakaran I, Remion E, Vogl T, Hoerauf A, Martin C, Hübner MP (2020) S100A8/S100A9 deficiency increases neutrophil activation and protective immune responses against invading infective L3 larvae of the filarial nematode *Litomosoides sigmodontis*. PLoS Negl Trop Dis 14:e0008119
Gallin M, Edmonds K, Ellner JJ, Erttmann KD, White AT, Newland HS, Taylor HR, Greene BM (1988) Cell-mediated immune responses in human infection with *Onchocerca volvulus*. J Immunol 140:1999–2007
Gandjui NVT, Njouendou AJ, Gemeg EN, Fombad FF, Ritter M, Kien CA, Chunda VC, Fru J, Esum ME, Hübner MP, Enyong PA, Hoerauf A, Wanji S (2021) Establishment of an in vitro culture system to study the developmental biology of *Onchocerca volvulus* with implications for anti-*Onchocerca* drug discovery and screening. PLoS Negl Trop Dis 15:e0008513
Garcia A, Abel L, Cot M, Richard P, Ranque S, Feingold J, Demenais F, Boussinesq M, Chippaux JP (1999) Genetic epidemiology of host predisposition microfilaraemia in human loiasis. Trop Med Int Health 4:565–574
Gardon J, Gardon-Wendel N, Demanga N, Kamgno J, Chippaux JP, Boussinesq M (1997) Serious reactions after mass treatment of onchocerciasis with ivermectin in an area endemic for *Loa loa* infection. Lancet 350:18–22
Gardon J, Boussinesq M, Kamgno J, Gardon-Wendel N, Demanga N, Duke BO (2002) Effects of standard and high doses of ivermectin on adult worms of *Onchocerca volvulus*: a randomised controlled trial. Lancet 360:203–210
Gelfand M, Wessels P (1964) *Acanthocheilonema perstans* in a European female. A discussion of its possible pathogenicity and a suggested new syndrome. Trans R Soc Trop Med Hyg 58:552–556
Gentil K, Lentz CS, Rai R, Muhsin M, Kamath AD, Mutluer O, Specht S, Hübner MP, Hoerauf A (2014) Eotaxin-1 is involved in parasite clearance during chronic filarial infection. Parasite Immunol 36:60–77

Gentilini M, Carme B (1981) Treatment of filariases in a hospital setting. Complications - results. Ann Soc Belg Med Trop 61:319–326

George PJ, Hess JA, Jain S, Patton JB, Zhan T, Tricoche N, Zhan B, Bottazzi ME, Hotez PJ, Abraham D, Lustigman S (2019) Antibody responses against the vaccine antigens Ov-103 and Ov-RAL-2 are associated with protective immunity to *Onchocerca volvulus* infection in both mice and humans. PLoS Negl Trop Dis 13:e0007730

Global Burden of Disease Study 2017 (2018) Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392: 1789–1858

Gobbi F, Bottieau E, Bouchaud O, Buonfrate D, Salvador F, Rojo-Marcos G, Rodari P, Clerinx J, Trevino B, Herrera-Avila JP, Neumayr A, Calleri G, Angheben A, Rothe C, Zammarchi L, Guerriero M, Bisoffi Z (2018) Comparison of different drug regimens for the treatment of loiasis-A TropNet retrospective study. PLoS Negl Trop Dis 12:e0006917

Gobbi F, Buonfrate D, Boussinesq M, Chesnais CB, Pion SD, Silva R, Moro L, Rodari P, Tamarozzi F, Biamonte M, Bisoffi Z (2020) Performance of two serodiagnostic tests for loiasis in a Non-Endemic area. PLOS Negl Trop Dis 14:e0008187

Graham AL, Lamb TJ, Read AF, Allen JE (2005) Malaria-filaria coinfection in mice makes malarial disease more severe unless filarial infection achieves patency. J Infect Dis 191:410–421

Greene BM, Taylor HR, Cupp EW, Murphy RP, White AT, Aziz MA, Schulz-Key H, D'Anna SA, Newland HS, Goldschmidt LP et al (1985) Comparison of ivermectin and diethylcarbamazine in the treatment of onchocerciasis. N Engl J Med 313:133–138

Grove DI (1990) A history of human helminthology. Bookcraft (Bath) Ltd UK, Oxon

Guderian RH, Proano R, Beck B, Mackenzie CD (1987) The reduction in microfilariae loads in the skin and eye after nodulectomy in Ecuadorian onchocerciasis. Trop Med Parasitol 38:275–278

Haapasalo K, Meri T, Jokiranta TS (2009) *Loa loa* Microfilariae evade complement attack in vivo by acquiring regulatory proteins from host plasma. Infect Immun 77:3886–3893

Haarbrink M, Terhell AJ, Abadi K, Asri M, de Medeiros F, Yazdanbakhsh M (1999) Anti-filarial IgG4 in men and women living in Brugia malayi-endemic areas. Trop Med Int Health 4:93–97

Habermann RT, Menges RW (1968) Filariasis (*Acanthocheilonema perstans*) in a gorilla. Vet Med Small Anim Clin 63:1040–1043

Hoerauf A (2008) Filariasis: new drugs and new opportunities for lymphatic filariasis and onchocerciasis. Curr Opin Infect Dis 21:673–681

Hoerauf A (2009) *Mansonella perstans*--the importance of an endosymbiont. N Engl J Med 361: 1502–1504

Hoerauf A (2011) Onchocerciasis. In: Guerrant RL, Walker DH, Weller PF (eds) Tropical infectious diseases: principles, pathogens and practice, 3rd edn. Saunders Elsevier, Philadelphia, pp 741–749

Hoerauf A, Brattig N (2002) Resistance and susceptibility in human onchocerciasis--beyond Th1 vs. Th2. Trends Parasitol 18:25–31

Hoerauf A, Nissen-Pahle K, Schmetz C, Henkle-Duhrsen K, Blaxter ML, Büttner DW, Gallin MY, Al-Qaoud KM, Lucius R, Fleischer B (1999) Tetracycline therapy targets intracellular bacteria in the filarial nematode *Litomosoides sigmodontis* and results in filarial infertility. J Clin Invest 103:11–18

Hoerauf A, Volkmann L, Hamelmann C, Adjei O, Autenrieth IB, Fleischer B, Büttner DW (2000) Endosymbiotic bacteria in worms as targets for a novel chemotherapy in filariasis. Lancet 355: 1242–1243

Hoerauf A, Mand S, Adjei O, Fleischer B, Büttner DW (2001) Depletion of wolbachia endobacteria in *Onchocerca volvulus* by doxycycline and microfilaridermia after ivermectin treatment. Lancet 357:1415–1416

Hoerauf A, Kruse S, Brattig NW, Heinzmann A, Mueller-Myhsok B, Deichmann KA (2002) The variant Arg 110Gln of human IL-13 is associated with an immunologically hyper-reactive form of onchocerciasis (sowda). Microbes Infect 4:37–42

Hoerauf A, Satoguina J, Saeftel M, Specht S (2005) Immunomodulation by filarial nematodes. Parasite Immunol 27:417–429

Hoerauf A, Specht S, Büttner M, Pfarr K, Mand S, Fimmers R, Marfo-Debrekyei Y, Konadu P, Debrah AY, Bandi C, Brattig N, Albers A, Larbi J, Batsa L, Taylor MJ, Adjei O, Büttner DW (2008) *Wolbachia* endobacteria depletion by doxycycline as antifilarial therapy has macrofilaricidal activity in onchocerciasis: a randomized placebo-controlled study. Med Microbiol Immunol 197:295–311

Hoerauf A, Specht S, Marfo-Debrekyei Y, Büttner M, Debrah AY, Mand S, Batsa L, Brattig N, Konadu P, Bandi C, Fimmers R, Adjei O, Büttner DW (2009) Efficacy of 5-week doxycycline treatment on adult *Onchocerca volvulus*. Parasitol Res 104:437–447

Hoerauf A, Pfarr K, Mand S, Debrah AY, Specht S (2011) Filariasis in Africa-treatment challenges and prospects. Clin Microbiol Infect 17:977–985

Holmes GK, Gelfand M, Boyt W, Mackenzie P (1969) A study to investigate the pathogenicity of a parasite resembling *Acanthocheilonema perstans*. Trans R Soc Trop Med Hyg 63:479–484

Hong WD, Benayoud F, Nixon GL, Ford L, Johnston KL, Clare RH, Cassidy A, Cook DAN, Siu A, Shiotani M, Webborn PJH, Kavanagh S, Aljayyoussi G, Murphy E, Steven A, Archer J, Struever D, Frohberger SJ, Ehrens A, Hübner MP, Hoerauf A, Roberts AP, Hubbard ATM, Tate EW, Serwa RA, Leung SC, Qie L, Berry NG, Gusovsky F, Hemingway J, Turner JD, Taylor MJ, Ward SA, O'Neill PM (2019) AWZ1066S, a highly specific anti-*Wolbachia* drug candidate for a short-course treatment of filariasis. Proc Natl Acad Sci U S A 116:1414–1419

Hooper PJ, Chu BK, Mikhailov A, Ottesen EA, Bradley M (2014) Assessing progress in reducing the at-risk population after 13 years of the global programme to eliminate lymphatic filariasis. PLoS Negl Trop Dis 8:e3333

Hotez PJ, Strych U, Lustigman S, Bottazzi ME (2016) Human anthelminthic vaccines: Rationale and challenges. Vaccine 34:3549–3555

Hübner MP, Stocker JT, Mitre E (2009) Inhibition of type 1 diabetes in filaria-infected non-obese diabetic mice is associated with a T helper type 2 shift and induction of Fox P3+ regulatory T cells. Immunology 127:512–522

Hübner MP, Torrero MN, Mitre E (2010) Type 2 immune-inducing helminth vaccination maintains protective efficacy in the setting of repeated parasite exposures. Vaccine 28:1746–1757

Hübner MP, Shi Y, Torrero MN, Mueller E, Larson D, Soloviova K, Gondorf F, Hoerauf A, Killoran KE, Stocker JT, Davies SJ, Tarbell KV, Mitre E (2012) Helminth Protection against Autoimmune Diabetes in Nonobese Diabetic Mice Is Independent of a Type 2 Immune Shift and Requires TGF-beta. J Immunol 188:559–568

Hübner MP, Martin C, Specht S, Koschel M, Dubben B, Frohberger SJ, Ehrens A, Fendler M, Struever D, Mitre E, Vallarino-Lhermitte N, Gokool S, Lustigman S, Schneider M, Townson S, Hoerauf A, Scandale I (2020) Oxfendazole mediates macrofilaricidal efficacy against the filarial nematode *Litomosoides sigmodontis* in vivo and inhibits *Onchocerca* spec. motility in vitro. PLoS Negl Trop Dis 14:e0008427

Hussain R, Hamilton RG, Kumaraswami V, Adkinson NF Jr, Ottesen EA (1981) IgE responses in human filariasis. I. Quantitation of filaria-specific IgE. J Immunol 127:1623–1629

Ichimori K, King JD, Engels D, Yajima A, Mikhailov A, Lammie P, Ottesen EA (2014) Global programme to eliminate lymphatic filariasis: the processes underlying programme success. PLoS Negl Trop Dis 8:e3328

Immanuel C, Ramanathan A, Balasubramaniyan M, Khatri VK, Amdare NP, Rao DN, Reddy MVR, Perumal K (2017) Immunoprophylaxis of multi-antigen peptide (MAP) vaccine for human lymphatic filariasis. Immunol Res 65:729–738

Jawaharlal JP, Madhumathi J, Prince RP, Kaliraj P (2014) Repeat region of *Brugia malayi* sheath protein (Shp-1) carries Dominant B epitopes recognized in filarial endemic population. Acta parasitologica 59:454–458

Jha R, Gangwar M, Chahar D, Setty Balakrishnan A, Negi MP, Misra-Bhattacharya S (2017) Humans from *Wuchereria bancrofti* endemic area elicit substantial immune response to proteins of the filarial parasite *Brugia malayi* and its endosymbiont *Wolbachia*. Parasit Vectors 10:40

Joardar N, Mondal C, Sinha Babu SP (2021) A review on the interactions between dendritic cells, filarial parasite and parasite-derived molecules in regulating the host immune responses. Scand J Immunol 93:e13001

Jungmann P, Figueredo-Silva J, Dreyer G (1991) Bancroftian lymphadenopathy: a histopathologic study of fifty-eight cases from northeastern Brazil. Am J Trop Med Hyg 45:325–331

Kalyanasundaram R, Khatri V, Chauhan N (2020) Advances in Vaccine Development for Human Lymphatic Filariasis. Trends Parasitol 36:195–205

Kamgno J, Gardon J, Gardon-Wendel N, Demanga N, Duke BO, Boussinesq M (2004) Adverse systemic reactions to treatment of onchocerciasis with ivermectin at normal and high doses given annually or three-monthly. Trans R Soc Trop Med Hyg 98:496–504

Kamgno J, Pion SD, Chesnais CB, Bakalar MH, D'Ambrosio MV, Mackenzie CD, Nana-Djeunga HC, Gounoue-Kamkumo R, Njitchouang GR, Nwane P, Tchatchueng-Mbouga JB, Wanji S, Stolk WA, Fletcher DA, Klion AD, Nutman TB, Boussinesq M (2017) A Test-and-Not-Treat strategy for onchocerciasis in *Loa loa*-endemic areas. N Engl J Med 377:2044–2052

Katawa G, Layland LE, Debrah AY, von Horn C, Batsa L, Kwarteng A, Arriens S, Taylor DW, Specht S, Hoerauf A, Adjobimey T (2015) Hyperreactive onchocerciasis is characterized by a combination of Th17-Th2 immune responses and reduced regulatory T cells. PLoS Negl Trop Dis 9:e 3414

Keiser PB, Nutman TB (2002) Update on Lymphatic Filarial Infections. Curr Infect Dis Rep 4:65–69

Keiser PB, Coulibaly Y, Kubofcik J, Diallo AA, Klion AD, Traore SF, Nutman TB (2008) Molecular identification of *Wolbachia* from the filarial nematode *Mansonella perstans*. Mol Biochem Parasitol 160:123–128

Kerr TS (1904) Calabar swelling and its relationship to Filaria loa and diurna. J Trop Med Hyg 7: 195

Khatri V, Chauhan N, Vishnoi K, von Gegerfelt A, Gittens C, Kalyanasundaram R (2018) Prospects of developing a prophylactic vaccine against human lymphatic filariasis - evaluation of protection in non-human primates. Int J Parasitol 48:773–783

Kilian HD (1988) The use of a topical Mazzotti test in the diagnosis of onchocerciasis. Trop Med Parasitol 39:235–238

Kilian HD, Nielsen G (1989a) Cell-mediated and humoral immune responses to BCG and rubella vaccinations and to recall antigens in onchocerciasis patients. Trop Med Parasitol 40:445–453

Kilian HD, Nielsen G (1989b) Cell-mediated and humoral immune response to tetanus vaccinations in onchocerciasis patients. Trop Med Parasitol 40:285–291

King CL, Nutman TB (1991) Regulation of the immune response in lymphatic filariasis and onchocerciasis. Immunol Today 12:A54–A58

King CL, Kumaraswami V, Poindexter RW, Kumari S, Jayaraman K, Alling DW, Ottesen EA, Nutman TB (1992) Immunologic tolerance in lymphatic filariasis. Diminished parasite-specific T and B lymphocyte precursor frequency in the microfilaremic state. J Clin Invest 89:1403–1410

King CL, Mahanty S, Kumaraswami V, Abrams JS, Regunathan J, Jayaraman K, Ottesen EA, Nutman TB (1993) Cytokine control of parasite-specific anergy in human lymphatic filariasis. Preferential induction of a regulatory T helper type 2 lymphocyte subset. J Clin Invest 92:1667–1673

King CL, Suamani J, Sanuku N, Cheng YC, Satofan S, Mancuso B, Goss CW, Robinson LJ, Siba PM, Weil GJ, Kazura JW (2018) A trial of a triple-drug treatment for lymphatic filariasis. N Engl J Med 379:1801–1810

Kipp W, Bamhuhiiga J (2002) Onchodermal skin disease in a hyperendemic onchocerciasis focus in western Uganda. Am J Trop Med Hyg 67:475–479

Kipp W, Bamuhiiga J, Rubaale T (2003) Simulium neavei-transmitted onchocerciasis: HIV infection increases severity of onchocercal skin disease in a small sample of patients. Trans R Soc Trop Med Hyg 97:310–311

Klion AD, Nutman TB (2011) Loiasis and *Mansonella* Infections. In: Guerrant RL, Walker DH, Weller PF (eds) Tropical infectious diseases: principles, pathogens and practice, 3rd edn. Saunders Elsevier, Philadelphia, pp 735–740

Klion AD, Massougbodji A, Sadeler BC, Ottesen EA, Nutman TB (1991) Loiasis in endemic and nonendemic populations: immunologically mediated differences in clinical presentation. J Infect Dis 163:1318–1325

Klion AD, Eisenstein EM, Smirniotopoulos TT, Neumann MP, Nutman TB (1992) Pulmonary involvement in loiasis. Am Rev Respir Dis 145:961–963

Klion AD, Massougbodji A, Horton J, Ekoue S, Lanmasso T, Ahouissou NL, Nutman TB (1993) Albendazole in human loiasis: results of a double-blind, placebo-controlled trial. J Infect Dis 168:202–206

Klion AD, Vijaykumar A, Oei T, Martin B, Nutman TB (2003) Serum immunoglobulin G4 antibodies to the recombinant antigen, Ll-SXP-1, are highly specific for *Loa loa* infection. J Infect Dis 187:128–133

Kluxen G, Hoerauf A (2008) The significance of some observations on African ocular onchocerciasis described by Jean Hissette (1888-1965). Bull Soc Belge Ophtalmol 307:53–58

Korten S, Büttner DW, Schmetz C, Hoerauf A, Mand S, Brattig N (2009) The nematode parasite *Onchocerca volvulus* generates the transforming growth factor-beta (TGF-beta). Parasitol Res 105:731–741

Korten S, Kaifi JT, Büttner DW, Hoerauf A (2010) Transforming growth factor-beta expression by host cells is elicited locally by the filarial nematode *Onchocerca volvulus* in hyporeactive patients independently from *Wolbachia*. Microbes Infect 12:555–564

Korten S, Hoerauf A, Kaifi JT, Büttner DW (2011) Low levels of transforming growth factor-beta (TGF-beta) and reduced suppression of Th2-mediated inflammation in hyperreactive human onchocerciasis. Parasitology 138:35–45

Kroidl I, Saathoff E, Maganga L, Makunde WH, Hoerauf A, Geldmacher C, Clowes P, Maboko L, Hoelscher M (2016) Effect of *Wuchereria bancrofti* infection on HIV incidence in southwest Tanzania: a prospective cohort study. Lancet 388:1912–1920

Kroidl I, Chachage M, Mnkai J, Nsojo A, Berninghoff M, Verweij JJ, Maganga L, Ntinginya NE, Maboko L, Clowes P, Hoelscher M, Saathoff E, Geldmacher C (2019) *Wuchereria bancrofti* infection is linked to systemic activation of CD4 and CD8 T cells. PLoS Negl Trop Dis 13: e0007623

Krücken J, Holden-Dye L, Keiser J, Prichard RK, Townson S, Makepeace BL, Hübner MP, Hahnel SR, Scandale I, Harder A, Kulke D (2021) Development of emodepside as a possible adulticidal treatment for human onchocerciasis-The fruit of a successful industrial-academic collaboration. PLoS Pathog 17:e1009682

Kumaraswami V (2000) The clinical manifestations of lymphatic filariasis. In: Nutman TB (ed) Lymphatic Filariasis. Imperial College Press, London, pp 103–125

Kurniawan A, Yazdanbakhsh M, van Ree R, Aalberse R, Selkirk ME, Partono F, Maizels RM (1993) Differential expression of IgE and IgG4 specific antibody responses in asymptomatic and chronic human filariasis. J Immunol 150:3941–3950

Kushwaha V, Tewari P, Mandal P, Tripathi A, Murthy PK (2019) Troponin 1 of human filarial parasite *Brugia malayi*: cDNA cloning, expression, purification, and its immunoprophylactic potential. Parasitol Res 118:1849–1863

Kwarteng A, Sylverken A, Asiedu E, Ahuno ST (2021) Genome editing as control tool for filarial infections. Biomed Pharmacother 137:111292

Lange AM, Yutanawiboonchai W, Scott P, Abraham D (1994) IL-4- and IL-5-dependent protective immunity to *Onchocerca volvulus* infective larvae in BALB/cBYJ mice. J Immunol 153:205–211

Leichsenring M, Troger J, Nelle M, Büttner DW, Darge K, Doehring-Schwerdtfeger E (1990) Ultrasonographical investigations of onchocerciasis in Liberia. Am J Trop Med Hyg 43:380–385

Lipner EM, Dembele N, Souleymane S, Alley WS, Prevots DR, Toe L, Boatin B, Weil GJ, Nutman TB (2006a) Field applicability of a rapid-format anti-Ov-16 antibody test for the assessment of onchocerciasis control measures in regions of endemicity. J Infect Dis 194:216–221

Lipner EM, Gopi PG, Subramani R, Kolappan C, Sadacharam K, Kumaran P, Prevots DR, Narayanan PR, Nutman TB, Kumaraswami V (2006b) Coincident filarial, intestinal helminth, and mycobacterial infection: helminths fail to influence tuberculin reactivity, but BCG influences hookworm prevalence. Am J Trop Med Hyg 74:841–847

Lloyd MM, Gilbert R, Taha NT, Weil GJ, Meite A, Kouakou IM, Fischer PU (2015) Conventional parasitology and DNA-based diagnostic methods for onchocerciasis elimination programmes. Acta Trop 146:114–118

Lobos E, Nutman TB, Hothersall JS, Moncada S (2003) Elevated immunoglobulin E against recombinant *Brugia malayi* gamma-glutamyl transpeptidase in patients with bancroftian filariasis: association with tropical pulmonary eosinophilia or putative immunity. Infect Immun 71: 747–753

Local Burden of Disease Neglected Tropical Diseases Collaborators (2020) The global distribution of lymphatic filariasis, 2000-18: a geospatial analysis. Lancet Glob Health 8:e1186–e1e94

Lustigman S, James ER, Tawe W, Abraham D (2002) Towards a recombinant antigen vaccine against *Onchocerca volvulus*. Trends Parasitol 18:135–141

Mackenzie CD, Homeida MM, Hopkins AD, Lawrence JC (2012) Elimination of onchocerciasis from Africa: possible? Trends Parasitol 28:16–22

Madhumathi J, Prince PR, Rao DN, Karande AA, Reddy MV, Kaliraj P (2017) Epitope mapping of *Brugia malayi* ALT-2 and the development of a multi-epitope vaccine for lymphatic filariasis. J Helminthol 91:43–54

Mahanty S, Nutman TB (1995) Immunoregulation in human lymphatic filariasis: the role of interleukin 10. Parasite Immunol 17:385–392

Mahanty S, Mollis SN, Ravichandran M, Abrams JS, Kumaraswami V, Jayaraman K, Ottesen EA, Nutman TB (1996) High levels of spontaneous and parasite antigen-driven interleukin-10 production are associated with antigen-specific hyporesponsiveness in human lymphatic filariasis. J Infect Dis 173:769–773

Malathi B, Mona S, Thiyagarajan D, Kaliraj P (2015) Immunopotentiating nano-chitosan as potent vaccine carter for efficacious prophylaxis of filarial antigens. Int J Biol Macromol 73:131–137

Mand S, Marfo-Debrekyei Y, Dittrich M, Fischer K, Adjei O, Hoerauf A (2003) Animated documentation of the filaria dance sign (FDS) in bancroftian filariasis. Filaria J 2:3

Mand S, Marfo-Debrekyei Y, Debrah A, Büttner M, Batsa L, Pfarr K, Adjei O, Hoerauf A (2005) Frequent detection of worm movements in onchocercal nodules by ultrasonography. Filaria J 4:1

Mand S, Supali T, Djuardi J, Kar S, Ravindran B, Hoerauf A (2006) Detection of adult *Brugia malayi* filariae by ultrasonography in humans in India and Indonesia. Trop Med Int Health 11: 1375–1381

Mand S, Pfarr K, Sahoo PK, Satapathy AK, Specht S, Klarmann U, Debrah AY, Ravindran B, Hoerauf A (2009) Macrofilaricidal activity and amelioration of lymphatic pathology in bancroftian filariasis after 3 weeks of doxycycline followed by single-dose diethylcarbamazine. Am J Trop Med Hyg 81:702–711

Mand S, Debrah AY, Klarmann U, Batsa L, Marfo-Debrekyei Y, Kwarteng A, Specht S, Belda-Domene A, Fimmers R, Taylor M, Adjei O, Hoerauf A (2012) Doxycycline improves filarial lymphedema independent of active filarial infection: a randomized controlled trial. Clin Infect Dis 55:621–630

Manguin S, Bangs MJ, Pothikasikorn J, Chareonviriyaphap T (2010) Review on global co-transmission of human *Plasmodium* species and *Wuchereria bancrofti* by *Anopheles* mosquitoes. Infect Genet Evol 10:159–177

Manoury B, Gregory WF, Maizels RM, Watts C (2001) Bm-CPI-2, a cystatin homolog secreted by the filarial parasite *Brugia malayi*, inhibits class II MHC-restricted antigen processing. Curr Biol 11:447–451

Manson P (1891) The sleeping sickness of Central Africa and Filaria sanguinis hominis minor. East London Institute for Home and Foreign Missions, London
Manson P (1897) On certain new speices of nematode haematozoa occurring in America. Br Med J 2:1837–1838
Manson P (1899) Parental Form of Filaria perstans. Br Med J 1:429
Marinkelle CJ, German E (1970) Mansonelliasis in the Comisaria del Vaupes of Colombia. Trop Geogr Med 22:101–111
Martin C, Le Goff L, Ungeheuer MN, Vuong PN, Bain O (2000a) Drastic reduction of a filarial infection in eosinophilic interleukin-5 transgenic mice. Infect Immun 68:3651–3656
Martin C, Al-Qaoud KM, Ungeheuer MN, Paehle K, Vuong PN, Bain O, Fleischer B, Hoerauf A (2000b) IL-5 is essential for vaccine-induced protection and for resolution of primary infection in murine filariasis. Med Microbiol Immunol 189:67–74
Martin-Prevel Y, Cosnefroy JY, Tshipamba P, Ngari P, Chodakewitz JA, Pinder M (1993) Tolerance and efficacy of single high-dose ivermectin for the treatment of loiasis. Am J Trop Med Hyg 48:186–192
McNeeley DF, Raccurt CP, Boncy J, Lowrie RC Jr (1989) Clinical evaluation of *Mansonella ozzardi* in Haiti. Trop Med Parasitol 40:107–110
Mediannikov O, Ranque S (2018) Mansonellosis, the most neglected human filariasis. New Microbes New Infect 26:S19–s22
Meyers WM, Connor DH, Harman LE, Fleshman K, Moris R, Neafie RC (1972) Human streptocerciasis. A clinico-pathologic study of 40 Africans (Zairians) including identification of the adult filaria. Am J Trop Med Hyg 21:528–545
Meyers WM, Moris R, Neafie RC, Connor DH, Bourland J (1978) Streptocerciasis: degeneration of adult *Dipetalonema streptocerca* in man following diethylcarbamazine therapy. Am J Trop Med Hyg 27:1137–1147
Michael E, Bundy DA, Grenfell BT (1996) Re-assessing the global prevalence and distribution of lymphatic filariasis. Parasitology 112:409–428
Mishra R, Panda SK, Sahoo PK, Bal MS, Satapathy AK (2017) Increased Fas ligand expression of peripheral B-1 cells correlated with CD4(+) T-cell apoptosis in filarial-infected patients. Parasite Immunol 39:12421
Mitre E, Chien D, Nutman TB (2008) CD4(+) (and not CD25+) T cells are the predominant interleukin-10-producing cells in the circulation of filaria-infected patients. J Infect Dis 197:94–101
MMWR (2013) Progress toward elimination of onchocerciasis in the Americas - 1993-2012. MMWR Morb Mortal Wkly Rep 62:405–408
Molina MA, Cabezas MT, Gimenez MJ (1999) *Mansonella perstans* filariasis in a HIV patient: finding in bone marrow. Haematologica 84:861
Molyneux DH, Bradley M, Hoerauf A, Kyelem D, Taylor MJ (2003) Mass drug treatment for lymphatic filariasis and onchocerciasis. Trends Parasitol 19:516–522
Mongin (1770) Observation on a worm found under conjunctiva in Maribou, Saint Domingue Island. J Med Chir Pharm Paris 32:338
Morales-Hojas R, Post RJ, Shelley AJ, Maia-Herzog M, Coscaron S, Cheke RA (2001) Characterisation of nuclear ribosomal DNA sequences from *Onchocerca volvulus* and *Mansonella ozzardi* (Nematoda: Filarioidea) and development of a PCR-based method for their detection in skin biopsies. Int J Parasitol 31:169–177
Moreno Y, Nabhan JF, Solomon J, Mackenzie CD, Geary TG (2010) Ivermectin disrupts the function of the excretory-secretory apparatus in microfilariae of *Brugia malayi*. Proc Natl Acad Sci U S A 107:20120–20125
Mourembou G, Fenollar F, Lekana-Douki JB, Ndjoyi Mbiguino A, Maghendji Nzondo S, Matsiegui PB, Zoleko Manego R, Ehounoud CH, Bittar F, Raoult D, Mediannikov O (2015) *Mansonella*, including a Potential New Species, as Common Parasites in Children in Gabon. PLoS Negl Trop Dis 9:e0004155

Muhsin M, Ajendra J, Gentil K, Berbudi A, Neumann AL, Klaas L, Schmidt KE, Hoerauf A, Hübner MP (2018) IL-6 is required for protective immune responses against early filarial infection. Int J Parasitol 48:925–935

Mukherjee S, Mukherjee S, Maiti TK, Bhattacharya S, Sinha Babu SP (2017) A Novel Ligand of Toll-like Receptor 4 From the Sheath of *Wuchereria bancrofti* Microfilaria Induces Proinflammatory Response in Macrophages. J Infect Dis 215:954–965

Mukherjee S, Karnam A, Das M, Babu SPS, Bayry J (2019) *Wuchereria bancrofti* filaria activates human dendritic cells and polarizes T helper 1 and regulatory T cells via toll-like receptor 4. Commun Biol 2:169

Murdoch ME, Hay RJ, Mackenzie CD, Williams JF, Ghalib HW, Cousens S, Abiose A, Jones BR (1993) A clinical classification and grading system of the cutaneous changes in onchocerciasis. Br J Dermatol 129:260–269

Murdoch ME, Asuzu MC, Hagan M, Makunde WH, Ngoumou P, Ogbuagu KF, Okello D, Ozoh G, Remme J (2002) Onchocerciasis: the clinical and epidemiological burden of skin disease in Africa. Ann Trop Med Parasitol 96:283–296

Nag JK, Shrivastava N, Gupta J, Misra-Bhattacharya S (2013) Recombinant translation initiation factor-1 of *Wolbachia* is an immunogenic excretory secretory protein that elicits Th2 mediated immune protection against Brugia malayi. Comp Immunol Microbiol Infect Dis 36:25–38

Neafie RC, Connor DH, Meyers WM (1975) *Dipetalonema streptocerca* (Macfie and Corson, 1922): description of the adult female. Am J Trop Med Hyg 24:264–267

Nelson GS (1991) Human onchocerciasis: notes on the history, the parasite and the life cycle. Ann Trop Med Parasitol 85:83–95

Nielsen NO, Simonsen PE, Dalgaard P, Krarup H, Magnussen P, Magesa S, Friis H (2007) Effect of diethylcarbamazine on HIV load, CD4%, and CD4/CD8 ratio in HIV-infected adult Tanzanians with or without lymphatic filariasis: randomized double-blind and placebo-controlled cross-over trial. Am J Trop Med Hyg 77:507–513

Njouendou AJ, Ritter M, Ndongmo WPC, Kien CA, Narcisse GTV, Fombad FF, Tayong DB, Pfarr K, Layland LE, Hoerauf A, Wanji S (2017) Successful long-term maintenance of *Mansonella perstans* in an in vitro culture system. Parasit Vectors 10:563

Njouendou AJ, Kien CA, Esum ME, Ritter M, Chounna Ndongmo WP, Fombad FF, Gandjui NVT, Njiokou F, Enyong P, Pfarr K, Turner J, Layland LE, Hoerauf A, Wanji S (2019) In vitro maintenance of *Mansonella perstans* microfilariae and its relevance for drug screening. Exp Parasitol 206:107769

Noireau F, Apembet JD, Nzoulani A, Carme B (1990) Clinical manifestations of loiasis in an endemic area in the Congo. Trop Med Parasitol 41:37–39

Numasaki M, Lotze MT, Sasaki H (2004) Interleukin-17 augments tumor necrosis factor-alpha-induced elaboration of proangiogenic factors from fibroblasts. Immunol Lett 93:39–43

Nutman TB, Kazura JW (2011) Lymphatic filariasis. In: Guerrant RL, Walker DH, Weller PF (eds) Tropical Infectious Diseases: principles, pathogens and practice, 3rd edn. Saunders Elsevier, Philadelphia, pp 729–734

Nutman TB, Kradin RL (2002) Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 1-2002. A 24-year-old woman with paresthesias and muscle cramps after a stay in Africa. N Engl J Med 346:115–122

Nutman TB, Kumaraswami V (2001) Regulation of the immune response in lymphatic filariasis: perspectives on acute and chronic infection with *Wuchereria bancrofti* in South India. Parasite Immunol 23:389–399

Nutman TB, Miller KD, Mulligan M, Ottesen EA (1986) Loa loa infection in temporary residents of endemic regions: recognition of a hyperresponsive syndrome with characteristic clinical manifestations. J Infect Dis 154:10–18

Nutman TB, Kumaraswami V, Ottesen EA (1987a) Parasite-specific anergy in human filariasis. Insights after analysis of parasite antigen-driven lymphokine production. J Clin Invest 79:1516–1523

Nutman TB, Nash TE, Ottesen EA (1987b) Ivermectin in the successful treatment of a patient with *Mansonella ozzardi* infection. J Infect Dis 156:662–665

Nutman TB, Reese W, Poindexter RW, Ottesen EA (1988) Immunologic correlates of the hyperresponsive syndrome of loiasis. J Infect Dis 157:544–550

Nutman TB, Parredes W, Kubofcik J, Guderian RH (1996) Polymerase chain reaction-based assessment after macrofilaricidal therapy in *Onchocerca volvulus* infection. J Infect Dis 173: 773–776

Opoku NO, Bakajika DK, Kanza EM, Howard H, Mambandu GL, Nyathirombo A, Nigo MM, Kasonia K, Masembe SL, Mumbere M, Kataliko K, Larbelee JP, Kpawor M, Bolay KM, Bolay F, Attah SK, Vaillant M, Halleux CM, Kuesel AC (2018) Single dose moxidectin versus ivermectin for *Onchocerca volvulus* infection in Ghana, Liberia, and the Democratic Republic of the Congo: a randomised, controlled, double-blind phase 3 trial. Lancet 392:1207–1216

O'Regan NL, Steinfelder S, Venugopal G, Rao GB, Lucius R, Srikantam A, Hartmann S (2014a) *Brugia malayi* microfilariae induce a regulatory monocyte/macrophage phenotype that suppresses innate and adaptive immune responses. PLoS Negl Trop Dis 8:e 3206

O'Regan NL, Steinfelder S, Schwedler C, Rao GB, Srikantam A, Blanchard V, Hartmann S (2014b) Filariasis asymptomatically infected donors have lower levels of disialylated IgG compared to endemic normals. Parasite Immunol 36:713–720

Orihel TC (1984) The tail of the *Mansonella streptocerca* microfilaria. Am J Trop Med Hyg 33: 1278

Osei-Atweneboana MY, Awadzi K, Attah SK, Boakye DA, Gyapong JO, Prichard RK (2011) Phenotypic evidence of emerging ivermectin resistance in *Onchocerca volvulus*. PLoS Negl Trop Dis 5:e998

Otabil KB, Tenkorang SB (2015) Filarial hydrocele: a neglected condition of a neglected tropical disease. J Infect Dev Ctries 9:456–462

Otsuji Y (2011) History, epidemiology and control of filariasis. Trop Med Health 39:3–13

Ottesen EA (2008) Immunopathology of Lymphatic Filariasis in Man. Springer Sem Immunopathol 2:373–385

Ottesen EA, Horton J (2020) Setting the stage for a Global Programme to Eliminate Lymphatic Filariasis: the first 125 years (1875-2000). International health 13:S3–s9

Ottesen EA, Weller PF, Heck L (1977) Specific cellular immune unresponsiveness in human filariasis. Immunology 33:413–421

Ottesen EA, Weller PF, Lunde MN, Hussain R (1982) Endemic filariasis on a Pacific Island. II. Immunologic aspects: immunoglobulin, complement, and specific antifilarial IgG, IgM, and IgE antibodies. Am J Trop Med Hyg 31:953–961

Ottesen EA, Skvaril F, Tripathy SP, Poindexter RW, Hussain R (1985) Prominence of IgG4 in the IgG antibody response to human filariasis. J Immunol 134:2707–2712

Ottesen EA, Hooper PJ, Bradley M, Biswas G (2008) The global programme to eliminate lymphatic filariasis: health impact after 8 years. PLoS Negl Trop Dis 2:e317

Pacque M, Munoz B, Greene BM, Taylor HR (1991) Community-based treatment of onchocerciasis with ivermectin: safety, efficacy, and acceptability of yearly treatment. J Infect Dis 163: 381–385

Padgett JJ, Jacobsen KH (2008) Loiasis: African eye worm. Trans R Soc Trop Med Hyg 102:983–989

Paleologo FP, Neafie RC, Connor DH (1984) Lymphadenitis caused by *Loa loa*. Am J Trop Med Hyg 33:395–402

Palumbo E (2008) Filariasis: diagnosis, treatment and prevention. Acta Biomed 79:106–109

Parkhouse RM, Bofill M, Gomez-Priego A, Janossy G (1985) Human macrophages and T-lymphocyte subsets infiltrating nodules of *Onchocerca volvulus*. Clin Exp Immunol 62:13–18

Patton JB, Bennuru S, Eberhard ML, Hess JA, Torigian A, Lustigman S, Nutman TB, Abraham D (2018) Development of *Onchocerca volvulus* in humanized NSG mice and detection of parasite biomarkers in urine and serum. PLoS Negl Trop Dis 12:e0006977

Pearlman E, Gillette-Ferguson I (2007) *Onchocerca volvulus*, *Wolbachia* and river blindness. Chem Immunol Allergy 92:254–265

Pedram B, Pasquetto V, Drame PM, Ji Y, Gonzalez-Moa MJ, Baldwin RK, Nutman TB, Biamonte MA (2017) A novel rapid test for detecting antibody responses to *Loa loa* infections. PLOS Negl Trop Dis 11:e0005741

Peel E (1946) Filariids of chimpanzees Pan paniscus and Pan satyrus in Belgian Congo. Ann Soc Belg Med Trop 26:117

Petersen HH, Nielsen NO, Monrad J, Magesa SM, Simonsen PE (2009) The effect of HIV on filarial-specific antibody response before and after treatment with diethylcarbamazine in Wuchereria bancrofti infected individuals. Parasitol Int 58:141–144

Pfarr KM, Debrah AY, Specht S, Hoerauf A (2009) Filariasis and lymphoedema. Parasite Immunol 31:664–672

Pilotte N, Torres M, Tomaino FR, Laney SJ, Williams SA (2013) A TaqMan-based multiplex real-time PCR assay for the simultaneous detection of *Wuchereria bancrofti* and *Brugia malayi*. Mol Biochem Parasitol 189:33–37

Pion SD, Montavon C, Chesnais CB, Kamgno J, Wanji S, Klion AD, Nutman TB, Boussinesq M (2016) Positivity of Antigen Tests Used for Diagnosis of Lymphatic Filariasis in Individuals Without *Wuchereria bancrofti* Infection But with High *Loa loa* Microfilaremia. Am J Trop Med Hyg 95:1417–1423

Pion SD, Nana-Djeunga H, Niamsi-Emalio Y, Chesnais CB, Deléglise H, Mackenzie C, Stolk W, Fletcher DA, Klion AD, Nutman TB, Boussinesq M, Kamgno J (2020) Implications for annual retesting after a test-and-not-treat strategy for onchocerciasis elimination in areas co-endemic with *Loa loa* infection: an observational cohort study. Lancet Infect Dis 20:102–109

Pionnier N, Brotin E, Karadjian G, Hemon P, Gaudin-Nome F, Vallarino-Lhermitte N, Nieguitsila A, Fercoq F, Aknin ML, Marin-Esteban V, Chollet-Martin S, Schlecht-Louf G, Bachelerie F, Martin C (2016) Neutropenic mice provide insight into the role of skin-infiltrating neutrophils in the host protective immunity against filarial infective larvae. PLoS Negl Trop Dis 10:e0004605

Pionnier NP, Sjoberg H, Chunda VC, Fombad FF, Chounna PW, Njouendou AJ, Metuge HM, Ndzeshang BL, Gandjui NV, Akumtoh DN, Tayong DB, Taylor MJ, Wanji S, Turner JD (2019) Mouse models of *Loa loa*. Nat Commun 10:1429

Pionnier N, Sjoberg H, Furlong-Silva J, Marriott A, Halliday A, Archer J, Steven A, Taylor MJ, Turner JD (2020) Eosinophil-Mediated Immune Control of Adult Filarial Nematode Infection Can Proceed in the Absence of IL-4 Receptor Signaling. J Immunol 205:731–740

Poole CB, Li Z, Alhassan A, Guelig D, Diesburg S, Tanner NA, Zhang Y, Evans TC Jr, LaBarre P, Wanji S, Burton RA, Carlow CK (2017) Colorimetric tests for diagnosis of filarial infection and vector surveillance using non-instrumented nucleic acid loop-mediated isothermal amplification (NINA-LAMP). PLoS One 12:e0169011

Prince-Guerra JL, Cama VA, Wilson N, Thiele EA, Likwela J, Ndakala N, Muzinga Wa Muzinga J, Ayebazibwe N, Ndjakani YD, Pitchouna NA, Mumba D, Tshefu AK, Ogawa G, Cantey PT (2018) Comparison of PCR Methods for *Onchocerca volvulus* Detection in Skin Snip Biopsies from the Tshopo Province, Democratic Republic of the Congo. Am J Trop Med Hyg 98:1427–1434

Prodjinotho UF, von Horn C, Debrah AY, Batsa Debrah L, Albers A, Layland LE, Hoerauf A, Adjobimey T (2017) Pathological manifestations in lymphatic filariasis correlate with lack of inhibitory properties of IgG4 antibodies on IgE-activated granulocytes. PLoS Negl Trop Dis 11: e0005777

Prodjinotho UF, Hoerauf A, Adjobimey T (2019) IgG4 antibodies from patients with asymptomatic bancroftian filariasis inhibit the binding of IgG1 and IgG2 to C1q in a Fc-Fc-dependent mechanism. Parasitol Res 118:2957–2968

Prost A, Nebout M, Rougemont A (1979) Lepromatous leprosy and onchocerciasis. Br Med J 1: 589–590

Puente S, Lago M, Subirats M, Sanz-Esteban I, Arsuaga M, Vicente B, Alonso-Sardon M, Belhassen-Garcia M, Muro A (2020) Imported *Mansonella perstans* infection in Spain. Infect Dis Poverty 9:105

Ramachandran CP (1981) The epidemiology and control of Brugian filariasis in South East Asia: an update. Ann Soc Belg Med Trop 61:257–268

Ramaiah KD, Ottesen EA (2014) Progress and impact of 13 years of the global programme to eliminate lymphatic filariasis on reducing the burden of filarial disease. PLoS Negl Trop Dis 8: e3319

Ramanathan A, Immanuel C, Rao DN, Kaliraj P (2015) Dissecting the immune response Elicited by WbALT-2, ALT MAP in clinical populations and mouse model: a prophylactic measure against lymphatic filariasis. Lymphat Res Biol 13:120–125

Rao RU, Moussa H, Weil GJ (2002) Brugia malayi: effects of antibacterial agents on larval viability and development in vitro. Exp Parasitol 101:77–81

Rao RU, Atkinson LJ, Ramzy RM, Helmy H, Farid HA, Bockarie MJ, Susapu M, Laney SJ, Williams SA, Weil GJ (2006) A real-time PCR-based assay for detection of *Wuchereria bancrofti* DNA in blood and mosquitoes. Am J Trop Med Hyg 74:826–832

Ravindran B, Satapathy AK, Sahoo PK, Mohanty MC (2003) Protective immunity in human lymphatic filariasis: problems and prospects. Med Microbiol Immunol 192:41–46

Rebollo MP, Bockarie MJ (2013) Toward the elimination of lymphatic filariasis by 2020: treatment update and impact assessment for the endgame. Expert Rev Anti Infect Ther 11:723–731

Ricciardi A, Nutman TB (2021) IL-10 and Its Related Superfamily Members IL-19 and IL-24 Provide Parallel/Redundant Immune-Modulation in *Loa loa* Infection. J Infect Dis 223:297–305

Ristimaki A, Narko K, Enholm B, Joukov V, Alitalo K (1998) Proinflammatory cytokines regulate expression of the lymphatic endothelial mitogen vascular endothelial growth factor-C. J Biol Chem 273:8413–8418

Ritter M, Tamadaho RSE, Feid J, Vogel W, Wiszniewsky K, Perner S, Hoerauf A, Layland LE (2017) IL-4/5 signalling plays an important role during *Litomosoides sigmodontis* infection, influencing both immune system regulation and tissue pathology in the thoracic cavity. Int J Parasitol 47:951–960

Ritter M, Ndongmo WPC, Njouendou AJ, Nghochuzie NN, Nchang LC, Tayong DB, Arndts K, Nausch N, Jacobsen M, Wanji S, Layland LE, Hoerauf A (2018) *Mansonella perstans* microfilaremic individuals are characterized by enhanced type 2 helper T and regulatory T and B cell subsets and dampened systemic innate and adaptive immune responses. PLoS Negl Trop Dis 12:e0006184

Ritter M, Osei-Mensah J, Debrah LB, Kwarteng A, Mubarik Y, Debrah AY, Pfarr K, Hoerauf A, Layland LE (2019) *Wuchereria bancrofti*-infected individuals harbor distinct IL-10-producing regulatory B and T cell subsets which are affected by anti-filarial treatment. PLoS Negl Trop Dis 13:e0007436

Rodrigo MB, Schulz S, Krupp V, Ritter M, Wiszniewsky K, Arndts K, Tamadaho RS, Endl E, Hoerauf A, Layland LE (2016) Patency of *Litomosoides sigmodontis* infection depends on Toll-like receptor 4 whereas Toll-like receptor 2 signalling influences filarial-specific CD4(+) T-cell responses. Immunology 147:429–442

Rougemont A, Boisson-Pontal ME, Pontal PG, Gridel F, Sangare S (1977) Tuberculin skin tests and B.C.G. vaccination in hyperendemic area of onchocerciasis. Lancet 1:309

Saeftel M, Volkmann L, Korten S, Brattig N, Al-Qaoud K, Fleischer B, Hoerauf A (2001) Lack of interferon-gamma confers impaired neutrophil granulocyte function and imparts prolonged survival of adult filarial worms in murine filariasis. Microbes Infect 3:203–213

Sahoo PK, Panda SK, Satapathy AK, Pati S (2018) Anti-filarial immunity blocks parasite development and plays a protective role. PLoS One 13:e0199090

Saint Andre A, Blackwell NM, Hall LR, Hoerauf A, Brattig NW, Volkmann L, Taylor MJ, Ford L, Hise AG, Lass JH, Diaconu E, Pearlman E (2002) The role of endosymbiotic *Wolbachia* bacteria in the pathogenesis of river blindness. Science 295:1892–1895

Sandri TL, Kreidenweiss A, Cavallo S, Weber D, Juhas S, Rodi M, Woldearegai TG, Gmeiner M, Veletzky L, Ramharter M, Tazemda-Kuitsouc GB, Matsiegui PB, Mordmüller B, Held J (2021) Molecular Epidemiology of Mansonella Species in Gabon. J Infect Dis 223:287–296

Satapathy AK, Sartono E, Sahoo PK, Dentener MA, Michael E, Yazdanbakhsh M, Ravindran B (2006) Human bancroftian filariasis: immunological markers of morbidity and infection. Microbes Infect 8:2414–2423

Satoguina J, Mempel M, Larbi J, Badusche M, Loliger C, Adjei O, Gachelin G, Fleischer B, Hoerauf A (2002) Antigen-specific T regulatory-1 cells are associated with immunosuppression in a chronic helminth infection (onchocerciasis). Microbes Infect 4:1291–1300

Satoguina JS, Weyand E, Larbi J, Hoerauf A (2005) T regulatory-1 cells induce IgG4 production by B cells: role of IL-10. J Immunol 174:4718–4726

Satoguina JS, Adjobimey T, Arndts K, Hoch J, Oldenburg J, Layland LE, Hoerauf A (2008) Tr1 and naturally occurring regulatory T cells induce IgG4 in B cells through GITR/GITR-L interaction, IL-10 and TGF-beta. Eur J Immunol 38:3101–3113

Sauerbrey M (2008) The Onchocerciasis Elimination Program for the Americas (OEPA). Ann Trop Med Parasitol 102(Suppl 1):25–29

Schiefer A, Hübner MP, Krome A, Lämmer C, Ehrens A, Aden T, Koschel M, Neufeld H, Chaverra-Muñoz L, Jansen R, Kehraus S, König GM, Pogorevc D, Müller R, Stadler M, Hüttel S, Hesterkamp T, Wagner K, Pfarr K, Hoerauf A (2020) Corallopyronin A for short-course anti-wolbachial, macrofilaricidal treatment of filarial infections. PLoS Negl Trop Dis 14: e0008930

Schonemeyer A, Lucius R, Sonnenburg B, Brattig N, Sabat R, Schilling K, Bradley J, Hartmann S (2001) Modulation of human T cell responses and macrophage functions by onchocystatin, a secreted protein of the filarial nematode *Onchocerca volvulus*. J Immunol 167:3207–3215

Schroeder JH, Simbi BH, Ford L, Cole SR, Taylor MJ, Lawson C, Lawrence RA (2012) Live *Brugia malayi* microfilariae inhibit transendothelial migration of neutrophils and monocytes. PLoS Negl Trop Dis 6:e1914

Semnani RT, Nutman TB (2004) Toward an understanding of the interaction between filarial parasites and host antigen-presenting cells. Immunol Rev 201:127–138

Semnani RT, Sabzevari H, Iyer R, Nutman TB (2001) Filarial antigens impair the function of human dendritic cells during differentiation. Infect Immun 69:5813–5822

Semnani RT, Liu AY, Sabzevari H, Kubofcik J, Zhou J, Gilden JK, Nutman TB (2003) *Brugia malayi* microfilariae induce cell death in human dendritic cells, inhibit their ability to make IL-12 and IL-10, and reduce their capacity to activate CD4+ T cells. J Immunol 171:1950–1960

Semnani RT, Law M, Kubofcik J, Nutman TB (2004) Filaria-induced immune evasion: suppression by the infective stage of *Brugia malayi* at the earliest host-parasite interface. J Immunol 172: 6229–6238

Sentongo E, Rubaale T, Büttner DW, Brattig NW (1998) T cell responses in coinfection with *Onchocerca volvulus* and the human immunodeficiency virus type 1. Parasite Immunol 20:431–439

Shenoy RK (2008) Clinical and pathological aspects of filarial lymphedema and its management. Korean J Parasitol 46:119–125

Shenoy RK, George LM, John A, Suma TK, Kumaraswami V (1998) Treatment of microfilaraemia in asymptomatic brugian filariasis: the efficacy and safety of the combination of single doses of ivermectin and diethylcarbamazine. Ann Trop Med Parasitol 92:579–585

Shenoy RK, John A, Hameed S, Suma TK, Kumaraswami V (2000) Apparent failure of ultrasonography to detect adult worms of *Brugia malayi*. Ann Trop Med Parasitol 94:77–82

Simonsen PE (2009) Filariasis. Manson's tropical diseases: expert comsult basic, 22e. In: Cook GC, Zumala A (eds) Philadelphia: Saunders, Elsevier. Section, vol 11, pp 1477–1513

Simonsen PE, Dunyo SK (1999) Comparative evaluation of three new tools for diagnosis of bancroftian filariasis based on detection of specific circulating antigens. Trans R Soc Trop Med Hyg 93:278–282

Simonsen PE, Mwakitalu ME (2013) Urban lymphatic filariasis. Parasitol Res 112:35–44

Simonsen PE, Onapa AW, Asio SM (2011) *Mansonella perstans* filariasis in Africa. Acta Trop 120 (Suppl 1):S109–S120

Singh PK, Kushwaha S, Rana AK, Misra-Bhattacharya S (2014) Cofactor independent phosphoglycerate mutase of *Brugia malayi* induces a mixed Th1/Th2 type immune response and inhibits larval development in the host. Biomed Res Int 2014:590281

Smith HL, Rajan TV (2000) Tetracycline inhibits development of the infective-stage larvae of filarial nematodes in vitro. Exp Parasitol 95:265–270

Soboslay PT, Dreweck CM, Hoffmann WH, Luder CG, Heuschkel C, Gorgen H, Banla M, Schulz-Key H (1992) Ivermectin-facilitated immunity in onchocerciasis. Reversal of lymphocytopenia, cellular anergy and deficient cytokine production after single treatment. Clin Exp Immunol 89: 407–413

Soboslay PT, Geiger SM, Weiss N, Banla M, Luder CG, Dreweck CM, Batchassi E, Boatin BA, Stadler A, Schulz-Key H (1997) The diverse expression of immunity in humans at distinct states of *Onchocerca volvulus* infection. Immunology 90:592–599

Soboslay PT, Luder CG, Riesch S, Geiger SM, Banla M, Batchassi E, Stadler A, Schulz-Key H (1999) Regulatory effects of Th1-type (IFN-gamma, IL-12) and Th2-type cytokines (IL-10, IL-13) on parasite-specific cellular responsiveness in *Onchocerca volvulus*-infected humans and exposed endemic controls. Immunology 97:219–225

Sondergaard J (1972) Filariasis caused by *Acanthocheilonema perstans*. Arch Dermatol 106:547–548

Specht S, Saeftel M, Arndt M, Endl E, Dubben B, Lee NA, Lee JJ, Hoerauf A (2006) Lack of eosinophil peroxidase or major basic protein impairs defense against murine filarial infection. Infect Immun 74:5236–5243

Specht S, Mand S, Marfo-Debrekyei Y, Debrah AY, Konadu P, Adjei O, Büttner DW, Hoerauf A (2008) Efficacy of 2- and 4-week rifampicin treatment on the *Wolbachia* of *Onchocerca volvulus*. Parasitol Res 103:1303–1309

Specht S, Brattig N, Büttner M, Büttner DW (2009) Criteria for the differentiation between young and old *Onchocerca volvulus* filariae. Parasitol Res 105:1531–1538

Specht S, Ruiz DF, Dubben B, Deininger S, Hoerauf A (2010) Filaria-induced IL-10 suppresses murine cerebral malaria. Microbes Infect 12:635–642

Specht S, Frank JK, Alferink J, Dubben B, Layland LE, Denece G, Bain O, Forster I, Kirschning CJ, Martin C, Hoerauf A (2011) CCL17 Controls Mast Cells for the Defense against Filarial Larval Entry. J Immunol 186:4845–4852

Specht S, Pfarr KM, Arriens S, Hübner MP, Klarmann-Schulz U, Koschel M, Sternberg S, Martin C, Ford L, Taylor MJ, Hoerauf A (2018) Combinations of registered drugs reduce treatment times required to deplete *Wolbachia* in the *Litomosoides sigmodontis* mouse model. PLoS Negl Trop Dis 12:e0006116

Sreenivas K, Kalyanaraman H, Babu S, Narayanan RB (2017) Recombinant *Brugia malayi* pepsin inhibitor (rBm33) exploits host signaling events to regulate inflammatory responses associated with lymphatic filarial infections. Microb Pathog 112:195–208

Steel C, Lujan-Trangay A, Gonzalez-Peralta C, Zea-Flores G, Nutman TB (1991) Immunologic responses to repeated ivermectin treatment in patients with onchocerciasis. J Infect Dis 164: 581–587

Steel C, Guinea A, McCarthy JS, Ottesen EA (1994) Long-term effect of prenatal exposure to maternal microfilaraemia on immune responsiveness to filarial parasite antigens. Lancet 343: 890–893

Steel C, Guinea A, Ottesen EA (1996) Evidence for protective immunity to bancroftian filariasis in the Cook Islands. J Infect Dis 174:598–605

Suma TK, Shenoy RK, Varghese J, Kuttikkal VV, Kumaraswami V (1997) Estimation of ASO titer as an indicator of streptococcal infection precipitating acute adenolymphangitis in brugian lymphatic filariasis. Southeast Asian J Trop Med Public Health 28:826–830

Takougang I, Meremikwu M, Wandji S, Yenshu EV, Aripko B, Lamlenn SB, Eka BL, Enyong P, Meli J, Kale O, Remme JH (2002) Rapid assessment method for prevalence and intensity of *Loa loa* infection. Bull World Health Organ 80:852–858

Talaat KR, Kumarasamy N, Swaminathan S, Gopinath R, Nutman TB (2008) Filarial/human immunodeficiency virus coinfection in urban southern India. Am J Trop Med Hyg 79:558–560

Tamarozzi F, Halliday A, Gentil K, Hoerauf A, Pearlman E, Taylor MJ (2011) Onchocerciasis: the role of *Wolbachia* bacterial endosymbionts in parasite biology, disease pathogenesis, and treatment. Clin Microbiol Rev 24:459–468

Tan KR, Magill AJ, Parise ME, Arguin PM (2011) Doxycycline for malaria chemoprophylaxis and treatment: report from the CDC expert meeting on malaria chemoprophylaxis. Am J Trop Med Hyg 84:517–531

Ta-Tang TH, Crainey JL, Post RJ, Luz SL, Rubio JM (2018) Mansonellosis: current perspectives. Res Rep Trop Med 9:9–24

Tawill SA, Gallin M, Erttmann KD, Kipp W, Bamuhiiga J, Büttner DW (1996) Impaired antibody responses and loss of reactivity to *Onchocerca volvulus* antigens by HIV-seropositive onchocerciasis patients. Trans R Soc Trop Med Hyg 90:85–89

Taylor HR (1992) Mazzotti test for onchocerciasis. Lancet 339:1549–1550

Taylor MJ, Bandi C, Hoerauf A (2005) *Wolbachia* bacterial endosymbionts of filarial nematodes. Adv Parasitol 60:245–284

Taylor MJ, Awadzi K, Basanez MG, Biritwum N, Boakye D, Boatin B, Bockarie M, Churcher TS, Debrah A, Edwards G, Hoerauf A, Mand S, Matthews G, Osei-Atweneboana M, Prichard RK, Wanji S, Adjei O (2009) Onchocerciasis control: vision for the future from a Ghanian perspective. Parasit Vectors 2:7

Taylor MJ, Hoerauf A, Townson S, Slatko BE, Ward SA (2013) Anti-*Wolbachia* drug discovery and development: safe macrofilaricides for onchocerciasis and lymphatic filariasis. Parasitology 141:1–9

Taylor MJ, von Geldern TW, Ford L, Hübner MP, Marsh K, Johnston KL, Sjoberg HT, Specht S, Pionnier N, Tyrer HE, Clare RH, Cook DAN, Murphy E, Steven A, Archer J, Bloemker D, Lenz F, Koschel M, Ehrens A, Metuge HM, Chunda VC, Ndongmo Chounna PW, Njouendou AJ, Fombad FF, Carr R, Morton HE, Aljayyoussi G, Hoerauf A, Wanji S, Kempf DJ, Turner JD, Ward SA (2019) Preclinical development of an oral anti-*Wolbachia* macrolide drug for the treatment of lymphatic filariasis and onchocerciasis. Sci Transl Med 11:313

Tchakoute VL, Graham SP, Jensen SA, Makepeace BL, Nfon CK, Njongmeta LM, Lustigman S, Enyong PA, Tanya VN, Bianco AE, Trees AJ (2006) In a bovine model of onchocerciasis, protective immunity exists naturally, is absent in drug-cured hosts, and is induced by vaccination. Proc Natl Acad Sci U S A 103:5971–5976

Tekle AH, Zouré HG, Noma M, Boussinesq M, Coffeng LE, Stolk WA, Remme JH (2016) Progress towards onchocerciasis elimination in the participating countries of the African Programme for Onchocerciasis Control: epidemiological evaluation results. Infect Dis Poverty 5:66

Tendongfor N, Wanji S, Ngwa JC, Esum ME, Specht S, Enyong P, Matthaei KI, Hoerauf A (2012) The human parasite *Loa loa* in cytokine and cytokine receptor gene knock out BALB/c mice: survival, development and localization. Parasit Vectors 5:43

Theodorides J (1994) Former observations of urinary bilharziasis and wuchereriosis. Bull Soc Pathol Exot 87:191–193

Thomsen EK, Sanuku N, Baea M, Satofan S, Maki E, Lombore B, Schmidt MS, Siba PM, Weil GJ, Kazura JW, Fleckenstein LL, King CL (2016) Efficacy, safety, and pharmacokinetics of coadministered diethylcarbamazine, albendazole, and ivermectin for treatment of bancroftian filariasis. Clin Infect Dis 62:334–341

Tielsch JM, Beeche A (2004) Impact of ivermectin on illness and disability associated with onchocerciasis. Trop Med Int Health 9:A45–A56

Timmann C, Fuchs S, Thoma C, Lepping B, Brattig NW, Sievertsen J, Thye T, Muller-Myhsok B, Horstmann RD (2004) Promoter haplotypes of the interleukin-10 gene influence proliferation of peripheral blood cells in response to helminth antigen. Genes Immun 5:256–260

Turner P, Copeman B, Gerisi D, Speare R (1993) A comparison of the Og4C3 antigen capture ELISA, the Knott test, an IgG4 assay and clinical signs, in the diagnosis of Bancroftian filariasis. Trop Med Parasitol 44:45–48

Turner JD, Mand S, Debrah AY, Muehlfeld J, Pfarr K, McGarry HF, Adjei O, Taylor MJ, Hoerauf A (2006) A randomized, double-blind clinical trial of a 3-week course of doxycycline plus albendazole and ivermectin for the treatment of *Wuchereria bancrofti* infection. Clin Infect Dis 42:1081–1089

Turner JD, Tendongfor N, Esum M, Johnston KL, Langley RS, Ford L, Faragher B, Specht S, Mand S, Hoerauf A, Enyong P, Wanji S, Taylor MJ (2010) Macrofilaricidal activity after doxycycline only treatment of *Onchocerca volvulus* in an area of *Loa loa* co-endemicity: a randomized controlled trial. PLoS Negl Trop Dis 4:e660

Turner JD, Pionnier N, Furlong-Silva J, Sjoberg H, Cross S, Halliday A, Guimaraes AF, Cook DAN, Steven A, Van Rooijen N, Allen JE, Jenkins SJ, Taylor MJ (2018) Interleukin-4 activated macrophages mediate immunity to filarial helminth infection by sustaining CCR3-dependent eosinophilia. PLoS Pathog 14:e1006949

Udall DN (2007) Recent updates on onchocerciasis: diagnosis and treatment. Clin Infect Dis 44:53–60

Volkmann L, Saeftel M, Bain O, Fischer K, Fleischer B, Hoerauf A (2001) Interleukin-4 is essential for the control of microfilariae in murine infection with the filaria *Litomosoides sigmodontis*. Infect Immun 69:2950–2956

Volkmann L, Bain O, Saeftel M, Specht S, Fischer K, Brombacher F, Matthaei KI, Hoerauf A (2003) Murine filariasis: interleukin 4 and interleukin 5 lead to containment of different worm developmental stages. Med Microbiol Immunol 192:23–31

Voronin D, Tricoche N, Jawahar S, Shlossman M, Bulman CA, Fischer C, Suderman MT, Sakanari JA, Lustigman S (2019) Development of a preliminary in vitro drug screening assay based on a newly established culturing system for pre-adult fifth-stage *Onchocerca volvulus* worms. PLoS Negl Trop Dis 13:e0007108

Walker M, Specht S, Churcher TS, Hoerauf A, Taylor MJ, Basanez MG (2015) Therapeutic efficacy and macrofilaricidal activity of doxycycline for the treatment of river blindness. Clin Infect Dis 60:1199–1207

Wammes LJ, Hamid F, Wiria AE, de Gier B, Sartono E, Maizels RM, Luty AJ, Fillie Y, Brice GT, Supali T, Smits HH, Yazdanbakhsh M (2010) Regulatory T cells in human geohelminth infection suppress immune responses to BCG and *Plasmodium falciparum*. Eur J Immunol 40:437–442

Wammes LJ, Hamid F, Wiria AE, Wibowo H, Sartono E, Maizels RM, Smits HH, Supali T, Yazdanbakhsh M (2012) Regulatory T cells in human lymphatic filariasis: stronger functional activity in microfilaremics. PLoS Negl Trop Dis 6:e1655

Wangala B, Gantin RG, Voßberg PS, Vovor A, Poutouli WP, Komlan K, Banla M, Köhler C, Soboslay PT (2019) Inflammatory and regulatory CCL and CXCL chemokine and cytokine cellular responses in patients with patent *Mansonella perstans* filariasis. Clin Exp Immunol 196: 111–122

Wanji S, Tendongfor N, Esum ME, Enyong P (2002) Chrysops silacea biting densities and transmission potential in an endemic area of human loiasis in south-west Cameroon. Trop Med Int Health 7:371–377

Wanji S, Tayong DB, Layland LE, Datchoua Poutcheu FR, Ndongmo WP, Kengne-Ouafo JA, Ritter M, Amvongo-Adjia N, Fombad FF, Njeshi CN, Nkwescheu AS, Enyong PA, Hoerauf A (2016) Update on the distribution of *Mansonella perstans* in the southern part of Cameroon: influence of ecological factors and mass drug administration with ivermectin. Parasit Vectors 9: 311

Wanji S, Tayong DB, Ebai R, Opoku V, Kien CA, Ndongmo WPC, Njouendou AJ, Ghani RN, Ritter M, Debrah YA, Layland LE, Enyong PA, Hoerauf A (2019) Update on the biology and ecology of Culicoides species in the South-West region of Cameroon with implications on the transmission of *Mansonella perstans*. Parasit Vectors 12:166

Ward DJ, Nutman TB, Zea-Flores G, Portocarrero C, Lujan A, Ottesen EA (1988) Onchocerciasis and immunity in humans: enhanced T cell responsiveness to parasite antigen in putatively immune individuals. J Infect Dis 157:536–543

Weil GJ, Steel C, Liftis F, Li BW, Mearns G, Lobos E, Nutman TB (2000) A rapid-format antibody card test for diagnosis of onchocerciasis. J Infect Dis 182:1796–1799

Weil GJ, Curtis KC, Fakoli L, Fischer K, Gankpala L, Lammie PJ, Majewski AC, Pelletreau S, Won KY, Bolay FK, Fischer PU (2013) Laboratory and field evaluation of a new rapid test for detecting *Wuchereria bancrofti* antigen in human blood. Am J Trop Med Hyg 89:11–15

Weil GJ, Bogus J, Christian M, Dubray C, Djuardi Y, Fischer PU, Goss CW, Hardy M, Jambulingam P, King CL, Kuttiat VS, Krishnamoorthy K, Laman M, Lemoine JF, O'Brian KK, Robinson LJ, Samuela J, Schechtman KB, Sircar A, Srividya A, Steer AC, Supali T, Subramanian S, Group DISS (2019) The safety of double- and triple-drug community mass drug administration for lymphatic filariasis: A multicenter, open-label, cluster-randomized study. PLoS Med 16:e1002839

WHO (2011) WHO: African Programme for Onchocerciasis Control: meeting of national task forces, September 2011. World Health Organization.; Weekly epidemiological record 2011, Geneva, pp 541–556

WHO (2012a) Provisional strategy for interrupting lymphatic filariasis transmission in loiasis-endemic countries. Report of the meeting on lymphatic filariasis, malaria and integrated vector management. Accra, Ghana, 5–9 March 2012. WHO/HTM/NTD/PCT/2012.6.

WHO (2017) Guideline – Alternative mass drug administration regimens to eliminate lymphatic filariasis. In: King J (ed) Lymphatic filariasis. World Health Organization, Geneva

WHO (2019) Progress in eliminating onchocerciasis in the WHO Region of the Americas: doxycycline treatment as an end-game strategy. Weekly Epidemiological Report 37:415–419

WHO (2020a) Global programme to eliminate lymphatic filariasis: progress report, 2019. Weekly Epidemiological Report 95:509–524

WHO (2020b) Ending the neglect to attain the sustainable development goals: a road map for neglected tropical diseases 2021–2030. Geneva. Available from: https://www.who.int/publications/i/item/WHO-UCN-NTD-2020.01

WHO (2020c) Progress report on the elimination of human onchocerciasis, 2019-2020. Weekly Epidemiological Report 95:545–556

Wildenburg G, Korten S, Büttner DW (1998) Mast cell distribution in nodules of *Onchocerca volvulus* from untreated patients with generalized onchocerciasis. Parasitology 116:257–268

Winkler S, Willheim M, Baier K, Aichelburg A, Kremsner PG, Graninger W (1999) Increased frequency of Th2-type cytokine-producing T cells in microfilaremic loiasis. Am J Trop Med Hyg 60:680–686

Wiseman RA (1967) *Acanthocheilonema perstans*, a cause of significant eosinophilia in the tropics: comments on its pathogenicity. Trans R Soc Trop Med Hyg 61:667–673

Wiszniewsky A, Ritter M, Krupp V, Schulz S, Arndts K, Weighardt H, Wanji S, Hoerauf A, Layland LE (2019) The central adaptor molecule TRIF influences *L. sigmodontis* worm development. Parasitol Res 118:539–549

Yimer M, Hailu T, Mulu W, Abera B (2015) Epidemiology of elephantiasis with special emphasis on podoconiosis in Ethiopia: A literature review. J Vector Borne Dis 52:111–115

Zamanian M, Fraser LM, Agbedanu PN, Harischandra H, Moorhead AR, Day TA, Bartholomay LC, Kimber MJ (2015) Release of small RNA-containing exosome-like vesicles from the human filarial parasite *Brugia malayi*. PLoS Negl Trop Dis 9:e0004069

Zoure HG, Wanji S, Noma M, Amazigo UV, Diggle PJ, Tekle AH, Remme JH (2011) The geographic distribution of Loa loa in Africa: results of large-scale implementation of the Rapid Assessment Procedure for Loiasis (RAPLOA). PLoS Negl Trop Dis 5:e1210

Zuidema PJ (1971) Renal changes in loiasis. Folia Med Neerl 14:168–172

Chapter 15
Dirofilaria Infections in Humans and Other Zoonotic Filariases

Claudio Genchi, Claudio Bandi, Laura Kramer, and Sara Epis

Abstract *Dirofilaria immitis* and *Dirofilaria* (*Nochtiella*) *repens*, the main *Filarioidea* of domestic and wild carnivores, are responsible for human infections worldwide. Other species of animal filariae that have frequently been reported in human patients include species from the genus *Dirofilaria* and nematodes from genera *Onchocerca*, *Brugia*, and *Molinema*. The higher frequency of human infection by *Dirofilaria* spp. compared to infections by other zoonotic filariae may be due to various factors. For example, awareness and attention of physicians for zoonotic filarial infection is higher in developed countries, where the dog represents an important reservoir for *Dirofilaria* worms. Climate change, together with the movement of infected dogs to previously unsuitable areas, is likely responsible for the increase in areas endemic for *D. immitis* and *D. repens*, with the consequence of an increased risk of infection for humans. Infection by *D. repens* is more frequent in Europe, where the documented human infections by *D. immitis* appear rather infrequent, but the situation is different in other countries, e.g., in the USA, where human infections by *D. immitis* are more frequently recorded. Infections by *Dirofilaria* worms are generally paucisymptomatic, but cases are also reported characterized by a severe clinical picture. The control of *Dirofilaria* infections in humans is essentially based on the control of the infection in dogs, and particular attention should be devoted to the transit of unprotected dogs (i.e., dogs that do not receive prophylactic treatment) from endemic areas, increasing the risk of acquiring filarial infections and of importing the infection in non-endemic areas.

C. Genchi · C. Bandi · L. Kramer (✉) · S. Epis
Department of Veterinary Sciences and Public Health, Universita` degli Studi di Milano, Milan, Italy

Department of Veterinary Science, Universita` degli Studi di Parma, Parma, Italy
e-mail: laurahelen.kramer@unipr.it

F. Bruschi (ed.), *Helminth Infections and their Impact on Global Public Health*,
https://doi.org/10.1007/978-3-031-00303-5_15

15.1 The Main Agents of Zoonotic Filariases: An Introduction

The main agents of zoonotic filarial infections are also the main agents of filariases in domestic and wild carnivores: *Dirofilaria immitis* Leidy 1856, the causative agent of canine and feline heartworm disease, and *D. repens* Railliet and Henry 1911, the causative agent of subcutaneous filarial infections (McCall et al. 2008; Capelli et al. 2018). While heartworm infection is distributed worldwide, *D. repens* has until now been found in Europe, Asia, and Africa. Both parasites are mosquito-transmitted nematodes belonging to the family Onchocercidae (Anderson 2000). Adult worms are thin and females are up to about 15–17 cm in *D. repens* and 25–30 cm in *D. immitis*. Circulating embryos (microfilariae) are found in the bloodstream of infected dogs that act as main reservoir (cats are generally amicrofilaremic) (McCall et al. 2008). In the USA, further than domestic dogs, coyotes are an important reservoir of heartworm infection (Lee et al. 2000).

Microfilariae are taken up by blood-sucking female mosquitoes mostly of the genera *Culex* and *Aedes* (McCall et al. 2008) and develop into the infective larval stage (L3) that is transmitted to the final host through the subsequent blood meal of the infected mosquito. Several mosquito species can act as competent vectors. In Europe, very efficient vectors of *Dirofilaria* spp. are *Culex pipiens* and *Aedes* (*Stegomyia*) *albopictus*; other species that might contribute to the transmission of these nematodes are *Aedes caspius*, *Ae. koreicus* (Genchi et al. 2011a; Montarsi et al. 2015), *Aedes caspius*, and *Coquillettidia richiardii* (Panarese et al. 2020). The mosquito species that take their blood meal on both humans and animals, and during both the day and the night (e.g., *C. pipiens* and *Ae. albopictus*), are those that are more likely to play a role in the transmission of the infection to human hosts. This might explain the high levels of seroprevalence for IgG antibodies against parasite antigens (up to about 30%) in humans living in endemic areas of Spain and Italy, where these mosquito genera are widely diffused (Simón et al. 2005; Torres-Chable et al. 2020; Savić et al. 2020).

The final location of *D. immitis* adult worms in animal hosts is the pulmonary arteries and the right heart ventricle, though in very severe infections adult worms can be found in the right atrium and in the caudal and cranial venae cavae (McCall et al. 2008). Circulating microfilariae are 290–330 μm in length. Ectopic localizations such as eye, brain, and testes are occasionally reported. According to Webber and Hawking (1955), the prepatent period lasts about 180 days. Heartworm infection is a severe/very severe life-threatening condition in both dogs and cats, although it is completely preventable by treating animals with antiparasitic macrocyclic lactones, such as ivermectin, milbemycin oxime, selamectin, and moxidectin throughout the mosquito transmission season (McCall et al. 2008). The infection prevalence in endemic or high endemic areas ranges from 5 to >40% in untreated dogs.

D. repens life cycle is similar to that of *D. immitis*, but adult worms are located mostly in subcutaneous tissues, although the parasite can be found in the abdominal cavity and along connective muscular fasciae (Genchi et al. 2011a; Capelli et al.

2018). According to Webber and Hawking (1955), Cancrini et al. (1989), and Genchi et al. (2013), the prepatent period is around 200 days. Ciuca et al. (2020) reported a range of 220–250 days in experimentally infected dogs. Circulating microfilariae are 300–370 μmin length.

D. repens infection often goes unnoticed. However, it has been reported that dogs with *D. repens* infection can present cutaneous disorders of varying severity, such as pruritus, dermal swelling, subcutaneous nodules containing the parasites, and ocular conjunctivitis (reviewed by Genchi et al. 2011a). Severe infections with allergic reactions likely due to sensitization toward microfilaria endosymbiont *Wolbachia* have also been reported. The infection prevalence in dogs in highly endemic areas ranges from around 30% in Italy to over 35% in Hungary (Genchi et al. 2011a).

In dogs, the diagnosis of *D. immitis* infection is based on clinical findings and is confirmed by testing for both blood microfilariae and for circulating antigens (from adult female) in the bloodstream. The searching for circulating microfilariae in the only method to confirm suspected *D. repens* infections (McCall et al. 2008; Capelli et al. 2018). Circulating microfilariae of the two species can be easily morphologically differentiated by microscopic observation after application of a modified Knott test (Knott 1939; Liotta et al. 2013; Magnis et al. 2013); histochemical staining or molecular methods can aid the identification (for more information, see Genchi et al. 2011b).

15.2 *Dirofilaria* Infections in Humans: Epidemiology and Clinical Manifestations

Both *D. immitis* and *D. repens* are zoonotic and are able to cause benign to severe/very severe conditions in humans (Theis 2005; Genchi et al. 2011a). However, in Europe, human *Dirofilaria* infections are caused mainly by *D. repens* (Genchi et al. 2011a; Simón et al. 2012; Capelli et al. 2018). In general, *D. immitis* human infections are characterized by pulmonary nodules that can be visualized by radiography or echography. However, other localizations have been described. For example, case of *D. immitis* conjunctival infection caused by *D. immitis* in Italy was described in 2011 by Avellis et al. (2011).

The number of human cases from the most endemic areas of canine *Dirofilaria* infections available in international data banks has been summarized by Simón et al. (2012). Even though caution is needed when comparing studies and clinical cases published by different authors along a range of over 50 years (also considering the different awareness of medical doctors in different countries and along the years), it is clear that the number of cases in Europe (>1400) is dramatically higher compared to the rest of the world, including the USA, where 110 cases have been recorded (Theis 2005; Lee et al. 2010), and Japan, with 390 cases (Akao 2011). Most cases in Europe have been attributed to *D. repens*. A rough estimation of the number of *D. immitis* human infections per year shows about 1.8 cases in the USA throughout

60 years and 7.1 cases in Japan throughout 39 years. In Europe, the average is about 39 cases throughout 37 years, attributed to *D. repens* infection. It must be noted that most cases in Europe have been diagnosed quite recently (roughly from 2000) and include the cases of individuals traveling or spending holidays in endemic areas of Southern Europe, such as Italy, Spain, and Greece (for a review, see Genchi et al. 2011a). Therefore, the number of cases in people living in areas previously not considered at risk has dramatically increased (Genchi et al. 2010; Simón et al. 2012; Masny et al. 2013; Genchi and Kramer 2020).

The increased number of human infections in Europe is most likely as a consequence of the changing climate, with increasing temperatures, which allows the survival and the expanding seasonal activity of mosquito vectors. Furthermore, the movement and relocation of microfilaremic dogs from the endemic areas toward the northern and eastern countries are introducing the parasite into new geographical areas. For instance, autochthonous cases of canine *D. immitis* and *D. repens* infections have been observed in Siberia (Yakutsk, 62°02′N 129°44′E). In spite of extremely cold winters (until −36 °C), the parasite has homoeothermic conditions within the host. During the summers, when transmission potentially occurs, the mean temperature in Yakutsk is 18.7 °C (July 1961–1990). Therefore, the 130 *Dirofilaria* Development Units degree days above 14 °C proposed to be required for the extrinsic development into the infective stage in mosquitoes can likely be reached within the mosquitos' lifespan (Pietikäinen et al. 2017).

The number of published *D. repens* human cases in Italy has increased from 4.5 per year from 1986 to 1998 to 15.6 per year in 1999 to 2009 (Pampiglione et al. 1995, 2009; Pampiglione and Rivasi 2000). Autochthonous human cases have been reported from Austria, France, Greece, Croatia, Hungary, Slovak Republic, Poland, Romania, Ukraine, Russia, Turkey, and in other countries such as Africa and Middle and Far East (reviewed by Genchi et al. 2011a; Genchi and Kramer 2020; Riebenbauer et al. 2021; Simón et al. 2012; Kartashev et al. 2011; Masny et al. 2013) (Table 15.1). Furthermore, the infection has been diagnosed in North American individuals traveling or spending their holidays in endemic European areas.

In most cases, the parasite is not able to develop to the adult, sexually mature stage, and infection is characterized by the presence of preadult stages located in subcutaneous tissues of the different body areas, near the point of the mosquito vector bite, although some cases of microfilaremic zoonotic infections have been reported in Europe and in the Middle East (reviewed by Genchi et al. 2011a). Very impressive is a case reported by Kłudkowska et al. (2018) of a Polish man with an intensive microfilaremia as a consequence of subcutaneous nodule caused by *D. repens*. Interestingly, in all the American individuals, who acquired the infection within the previous 8 months to 8 years, adult female worms were found. In two cases, worms were still viable (see Genchi et al. 2011a). Women are more commonly affected than men, although there is no statistical difference (Pampiglione and Rivasi 2000). To note that, of more than 1400 reported cases, the parasite was localized in the ocular region (e.g., orbital region, eyelid, subconjunctival, and intravitreal) in about 23% of cases (Table 15.1), probably as a consequence of the perception of a

Table 15.1 Human dirofilarial infection in Europe and other countries of the Old World (Modified from Genchi et al. 2011a)

Country	No. of cases	Ocular	Pulmonary	Other unusual and serious localizations
Austria	>16			Abdominal cavity, spermatic cord, spermatic duct, scrotum, epididymis associated with meningoencephalitis (surgery in Germany)
France	91	22	2	
Greece	38	7	3	
Hungary	39	19		
Italy	341	68	22	
Spain	16		8	
Russia	624	54		
Ukraine	51	18	2	
Turkey	22	12	3	
Other[a]	192	134	2	
Total	1430	334	42	

[a]Albania, Bulgaria, Croatia, Georgia, Kazakhstan, Poland, Romania, Serbia, Montenegro, and Slovenia

"foreign body" by the human and possibly also for the easier observation by the oculist, compared to the deeper localizations of the worm.

Impaired vision and floater-like mobile shadows seem to be the most frequent symptoms, but the infection is seldom accompanied by loss of vision or serious ocular complications. Intravitreal ocular infection is quite rare, but at least three cases have been reported in Europe (Angunawela et al. 2003; Gorezis et al. 2006; Gungel et al. 2009).

Besides subcutaneous and ocular localization, *Dirofilaria* spp. have been shown to infect viscera (the lungs and mesentery) as well as the female breast and male genitalia (e.g., scrotum, verga, spermatic cord, epididymis). At least 27 cases of pulmonary localization have been reported from 1981 to 2010. The lesions are usually identified by X-ray as a coin lesion. To note visceral and lung localization usually lead to suspect a malignant tumor, thus requiring biopsy or more invasive surgery for differential diagnosis through histology and morphologic identification of the parasite. In some cases, infections have been described as mimicking either cervical intradural Langerhans cell histiocytic tumor (Perret-Court et al. 2009) or scrotal tumors (Fleck et al. 2009); intraperitoneal localizations are also recorded, causing severe consequences (Abbas et al. 2006).

Human dirofilariasis is currently considered an emerging zoonosis in Italy (Genchi et al. 2011a), France (Raccurt 1999), Hungary (Szénási et al. 2008), in Central and Eastern Europe (Masny et al. 2013), and in Russia (Kartashev et al. 2011). Importantly, many infections, mainly the benign forms, likely go unnoticed due to a lack of awareness among the medical profession and to diagnostic uncertainty. In fact, until the recent introduction of molecular methods based on PCR and sequencing, diagnosis was usually carried out after surgery and examination of histological sections of the infected tissue, except for some subconjunctival cases where it was possible to see and remove the parasite. Serology, using crude,

secretory/excretory *Dirofilaria* antigens, and recombinant antigens or surface proteins of *Wolbachia*, the bacterial endosymbiont of filarial worms, is still not fully reliable because of insufficient specificity, i.e., these antigens do not allow to distinguish infections by *D. immitis* or *D. repens* (or other *Dirofilaria* spp.). However, detection of anti-*Dirofilaria* antibodies can anyway be useful, even with the current antigens, in at least two situations: (1) both pulmonary and subcutaneous *Dirofilaria* nodules can lead to the suspicion of a malignant tumor; detection of anti-*Dirofilaria* antibodies can help to discard the malignant origin of the nodule (Simón et al. 2003), being the specific diagnosis of the *Dirofilaria* species involved an irrelevant matter, and (2) in epidemiological surveys, where serology of *Dirofilaria* antibodies can be an adequate way to evaluate the risk of infection in human populations living in endemic areas. For example, the recent seroepidemiological survey of human dirofilariasis carried out in Spain that revealed that 11% of the sera were positive was performed using the crude antigen of adult *D. immitis* (Morchón et al. 2010).

15.3 Is the Spread of *Dirofilaria* Infection an Actual Trend?

In North America, where *D. immitis* is endemic, canine heartworm infection has gradually expanded its geographical range since 1950 from hyperendemic foci (e.g., Mississippi River coastal area) to more northern areas. At that time, the cause was attributed to two main factors: (1) movement of dogs for hunting, breeding, and shows and (2) improved awareness of the infection by veterinarians. Nowadays, heartworm is endemic in all 50 states of the USA (Lee et al. 2000), and the infection risk has increased, at least at the regional level, due to the exportation of heavily infected dogs (prevalence 34–51%) from the New Orleans area and other areas near Louisiana to northern states and Canada in the aftermath of Hurricane Katrina in August 2005 (Levy et al. 2007).

In Europe, until the second half of the last century, both filarial infections (*D. immitis* and *D. repens*) were diagnosed mainly in southern regions, and the highest endemic area was the Po River Valley in Italy. At that time, no autochthonous cases were found in Northern Europe, even though several cases of heartworm infection were diagnosed in dogs which had visited endemic areas. After the introduction of the Pet Travel Scheme in 2000, which allows an easier movement of animals throughout the European Union, the risk of *Dirofilaria* is spreading.

Besides movement of infected dogs, climate plays a critical role in the transmission and spread of *Dirofilaria* infections. The latest report by the Intergovernmental Panel on Climate Change (IPCC 2019) estimates current global warming to be almost 1.5 °C above preindustrial levels and foresees a further rise of 1.1–6.4 °C by 2100. Global warming is defined as an average increase in the temperature of the atmosphere near the Earth's surface and in the troposphere, which can contribute to changes in global climate patterns. There is now strong scientific consensus that (1) global warming is occurring, (2) it is largely attributable to human emission of

greenhouse gases, (3) the effects are now observable, (4) further warming will occur, and (5) climate change has a potentially serious impact on public and animal health (Bernardi 2008). By altering the global environment, climate change has the significant potential to intensify certain diseases, particularly those transmitted by vectors (Khasnis and Nettleman 2005). Global climate change can affect disease vector behavior, which in turn may alter the current patterns of vector-borne diseases transmitted by the bite of hematophagous arthropods (Rogers and Randolph 2006). Important examples are canine leishmaniosis and dirofilariasis in Italy: both these arthropod-borne infections have changed distribution patterns; *Leishmania infantum* was endemic in southern areas of the country until the late 1990s, but it is now increasingly diagnosed in northern areas. *Dirofilaria*sis, which was endemic in canine populations in northern Italy, is now spreading all over the country (Otranto et al. 2009; Traversa et al. 2010).

Mosquitoes, intermediate hosts and vectors of *Dirofilaria* spp., are cold-blooded animals, meaning that their internal temperature is affected by the temperature of their environment. Thus, for many terrestrial arthropod species, a northward range expansion can be expected in response to projected climate change as recently observed in Germany (Sassnau and Genchi 2013). An example is the mosquitoes accidentally introduced in Europe from the Far East and America, such as the case of Asian tiger mosquito *Ae. albopictus* that was introduced into Italy in 1990 and then spread throughout Europe as far as the Netherlands (Scholte et al. 2008), and more recently *Ae. koreicus* and *Ae. japonica* (Montarsi et al. 2015, 2019).

Vector-borne pathogens are sensitive to climate, and there is some evidence that anthropogenic climate change can play a role in increasing their incidence and intensity (Purse et al. 2005). Transmission of dirofilariasis is dependent on the presence of (1) sufficient numbers of microfilaremic dogs (microfilaremia is usually absent in cats and their role as reservoirs is not relevant), (2) susceptible mosquitoes, and (3) a suitable climate to permit extrinsic incubation of *Dirofilaria* in the mosquito intermediate host (Genchi et al. 2011b). Temperature, precipitation, and relative humidity are the main factors that determine the abundance of mosquitoes and the prevalence of mosquito-borne diseases such as filarial infection, and there is a strong temperature dependence for the development of the parasites within the mosquito vectors. Even though a holistic approach of vector-borne diseases should consider, besides temperature, other factors such as human activity and the ecology and behavior of both hosts and the vectors, models based on temperature have shown to be able to predict the spread of *Dirofilaria* infection in Europe (Genchi et al. 2005, 2011b; Mortarino et al. 2008). Climate-based forecast systems usually employ the concept of growing degree days, i.e., 1 degree day occurs when the mean temperature for the day is 1 °C above the threshold temperature. For *D. immitis* infections, climate-based models that determine the effect of temperature on the extrinsic incubation of larval stages are based on the study of Fortin and Slocombe (1981). The rationale of this model is that climate dictates the seasonal occurrence of *Dirofilaria* transmission and there is a threshold of about 14 °C below which development will not proceed. The authors demonstrated that at 30 °C, the development of *D. immitis* microfilariae to infective L3 larvae was completed in 8–9 days

in the mosquitoes. This increased to 10–14 days at 26 °C, 17 days at 22 °C, and 29 days at 18 °C. The seasonal transmission model assumes a requirement of 130 *D. immitis* Development Units (DiDUs) for larvae to reach infectivity and a maximum life expectancy of 30 days for a vector mosquito (Slocombe et al. 1989; Lok and Knight 1998). Based on these assumptions, climate-based models have been used in order to predict the occurrence and seasonality of *Dirofilaria* in Europe (Genchi et al. 2009, 2011a), in the UK (Medlock et al. 2006), and in Argentina (Vezzani and Carbajo 2006). For *D. repens*, the development times of microfilariae to the infective stage at the different temperatures are quite similar: 8–13 days at 28–30 °C, 10–11 days at 26 °C, and 16–20 days at 22 °C. In *Ae. albopictus*, the development from the microfilarial stage to infective larvae takes 14–18 days at 26 °C for *D. immitis* and 16–18 days for *D. repens* (reviewed by Genchi et al. 2011a). In a recent study (Genchi et al. 2009), a threshold value of 130 cumulative Development Units (DUs) was accepted for both *Dirofilaria* species only if it was reached in 30 consecutive days, and the data was interpolated utilizing the linear kriging function of a geographical information system (GIS) to calculate the number of *Dirofilaria* generations. The input of the model was based on the average temperature of the last 15 years, for a total 5475 temperature measures per station and above 19,000,000 values processed. The outputs of this model were predictive maps which assessed the duration of the *Dirofilaria* transmission risk period and monthly maps showing the stations that reached the 130 *Dirofilaria* DUs (DDUs). Most stations located in Southern, Central, and Eastern Europe have reached the 130 DDUs at least once in the years studied. Note that, previously, this model had correctly predicted the spread of *Dirofilaria* infections into several Eastern European countries. Indeed, studies from Hungary, the Czech Republic, Slovakia, and northern Serbia confirmed that areas formerly free of *Dirofilaria* infection are now endemic (Genchi et al. 2011b). Further empirical data has confirmed such a trend, and recently autochthonous *D. repens* infections in dogs have been reported from Northern Germany, Austria, and the Netherlands (reviewed by Genchi et al. 2011a, 2013). Interestingly, most of these studies report the presence of *D. repens* both in animals and in humans, and, when *D. immitis* is also present in dogs, *D. repens* shows higher prevalences. It is thus of interest to try to understand why *D. repens* is spreading more rapidly than *D. immitis*. A possible explanation could be that most *D. repens* infections in dogs are asymptomatic, while heartworm infections usually cause severe clinical disease. It is thus likely that dogs which have traveled to endemic areas of Southern Europe become infected and when they return to northern areas, having no apparent symptoms, act as donors of microfilariae to local mosquito populations. On the contrary, dogs with heartworm infection are usually referred to veterinary clinics and cured. If such a hypothesis is confirmed, considering that an increasing number of dogs travel for holidays or relocation and that pet travel is now facilitated by the new schemes in many European countries, subcutaneous dirofilarial infection could continue its spread. Furthermore, many in clinic rapid kits for the serological diagnosis of *D. immitis* are available on the veterinary market, while it does not exist for *D. repens*. More recently, a conditional autoregressive

model has been proposed by Bowman et al. (2016) to forecasting the prevalence of canine heartworm in the USA.

15.4 Prospects for the Control of *Dirofilaria* Infections in Humans

During the recent years, Europe has experienced the introduction of vector-borne diseases from tropical regions such as the outbreak of chikungunya virus epidemics (Rezza et al. 2007) and West Nile virus (Sambri et al. 2013) in Italy or, as it is the case of *Dirofilaria*, the spread of the infection from southern Mediterranean regions toward northern and eastern areas. Although it has been argued that climate change is the key factor responsible for the more northerly distribution of vectors and their possibility to transmit pathogens, other drivers, notably travel and trade and insecticide resistance, are also likely to have played a role in these processes (Knols and Takken 2007). Transport networks continue to expand so that pathogens and their vectors and animal reservoirs can now move further and faster than ever before (Tatem et al. 2006). Thus, in addition to climate changes and global warming, it is also important to consider the effects of global movement as an important factor inducing the spread of vector-borne diseases, such as *Dirofilaria* infection, whose epidemiology has now the following characteristics: (1) the spread of the infection has increased in endemic areas. (2) Areas formerly free from the infection are now endemic. In dogs not treated with preventive drugs, both the abundance and the incidence of *Dirofilaria* infections have increased (Genchi et al. 2007). (3) *Ae. albopictus* is now considered an important, competent vector of *Dirofilaria* infections. This mosquito species could spread from Southern to Northern European countries in the near future (Medlock et al. 2006; Takumi et al. 2009), changing the epidemiological patterns of dirofilariasis in both humans and animals. (4) Human infections have dramatically increased; the infection is more and more frequently diagnosed, and severe/very severe conditions have been reported. From a practical point of view, to prevent the further spread and endemicity of *Dirofilaria* infections and to control the risk of human infections, epidemiological surveys should now be carried out in different European countries to assess the actual prevalence values, such as that done in Germany (Pantchev et al. 2009). Furthermore, all traveling dogs should be examined for circulating microfilariae, treated with preventative drugs (Genchi et al. 2010, 2013) or microfilaricidal drugs (Fok et al. 2010) when visiting at-risk areas, and reexamined for circulating microfilariae 6–7 months after their stay abroad. European guidelines for the control and prevention of *Dirofilaria* infections in pets are available at http://www.esccap.org.

15.5 Other Species of Filarial Nematodes Infecting Humans

The most comprehensive review of zoonotic filarial infections is by Orihel and Eberhard (1998), who report other zoonotic filariae of livestock and/or wild animals in various parts of the world. Even though unequivocal identification of the worms has not always been possible, the geographical location of affected individuals and the histological features, when available, almost always allowed genus, if not species, identification. The genus *Dirofilaria* contains other species besides *D. immitis* and *D. repens* that have been reported as infecting humans, including *D. ursi*, which parasitizes bears, and *D. subdermata*, a parasite of porcupines in the northern USA and Canada (Beaver et al. 1987; Gutierrez 1990; Orihel and Isbey 1990). Most infections regard the subcutaneous tissue and the eye.

Worms of the genus *Onchocerca* can also cause zoonotic infections. *Onchocerca volvulus* is the most widespread species of the genus and is a parasite of humans. However, many other species of *Onchocerca* are natural parasites of animals including horses and cattle worldwide, and several cases of human infections with these species have been reported. In fact, zoonotic onchocerciasis has been described in the USA, Canada, Switzerland, Russia, and Japan. Infections usually present as firm subcutaneous nodules in different locations on the body or in the eye (Azarova et al. 1965; Siegenthaler and Gubler 1965; Beaver et al. 1974, 1989; Ali-Khan 1977; Takaoka et al. 1996). Nematodes of the genus *Brugia* include species that are parasites of human (*B. malayi*, *B. timori*), together with several species infecting a wide variety of animals across the globe. For example, there are species of *Brugia* which infect monkeys in Southeast Asia and others that are parasites of raccoons and rabbits in the USA. There have been nearly 30 recognized cases of zoonotic infections by *Brugia* in the USA and several more from different countries around the world, including Colombia, Brazil, Peru, and Ethiopia (Baird and Neafie 1988; Menendez and Bouza 1988; Orihel and Beaver 1989; Elenitoba-Johnson et al. 1996). The localization of adult worms varies in affected patients, and the lymph nodes of the groin, head/neck, and torso may be involved. Several cases of ocular infection by zoonotic nematodes of the genera *Molinema* (*Dipetalonema*) (*M. arbuta* and *M. sprenti*; Beaver et al. 1980) and *Loaina* (*Pelicitus*) (*L. uniformis*, *L. scapiceps*, *L. roemeri*; Botero et al. 1984) have also been reported in the literature. The apparent tropisms of these parasites in human infections likely mimic their biological behavior in the natural host (beaver, raccoon, kangaroo, etc.).

Finally, there have been several reports of "zoonotic microfilariae" of unknown origin and without definitive identification of the parasite (Orihel and Eberhard 1998). While the prevention of zoonotic infections with *D. immitis* and *D. repens* is feasible and requires adequate preventive treatment of the definitive host, the other filarial species which are parasites of livestock and/or wild animals that may infect humans are nearly impossible.

References

Abbas KF, El-Monem SG, Malik Z et al (2006) Surgery still opens an unexpected bag of worms. An intraperitoneal live female *Dirofilaria* worms: case report and review of the literature. Surg Infect 7:323–324

Akao N (2011) Human dirofilariasis in Japan. Trop Med Heath 39:65–71

Ali-Khan Z (1977) Tissue pathology and comparative microanatomy of *Onchocerca* from a resident of Ontario and other enzootic *Onchocerca* species from Canada and the USA. Ann Trop Med Parasitol 71:469–482

Anderson RC (2000) Nematode parasites of vertebrates; their development and transmission. CABI Publishing, Wallingford

Angunawela RI, Atullah S, Whitehead KJ et al (2003) Dirofilarial infection of the orbit. Orbit 22: 41–46

Avellis FO, Kramer LH, Mora P et al (2011) A case of human conjunctival dirofilariasis by *Dirofilaria immitis* in Italy. Vector Borne Zoonotic Dis 11:451–452

Azarova NS, Miretskij OY, Sonin MD (1965) The first discovered case of nematode parasitism in the USSR in a human being (genus *Onchocerca* Diesing, 1841). Med Parazytol (Moscow) 34: 156–158

Baird KJ, Neafie RC (1988) South American brugian filariasis: report of a human infection acquired in Peru. Am J Trop Med Hyg 39:185–188

Beaver PC, Horner GS, Bilos JZ (1974) Zoonotic onchocercosis in a resident of Illinois and observations on the identification of *Onchocerca* species. Am J Trop Med Hyg 23:595–607

Beaver PC, Meyers EA, Jarroll EL et al (1980) *Dipetalonema* from the eye of a man in Oregon. Am J Trop Med Hyg 29:369–372

Beaver PC, Wolfson JS, Waldron MA et al (1987) *Dirofilaria ursi*-like parasites acquired by humans in the northern United States and Canada: report of two cases and brief review. Am J Trop Med Hyg 37:357–362

Beaver PC, Yoshimura H, Takayasu S et al (1989) Zoonotic *Onchocerca* in a Japanese child. Am J Trop Med Hyg 40:298–300

Bernardi M (2008) Global climate change–a feasibility perspective of its effect on human health at a local scale. Geospat Health 2(2):137–150

Botero D, Aguledo LM, Uribe FJ et al (1984) Intraocular filaria, a *Loaina* species, from man in Colombia. Am J Trop Med Hyg 33:578–582

Bowman DD, Liu Y, McMahan CS, Nordone SK et al (2016) Forecasting United States heartworm *Dirofilaria immitis* prevalence in dogs. Parasit Vectors 9(1):540. https://doi.org/10.1186/s13071-016-1804-y

Cancrini G, Tassi P, Coluzzi M (1989) Ivermectin against larval stages of *Dirofilaria repens* in dogs. Parassitologia 31:177–182

Capelli G, Genchi C, Baneth G et al (2018) Recent advances on *Dirofilaria repens* in dogs and humans in Europe. Parasit Vectors 11:663. https://doi.org/10.1186/s13071-018-3205-x

Ciuca L, Vismarra A, Lebon W, Beugnet F, Morchon R, Rinaldi L, Cringoli G, Kramer L, Genchi M (2020) New insights into the biology, diagnosis and immune response to *Dirofilaria repens* in the canine host. Vet Parasitol X 5(4):100029. https://doi.org/10.1016/j.vpoa.2020.100029

Elenitoba-Johnson KSJ, Eberhard ML, Dauphinais RM et al (1996) Zoonotic brugian lymphadenitis: an unusual case with florid monocytoid B-cell proliferation. Am J Clin Pathol 105:384–387

ESCCAP Guideline No 1 (2020) Worm control in dogs and cats and cats. http://www.esccap.org/

Fleck R, Kurz W, Quade B et al (2009) Human dirofilariasis due to *Dirofilaria repens* mimicking a scrotal tumor. Urology 73:e1–e3

Fok E, Jacso O, Szebeni Z et al (2010) Elimination of *Dirofilaria* (syn. *Nochtiella*) *repens* microfilariae in dogs with monthly treatments of moxidectin 2.5 %/imidacloprid 10 % (Advocate®, Bayer) spot-on. Parasitol Res 106:1141–1149

Fortin JF, Slocombe JOD (1981) Temperature requirements for the development of *Dirofilaria immitis* in *Aedes triseriatus* and *Ae. vexans*. Mosq News 41:625–633

Genchi C, Kramer LH (2020) The prevalence of *Dirofilaria immitis* and *D. repens* in the Old World. Vet Parasitol 280:108995. https://doi.org/10.1016/j.vetpar.2019.108995

Genchi C, Rinaldi L, Cascone C et al (2005) Is heartworm really spreading in Europe? Vet Parasitol 133:137–148

Genchi M, Ferrari N, Sirtori M et al (2007) Can a human shelter for dogs be a risk factor for spreading of canine filarial (*Dirofilaria immitis* and *D. repens*) infection? First European *Dirofilaria* Days Abstract book, Zagreb, February 2007, p 8

Genchi C, Rinaldi L, Mortarino M et al (2009) Climate and *Dirofilaria* in Europe. Vet Parasitol 163: 286–292

Genchi M, Pengo G, Genchi C (2010) Efficacy of moxidectin microsphere sustained release formulation for the prevention of subcutaneous filarial (*Dirofilaria repens*) infection in dogs. Vet Parasitol 170:167–169

Genchi C, Kramer LH, Rivasi F (2011a) *Dirofilaria* infections in Europe. Vector Borne Zoonotic Dis 11:1307–1317

Genchi C, Mortarino M, Rinaldi L et al (2011b) Changing climate and changing vector-borne disease distribution: the example of *Dirofilaria* in Europe. Vet Parasitol 176:295–299

Genchi C, Genchi M, Petry G et al (2013) Evaluation of the efficacy of imidacloprid 10%/ moxidectin 2.5% (Advocate®, Advantage® Multi, Bayer) for the prevention of *Dirofilaria repens* infection in dogs. Parasitol Res 112(1):81–89

Gorezis S, Psilla M, Asproudis I et al (2006) Intravitreal dirofilariasis: a rare ocular infection. Orbit 25:57–59

Gungel H, Kara N, Pinarci EY et al (2009) An uncommon case with intravitreal worm. Br J Ophthalmol 93:573–574

Gutierrez Y (1990) Diagnostic pathology of parasitic infections with clinical correlations. Lea & Febiger, Philadelphia, PA

IPCC (2019) Climate change 2019: synthesis report glossary. Sixth Assessment Report (AR6)

Kartashev V, Batashove I, Kartashov S et al (2011) Canine and human dirofilariasis in the Rostov region (Southern Russia). Vet Med Int. https://doi.org/10.4061.2011.6857.3

Khasnis AA, Nettleman MD (2005) Global warming and infectious disease. Arch Med Res 36:689–696

Kłudkowska M, Pielok Ł, Frąckowiak K et al (2018) Dirofilaria repens infection as a cause of intensive peripheral microfilaremia in a Polish patient: process description and cases review. Acta Parasitol 63(3):657–663. https://doi.org/10.1515/ap-2018-0077. PMID: 29975647

Knols BGJ, Takken W (2007) Alarm bells ringing: more of the same, and new and novel diseases and pets. In: Takken BGJ, Knols W (eds) Emerging pests and vector-borne diseases in Europe. Wageningen Academic, Wageningen, pp 13–19

Knott J (1939) A method for making microfilarial survey on day blood. Trans R Soc Med Hyg 33: 191

Lee ACY, Montgomery SP, Theis JH et al (2000) Public health issues concerning the widespread distribution of canine heartworm disease. Trends Parsitol 26:168–173

Lee ACY, Montgomery SP, Theis JH et al (2010) Public health issues concerning the widespread distribution of canine heartworm disease. Trends Parasitol 26:168–173

Levy JK, Edinboro CH, Glotfelty C-S et al (2007) Seroprevalence of *Dirofilaria immitis*, feline leukemia virus, and feline immunodeficiency virus infection among dogs and cats exported from the 2005 Gulf Coast hurricane disaster area. J Am Vet Med Assoc 231:218–225

Liotta JL, Sandhu GK, Rishniw M et al (2013) Differentiation of the microfilariae of *Dirofilaria immitis* and *Dirofilaria repens* in stained blood films. J Parasitol 99(3):421–425. https://doi.org/10.1645/12-10.1

Lok JB, Knight DH (1998) Laboratory verification of a seasonal heartworm transmission model. In: Seward RL (ed) Advances in heartworm disease. American Heartworm Society, Batavia, IL, pp 15–20

Magnis J, Lorentz S, Guardone L et al (2013) Morphometric analyses of canine blood microfilariae isolated by Knott's test enable *Dirofilaria immitis* and *D. repens* species-specific and *Acanthocheilonema* (syn. *Dipetalonema*) genus-specific diagnosis. Parasit Vectors 6:48. https://doi.org/10.1186/1756-3305-6-48. PMID: 23442771

Masny A, Goła¸b E, Cielka D et al (2013) Vector-borne of dogs and humans—focus in central and eastern part of Europe. Parasit Vectors 6:38–39

McCall JW, Genchi C, Kramer LH et al (2008) Heartworm disease in animals and humans. Adv Parasitol 66:193–285

Medlock JM, Avenell D, Barras I et al (2006) Analysis of the potential for survival and seasonal activity of *Aedes albopictus* (Diptera: Culicidae) in the United Kingdom. J Vector Ecol 31:292–304

Menendez MC, Bouza M (1988) *Brugia* species in a man from western Ethiopia. Am J Trop Med Hyg 39:189–190

Montarsi F, Ciocchetta S, Devine G et al (2015) Development of *Dirofilaria immitis* within the mosquito *Aedes* (*Finlaya*) *koreicus*, a new invasive species for Europe. Parasit Vectors 23(8): 177. https://doi.org/10.1186/s13071-015-0800-y. PMID: 25884876

Montarsi F, Martini S, Michelutti A et al (2019) The invasive mosquito *Aedes japonicus japonicus* is spreading in northeastern Italy. Parasit Vectors 12(1):120. https://doi.org/10.1186/s13071-019-3387-x. PMID: 30909981

Morchón R, Moya I, Gonzalez-Miguel J et al (2010) Zoonotic *Dirofilaria immitis* infections in a province of Northern Spain. Epidemiol Infect 138:380–383

Mortarino M, Musella V, Costa V et al (2008) GIS modeling for canine dirofilariasis risk assessment in central Italy. Geospat Health 2:253–261

Orihel TC, Beaver PC (1989) Zoonotic *Brugia* infections in North and South America. Am J Trop Med Hyg 40:638–647

Orihel TC, Eberhard ML (1998) Zoonotic filariasis. Clin Microbiol Rev 11:366–381

Orihel TC, Isbey EK (1990) *Dirofilaria striata* infection in a North Carolina child. Am J Trop Med Hyg 42:124–126

Otranto D, Capelli G, Genchi C (2009) Changing distribution patterns of canine vector borne diseases in Italy: leishmaniosis vs dirofilariasis. Parasit Vectors Suppl 1:S2. https://doi.org/10.1186/1756-3305-2-S1-S2

Pampiglione S, Rivasi F (2000) Human dirofilariasis due to *Dirofilaria* (*Nochtiella*) *repens*: an update of world literature from 1995 to 2000. Parassitologia 42:235–254

Pampiglione S, Canestri Trotti G, Rivasi F (1995) Human dirofilariasis due to *Dirofilaria* (*Nochtiella*) *repens*: a review of world literature. Parassitologia 37:149–193

Pampiglione S, Rivasi F, Gustinelli A (2009) Dirofilarial human cases in the Old World, attributed to *Dirofilaria immitis*: a critical analysis. Histopathology 54:192–204

Panarese R, Iatta R, Latrofa MS, Zatelli A, Ćupina AI, Montarsi F, Pombi M, Mendoza-Roldan JA, Beugnet F, Otranto D (2020) Hyperendemic Dirofilaria immitis infection in a sheltered dog population: an expanding threat in the Mediterranean region. Int J Parasitol 50(8):555–559

Pantchev N, Norden N, Lorentzen L et al (2009) Current surveys on the prevalence and distribution of *Dirofilaria* spp. in dogs in Germany. Parasitol Res 105:S63–S74

Perret-Court A, Coulibaly B, Ranque S et al (2009) Intradural dirofilariasis mimicking a Langerhans cell histiocytosis tumor. Pediatr Blood Cancer 53:485–487

Pietikäinen R, Nordling S, Jokiranta S et al (2017) *Dirofilaria repens* transmission in southeastern Finland. Parasit Vectors 10(1):561. https://doi.org/10.1186/s13071-017-2499-4

Purse BV, Mellor PS, Rogers DJ et al (2005) Climate change and the recent emergence of bluetongue in Europe. Nat Rev Microbiol 53:171–181

Raccurt CP (1999) La dirofilariose, zonnose émergente et me´connue en France. Med Trop (Mars) 59:389–400

Rezza G, Nicoletti L, Romi R et al (2007) Infection with chikungunya virus in Italy: an outbreak in a temperate region. Lancet 370:1840–1846

Riebenbauer K, Weber PB, Walochnik J et al (2021) Human dirofilariosis in Austria: the past, the present, the future. Parasit Vectors 14:227. https://doi.org/10.1186/s13071-021-04696-4

Rogers DJ, Randolph SE (2006) Climate change and vector-borne diseases. Adv Parasitol 62:345–381

Sambri V, Capobianchi M, Chanell R et al (2013) West Nile virus in Europe: emergence, epidemiology, diagnosis, treatment, and prevention. Clin Microbiol Infect. https://doi.org/10.1111.1469.0691.12211

Sassnau R, Genchi C (2013) Qualitative risk assessment for the endemisation of *Dirofilaria repens* in the state of Brandenburg (Germany) based on temperature-dependent vector competence. Parasitol Res 112:2647–2652

Savić S, Stosic MZ, Marcic D, Hernández I, Potkonjak A, Otasevic S, Ruzic M, Morchón R (2020) Seroepidemiological study of canine and human dirofilariasis in the endemic region of Northern Serbia. Front Vet Sci 29(7):571

Scholte EJ, Dijkstra E, Blok H et al (2008) Accidental importation of the mosquito *Aedes albopictus* into The Netherlands: a survey of mosquito distribution and the presence of dengue virus. Med Vet Entomol 22:352–358

Siegenthaler R, Gubler R (1965) Para-articulares nematode granuloma (indigenous *Onchocerca*). Schweiz Med Wochenschr 95:1102–1104

Simón F, Prieto G, Morchón R et al (2003) Immunoglobulin G antibodies against the endosymbionts of filarial nematodes (Wolbachia) in patients with pulmonary dirofilariasis. Clin Diagn Lab Immunol 10:180–181

Simón F, Lopez-Belmonte J, Marcos-Atxutegi C et al (2005) What is happening outside North America regarding human dirofilariasis? Vet Parasitol 133:181–189

Simón F, Siles-Lucal M, Morchón R et al (2012) Human and animal dirofilariasis: the emergence of a zoonotic mosaic. Clin Microbiol Rev 25:507–543

Slocombe JOD, Surgeoner GA, Srivastava B (1989) Determination of the heartworm transmission period and its use in diagnosis and control. In: Otto GF (ed) Proceedings of the heartworm symposium'89. American Heartworm Society, Batavia, IL, pp 19–26

Szenasi Z, Kovacs H, Pampiglione S et al (2008) Human dirofilariasis in Hungary: an emerging zoonosis in central Europe. Wien Klin Wochenschr 120:96–102

Takaoka H, Bain O, Tajimi S et al (1996) Second case of zoonotic *Onchocerca* infection in a resident of Oita in Japan. Parasite 3:179–182

Takumi K, Scholte E-J, Braks M et al (2009) Introduction, scenarios for establishment and seasonal activity of *Aedes albopictus* in The Netherlands. Vector Borne Zoonotic Dis 9:191–196

Tatem AJ, Rogers DJ, Hay SI (2006) Global transport networks and infectious disease spread. Adv Parasitol 62:293–343

Theis JH (2005) Public health aspects of dirofilariasis in the United States. Vet Parasitol 133:157–180

Torres-Chable OM, Brito-Argaez LG, Islas-Flores IR, Zaragoza-Vera CV, Zaragoza-Vera M, Arjona-Jimenez G, Baak-Baak CM, Cigarroa-Toledo N, Gonzalez-Garduño R, Machain-Williams CI, Garcia-Rejon JE (2020) *Dirofilaria immitis* proteins recognized by antibodies from individuals living with microfilaremic dogs. J Infect Dev Ctries 14(12):1442–1447

Traversa D, Aste G, Milillo P et al (2010) Autochthonous foci of canine and feline infections by *Dirofilaria immitis* and *Dirofilaria repens* in central Italy. Vet Parasitol 169:128–132

Vezzani D, Carbajo AE (2006) Spatial and temporal transmission risk of *Dirofilaria immitis* in Argentina. Int J Parasitol 36:1463–1472

Webber WAF, Hawking F (1955) Experimental maintenance of *Dirofilaria repens* and *D. immitis* in dogs. Exp Parasitol 4:143–164

Chapter 16
Toxocariasis

Ayako Yoshida, Clare M. Hamilton, Elena Pinelli, and Celia V. Holland

Abstract *Toxocara canis* and *Toxocara cati* are ubiquitous gastrointestinal parasites of dogs and cats, respectively, worldwide. Due to widespread environmental contamination with their eggs, which are shed in the faeces of infected animals, other hosts such as humans can become infected. In these accidental hosts, the parasites do not develop into adults but remain as larvae, migrating through different organs of the body giving rise to a number of clinical syndromes including visceral larva migrans, ocular larva migrans and neurotoxocariasis. Seroprevalence studies indicate high levels of human exposure worldwide, yet the risks of *Toxocara* spp. infection remain relatively unknown among the general public, and toxocariasis is considered a classic neglected disease. This chapter reviews the life cycles and transmission routes of *T. canis* and *T. cati*, along with the different clinical syndromes that manifest during infection. Current diagnostic methods are reviewed, and the drawbacks and need for standardisation are discussed, particularly with reference to the present difficulties in distinguishing between *T. canis* and *T. cati* infections.

16.1 Introduction

Toxocariasis is the clinical term used to describe human infection with the parasitic roundworms *Toxocara canis* and *Toxocara cati*, commonly found in the intestines of dogs and cats, respectively. Although there are a number of other species in the genera (including *T. malayiensis* of cats; *T. vitulorum* of cattle, buffalo and other

A. Yoshida (✉)
Centre for Animal Disease Control, University of Miyazaki, Miyazaki, Japan
e-mail: kukuri@med.miyazaki-u.ac.jp

C. M. Hamilton
Moredun Research Institute, Edinburgh, UK

E. Pinelli
National Institute of Public Health and Environment, Bilthoven, Netherlands

C. V. Holland
Department of Zoology, Trinity College Dublin, Dublin, Ireland

F. Bruschi (ed.), *Helminth Infections and their Impact on Global Public Health*,
https://doi.org/10.1007/978-3-031-00303-5_16

ruminants; and *T. pteropodis* of bats), their zoonotic potential is limited (Moorhouse 1982; Iddawela et al. 2003) or as yet undetermined, and therefore only *T. canis* and *T. cati* are the focus of this chapter.

Adult *Toxocara* spp. worms reside in the small intestines of their definitive hosts, and eggs are passed into the environment via the faeces (Overgaauw 1997b). After a period of embryonation, the eggs become infective to humans (and paratenic hosts) and can cause infection after they have been accidentally ingested via contaminated hands or food (Overgaauw 1997a). Children are particularly prone to infection because they can become exposed to infective eggs when playing in sandboxes and playgrounds where cats and dogs commonly defecate. Once eggs are ingested, the larvae hatch in the intestine and migrate throughout the soft tissues of the body, including the liver, lungs, eyes and central nervous system (CNS) for prolonged periods of time but fail to develop into adult worms. The migration pathways, as well as ensuing inflammatory immune response, give rise to various clinical manifestations of toxocariasis, including visceral larva migrans (VLM), ocular larva migrans (OLM), covert (or subclinical) toxocariasis (CT) and the more recently defined neurotoxocariasis (NT).

T. cati and *T. canis* have a worldwide distribution, and the high fecundity of adult worms results in extensive environmental contamination with infective eggs and thus the risk of infection to humans. Seroprevalence studies indicate high levels of exposure in the human population (Smith and Noordin 2006), and, in fact, toxocariasis is now considered to be the most important zoonotic infection in the USA, particularly among the socioeconomically disadvantaged (Hotez and Wilkins 2009). Given the high prevalence of toxocariasis in areas of poor hygiene and the lack of awareness among the general public (Wells 2007), the true magnitude and global importance of *Toxocara* spp. infection are likely to be significantly underestimated (Hotez and Wilkins 2009).

16.2 Life Cycle and Transmission Routes

The life cycle of *Toxocara canis* is complex, with numerous modes of transmission to the definitive host (Fig. 16.1). Adult worms reside in the small intestine of dogs where females can produce up to 200,000 unembryonated eggs per day which are passed into the environment in the faeces, 4–5 weeks after initial infection (Overgaauw 1997b) (Fig. 16.2). Under optimum conditions, eggs will embryonate and become infective within 6 weeks, but this can be delayed for several months at lower temperatures (Moore and McCarthy 2006). Following ingestion from the environment by a canine host, *T. canis* larvae hatch in the small intestine, burrow through the intestinal mucosa, enter the bloodstream and travel via the liver to the lungs (Overgaauw 1997b). From here, the larvae either migrate up the trachea where they are swallowed and returned to the small intestine to develop into adult worms or undergo somatic migration and enter a wide range of tissues including the liver, lungs, heart, brain and muscle (Glickman and Schantz 1981). Tracheal migration and

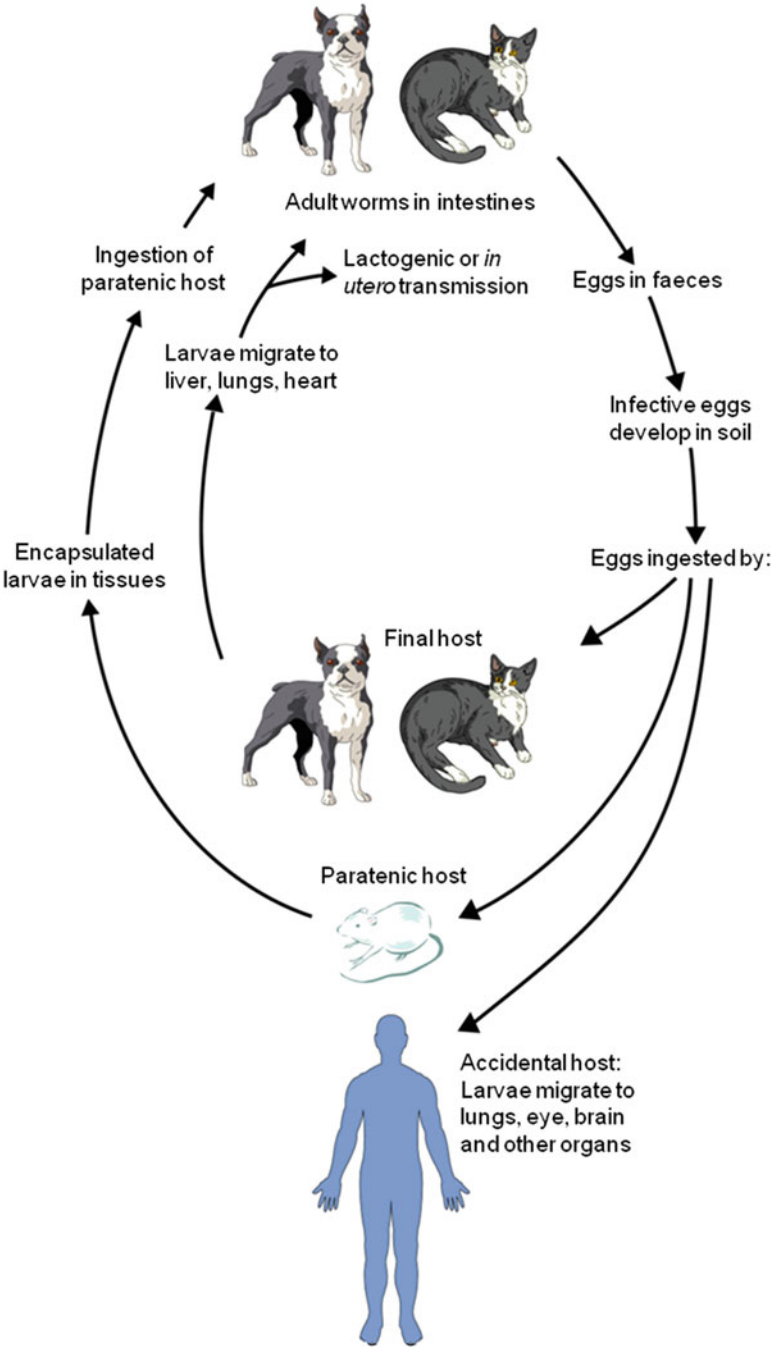

Fig. 16.1 Life cycle of *Toxocara* spp.

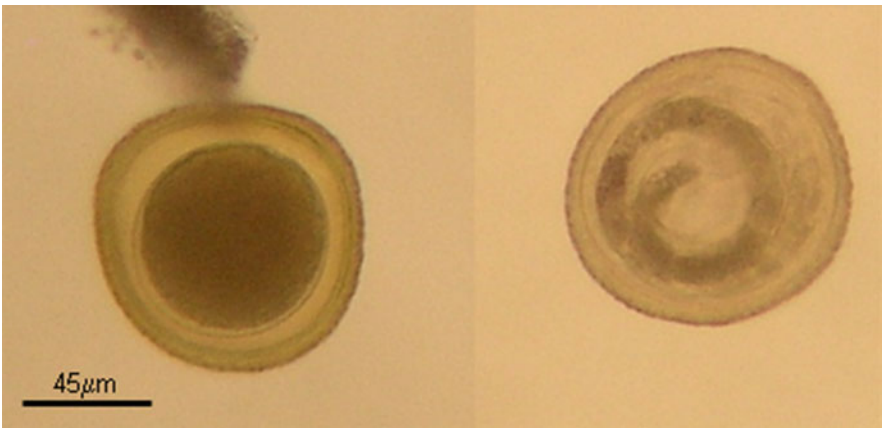

Fig. 16.2 Unembryonated (left) and embryonated (right) *Toxocara canis* eggs (90 × 75 µm)

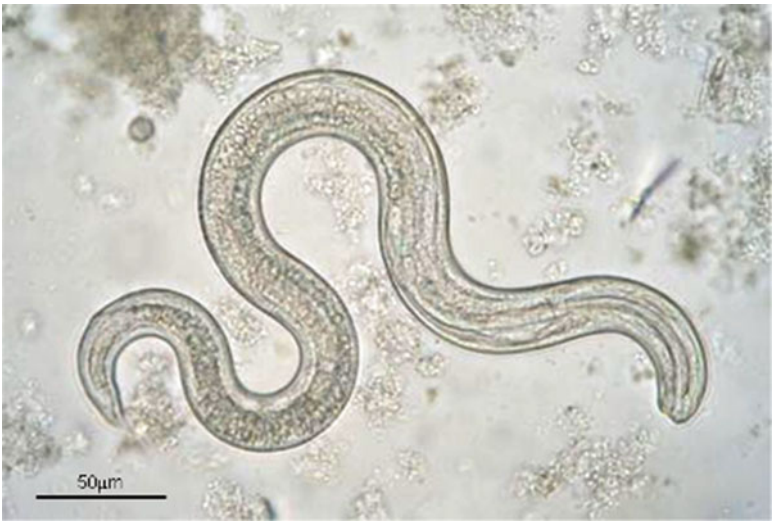

Fig. 16.3 *Toxocara canis* L3 larva (400 × 20 µm) isolated from the brain of an infected BALB/c mouse

the development of a patent infection are more common in young dogs (Greve 1971); however, recent research has shown that adult dogs infected with low numbers of infective eggs may also develop patent infections, highlighting their importance as reservoirs of infection (Fahrion et al. 2008). Where somatic migration occurs, the larvae do not develop into adult worms, and they can remain in a state of arrested development in the tissues of the host for many years (Overgaauw 1997b) (Fig. 16.3). This phenomenon offers two further possible modes of transmission if the dog becomes pregnant: (1) the larvae can become mobilised from the tissues and

migrate across the placenta infecting puppies in utero, leading to tracheal migration in the pup and eggs being shed in the faeces 2–3 weeks after birth, or (2) they can migrate to the mammary glands and infect puppies during lactation in which case there is no tracheal migration and the larvae develop to adults in the intestines (Overgaauw 1997b). Some studies have suggested that patent infections occur more commonly in male dogs, whereas females harbour arrested larvae in their tissues that can go on to infect their offspring (Overgaauw 1997b; Webster 1958). While this theory would offer an evolutionary advantage to the parasite, most prevalence studies have not reported a difference in the number of patent infections between male and female dogs, so a gender influence is unlikely (Overgaauw and van Knapen 2013; Schnieder et al. 2011). Dogs of any age may ingest *T. canis* larvae in the tissues of paratenic hosts such as mice or birds and develop a patent infection without tracheal migration (Warren 1969).

The life cycle of *Toxocara cati* is slightly less complex (Fig. 16.1). Following ingestion of infective eggs from the environment, the larvae hatch and undergo a similar hepato-tracheal migration pattern to that described above, developing to adults in the small intestine (Overgaauw 1997b). Unembryonated eggs are passed in the faeces 8 weeks postinfection. In contrast to the dog, tracheal migration and the development of a patent infection can remain high in older cats although it is still less frequent than in younger cats (O'Lorcain 1994). Some of the larvae also undergo somatic migration and can remain in the tissues of the cat for long periods of time (Overgaauw 1997b). If the cat is pregnant, the larvae can migrate to the mammary glands and infect kittens during lactation, although this has been shown to be more likely to occur during an acute infection where the queen is infected in late gestation (Coati et al. 2004). Following this mode of transmission, the larvae undergo full development in the intestines without tracheal migration. There is no transplacental transmission with *T. cati*. Cats can also become infected with *T. cati* through the ingestion of larvae in the tissues of paratenic hosts—which is probably a more significant route of transmission than in dogs, given the predatory nature of cats.

Due to the highly successful transmission routes of these parasites and the high level of fecundity of the female worms, the environment can become extensively contaminated with infective eggs, posing a risk of infection to a wide variety of paratenic and accidental hosts including mice, birds and, most significantly, humans. If these hosts ingest infective eggs, the larvae hatch in the intestine and migrate through the somatic tissues for months before becoming immobilised in the tissues in a long-term state of developmental arrest (Moore and McCarthy 2006). There have been some reports of human infection with adult *T. cati* worms, but most of these are thought to be erroneous (Eberhard and Alfano 1998).

Humans become infected with *Toxocara* spp. through the ingestion of infective eggs directly from the soil or indirectly through soil-contaminated hands or food (Glickman and Schantz 1981; Glickman and Shofer 1987). Children are particularly at risk of infection due to the areas they play in (e.g. sandboxes and playgrounds, where cats and dogs may defecate) and their propensity to ingest non-food substances such as sand or earth (geophagia) (Lewis 2006). Humans may also become infected through the ingestion of encapsulated larvae in the raw or undercooked

tissues of paratenic hosts such as cows, ostrich, chickens and pigs (Nagakura et al. 1989; Yoshikawa et al. 2008; Noh et al. 2012) or through unwashed contaminated fruit and vegetables (Klapec and Borecka 2012). The larvae have been shown to remain infective in paratenic host tissues for up to 6 months highlighting the potential risk of this route of transmission (Taira et al. 2011). Recently, it was suggested that direct contact with contaminated dog hair may pose a potentially important source of infection of *T. canis* to humans (Wolfe and Wright 2003; Roddie et al. 2008); however, a recent survey of owned dogs demonstrated a low prevalence of *Toxocara* spp. eggs on fur (and these were all unembryonated) (Keegan and Holland 2010), suggesting that direct contact with well-cared dogs poses a low risk of infection (Keegan and Holland 2013).

16.3 Clinical Syndromes

The degree of host damage and concomitant manifestation of clinical signs and symptoms are determined by the size of the inoculum, the location of the invading larvae and the host immune response (Despommier 2003; Pawlowski 2001). Most *Toxocara* spp. infections are thought to be asymptomatic; however, high parasite burdens can lead to toxocariasis which can be clinically divided into four different syndromes: visceral larva migrans (VLM), ocular larva migrans (OLM), covert toxocariasis (CT) and neurotoxocariasis (NT). Although it is believed that *T. canis* is the causative agent most commonly associated with these syndromes, most current diagnostic procedures do not differentiate between *T. canis* and *T. cati*, so the zoonotic potential of *T. cati* should not be underestimated (Fisher 2003).

16.3.1 Visceral Larva Migrans

Visceral larva migrans was first described by Beaver et al. (1952) and refers to the migration of larvae through the somatic tissues and the associated pathogenesis. It is thought to be associated with a larger infectious dose and multi-systemic disorder. This syndrome is classically characterised by persistent eosinophilia, fever and hepatomegaly and is mainly diagnosed in children between 2 and 7 years of age with a history of pica and contact with puppies (Magnaval et al. 2001; Pawlowski 2001). The full clinical spectrum of VLM, associated with hepatic and pulmonary larval migration, includes hepatomegaly, abdominal pain, loss of appetite, hypergammaglobulinemia, wheezing and coughing (Rubinsky-Elefant et al. 2010; Taylor et al. 1988). Pulmonary involvement, which may appear as acute bronchiolitis, asthma or pneumonitis, is common, but severe respiratory distress is rare (Glickman and Schantz 1981; Bartelink et al. 1993). Wheezing can be a common presenting feature of VLM, and some studies have found a positive association between *Toxocara* spp. seropositivity and asthma (Walsh 2011) (see Sect. 16.5).

Granulomatous lesions, induced by the immune response to secreted larval antigens, in the liver and lungs of infected patients have occasionally been mistaken for metastatic cancer (Ota et al. 2009). Larval migration to the heart may result in myocarditis, but this has rarely been reported (Kim et al. 2012).

Many studies have reported cutaneous manifestations of toxocariasis. Upon analysis of 350 cases of VLM, skin symptoms such as transient rash, urticaria and hypodermic nodules were the most frequently noted, reaching 23% in children and 29% in adults (Gavignet et al. 2008). Other cutaneous symptoms such as chronic pruritus, chronic prurigo, miscellaneous eczema and vasculitis have also been observed with toxocariasis, and in some cases, dermatological symptoms were the only indication of the disease's presence (Gavignet et al. 2008). *Toxocara* spp. seropositivity has been shown to be significantly associated with prurigo (Humbert et al. 2000) and urticaria (Ismail and Khalafallah 2005) but not with pruritus or eczema (Humbert et al. 2000). Increased clinical awareness of cutaneous manifestations may result in improved recognition of human toxocariasis (Smith et al. 2009). Consequently, *Toxocara* spp. infection should be considered along with bacterial or viral causes in patients presenting with a skin manifestation of unknown aetiology (Piarroux et al. 2006).

16.3.2 Ocular Larva Migrans

Ocular larva migrans (OLM) was first described by Wilder (1950), who reviewed children's eyes enucleated because of endophthalmitis and/or suspected retinoblastoma and reported the presence of nematode larvae in 24 of 46 eyes examined. Later, Nichols (1956) identified *T. canis* larvae in four out of five eyes examined. OLM typically occurs in older children (mean age 8 years), although it is also reported in adults, and results from the migration of as few as a single larva in the eye (Raistrick and Hart 1976; Rubinsky-Elefant et al. 2010). It is generally characterised by unilateral vision impairment, strabismus and leukocoria, although the clinical presentation can vary depending on the site of involvement and immune response of the host (Schantz 1989; Sakai et al. 1998; Pivetti-Pezzi 2009). The peripheral retina and vitreous are most commonly involved (Taylor 2006). Granuloma formation around trapped larvae can drag on the retina causing a distortion, heterotopia or detachment of the macula (Despommier 2003). Total blindness in one or both eyes can occur in severe cases, but this is rare (Taylor 2001). In a study of over 120,000 Irish school children, the prevalence of definitive consultant-diagnosed ocular toxocariasis was 6.6 cases per 100,000 (Good et al. 2004). A recent web-based survey completed by ophthalmologists and ophthalmology societies in the USA reported 68 cases of ocular toxocariasis diagnosed between September 2009 and September 2010 (Woodhall et al. 2012). Of 30 patients with full clinical data available, 83% reported vision loss, and in 68% of these, the loss of vision was permanent. Hallmarks of VLM, such as hepatomegaly, hypereosinophilia and pulmonary symptoms, are usually absent in OLM, and this is thought to reflect a lower infectious dose. OLM

patients have lower antibody titres compared to VLM patients (Fan et al. 2013) which possibly allows for the persistence of larvae in the tissues for years with periodical migration (Overgaauw 1997a). This longer "incubation period" has been suggested as a possible explanation for the higher mean age of OLM patients in comparison to those with VLM (Overgaauw 1997a). Risk factors associated with OLM have been reported to include history of convulsions, geophagia, close contact or ownership of a dog and the ingestion of raw meat (Taylor 2006; Lee et al. 2010).

16.3.3 Neurotoxocariasis

Neurotoxocariasis, or cerebral toxocariasis, is a much less well established clinical syndrome than the others, and its effects in humans are significantly less well understood or appreciated (Holland and Hamilton 2006).

Humans are known to carry *Toxocara* spp. larvae in the brain. Some of the earliest studies describe the presence of larvae within granulomas in the CNS, discovered accidentally at autopsy when the patient had died from another cause (Dent et al. 1956; Hill et al. 1985; Nelson et al. 1990). To our knowledge, there are approximately 200 cases of neurological toxocariasis reported in the literature to date, as determined by the presence of *Toxocara* spp. larvae in the brain, seropositivity to *Toxocara*, and/or amelioration of clinical and radiological symptoms upon anthelmintic treatment (Deshayes et al. 2016; Docu Axelerad et al. 2021; Yoshida et al. 2016). Although comparatively rare compared to other clinical syndromes, more than 80% of these neurological cases have been reported since the year 2000 indicating an enhanced awareness of clinical presentation and also improved diagnosis. The predominant clinical pictures are myelitis, encephalitis and/or meningitis, but blood eosinophilia and hyperIgEemia is inconstant (Yoshida et al. 2016; Deshayes et al. 2016). Interestingly, NT may be a clinical syndrome more associated with adults rather than children, considering the age distribution of the reported patients (Yoshida et al. 2016; Deshayes et al. 2016).

The degree of neurological symptoms is likely to depend on the number and location of the larvae in the brain, the immune response directed against them and the resulting pathology (Despommier 2003). The mouse model has been an invaluable tool in studying the consequences of cerebral *T. canis* infection (Holland and Hamilton 2013), and studies have highlighted a number of issues that may have implications for human health: larval distribution in the brain may not be random (Burren 1971; Good et al. 2001); behavioural alterations are dose-dependent (Cox and Holland 2001); cerebral infection can lead to deficits in learning and memory (Hamilton et al. 2006); and infection induces inflammatory and immune cerebral responses (Hamilton et al. 2008; Liao et al. 2008) that may be correlated with behavioural deficits (Holland and Hamilton 2013).

Unfortunately, the impact of *Toxocara* spp. infection on cognitive development in humans is sorely lacking, with only a handful of studies having examined the relationship between *Toxocara* spp. seropositivity and neuropsychological

parameters. In particular, some researchers have described the effect of *Toxocara* infection as follows: lack of developmental progress and speech (Fortenberry et al. 1991), depressive symptoms and cognitive deficits possibly indicative of dementia (Richartz and Buchkremer 2002), mental confusion and cognitive impairment (Salvador et al. 2010), slowed cognitive information processing, impairments of short-term and working memory spans and mental fluency (Scheid et al. 2008). In order to fully examine the etiological connection between toxocariasis and impaired cognitive function more appropriately, further investigations are required.

16.3.4 Covert Toxocariasis

Covert toxocariasis was first put forward by Taylor et al. (1987) to describe a series of mild, non-specific symptoms which did not fall within the categories of VLM or OLM yet were recognisable as *Toxocara* spp. infection. That such a disease might exist had previously been suggested by Bass et al. (1983). Symptoms such as hepatomegaly, splenomegaly, coughing, sleep disturbances, abdominal pains, anorexia, nausea and headaches, with or without eosinophilia, were associated with raised *Toxocara* spp. antibodies, particularly in children beyond the toddler stage (Taylor et al. 1987). Around the same time, Glickman et al. (1987) described a similar clinical syndrome in a group of French adults, comprising weakness, pruritus, difficulty in breathing, abdominal pain, eosinophilia and increased levels of IgE, and subsequently named it "common toxocariasis". It seems likely that "covert" and "common" toxocariasis represent slight variations of the same, mild syndrome (CT) depending on whether the patient is an adult or a child (Smith et al. 2009).

16.4 Epidemiology

Toxocara cati and *T. canis* are ubiquitous parasites of cats and dogs, respectively, worldwide. Infection rates, as determined by the presence of eggs in faeces, vary from 8% to 91% for *T. cati* in cats and from 0.7% to 82.6% for *T. canis* in dogs (Epe 2006; O'Lorcain 1994). The broad prevalence ranges are reflective of different environmental and biological factors (i.e. pet owned or stray animals, rural or urban location, age) as well as differences in the sensitivity of the detection methods used (Overgaauw and van Knapen 2013). For *T. canis*, worm burdens and patent infections tend to be highest in dogs under 6 months of age and stray dogs (Roddie et al. 2008; Overgaauw and van Knapen 2013), whereas for *T. cati* the highest prevalence of patent infections occurs in cats 2- to 6-month-old (Lightner et al. 1978). Urban and rural foxes can carry patent *T. canis* infections, with prevalence as high as 79% in some areas (Richards et al. 1993); however, their contribution to environmental contamination is thought to be lower than that of dogs (Morgan et al. 2013). For both *T. canis* and *T. cati*, paratenic hosts can act as important reservoirs of

infection for the circulation and maintenance of the parasites in the environment (Dubinsky et al. 1995).

Due to the high fecundity of adult *Toxocara* spp. worms, large numbers of eggs can be excreted in the faeces of infected cats and dogs leading to widespread contamination of the environment. When eggs are initially passed in the faeces, they are not infective and require a period of embryonation which, depending on the soil type and environmental conditions such as humidity and temperature, can take from 3 weeks to several months (Overgaauw 1997b). *Toxocara* spp. eggs are highly resistant to environmental extremes and can remain viable in the soil for several years posing an infection risk to humans (Mizgajska-Wiktor and Uga 2006).

The one of main infection route to humans is through the ingestion of eggs from contaminated soil. Surveys carried out on samples taken from public and private parks and gardens have revealed worldwide contamination with *Toxocara* spp. eggs, with prevalence ranging from 13% to 87% in Europe, 14.4% to 20.6% in the USA, 6.6% to 63.3% in Asia, and 17.4% to 60.3% in Brazil (Rubinsky-Elefant et al. 2010). Furthermore, 51–95% of eggs in soil samples collected from temperate climates such as Ireland and Poland were fully embryonated and therefore infective to humans (Rubinsky-Elefant et al. 2010). Most surveys fail to discriminate between *T. canis* and *T. cati*, but it has been suggested that *T. canis* eggs are more common in public parks, whereas *T. cati* eggs are more common in private backyards and sandboxes (Overgaauw 1997b; Fisher 2003; Macuhova et al. 2012). In a recent study, Morgan and colleagues evaluated the relevant contribution of cats, dogs and foxes to environmental contamination with *Toxocara* spp. eggs, using the city of Bristol (UK) as a case study (Morgan et al. 2013). The authors demonstrated that dogs, especially those less than 12 weeks of age, dominated total egg output in the environment, but where the level of faecal removal by owners was high, foxes could take over as the primary source of eggs. They also noted, however, that Bristol has a low stray dog population and therefore this dynamic could change in areas where stray cats/dogs are more frequent. Nonetheless, it highlights the relative contribution of host animals to environmental contamination and therefore the risk of human infection.

Since humans are accidental hosts of *T. canis* and *T. cati* and do not develop patent infections, exposure must be estimated using seroprevalence studies—although these have their limitations and drawbacks, as discussed below. Seroprevalence of *Toxocara* spp. varies widely worldwide. A meta-analysis based on seroprevalence studies published from 1 January 1980 to 15 March 2019 revealed that estimated global seroprevalence of anti-*Toxocara* antibodies in healthy population was 19.0% and seroprevalences in WHO-defined regions were 22.8% in the Americas, 10.5% in Europe, 8.2% in Eastern Mediterranean countries, 24.2% in the Western Pacific, 37.7% in Africa and 34.1% in the Southeast Asia region (Rostami et al. 2019). This statistical method has recently been applied to a variety of areas in medical research. However, attention should be paid to the limitations of the analysis due to heterogeneity between studies meta-analysed, the quality of included studies and the publication bias. At the country level, the highest prevalence of 92.8% was reported on the island of La Reunion (Indian Ocean) in a study of

Table 16.1 Risk factors for human toxocariasis

Risk factors	Refs.
Ingestion of contaminated soil (geophagia)	Negri et al. (2013), Won et al. (2008), Holland et al. (1995)
Consumption of contaminated vegetables	Uga et al. (2009), Klapec and Borecka (2012)
Consumption of raw or undercooked meat	Nagakura et al. (1989), Yoshikawa et al. (2008), Noh et al. (2012), Yoshida et al. (2016)
Exposure to dogs	Holland et al. (1995), Jarosz et al. (2010), Fan et al. (2005)
Poverty	Hotez and Wilkins (2009), Congdon and Lloyd (2011)
Age	Holland et al. (1995), Fan et al. (2004)
Male gender	Santarem et al. (2011), Holland et al. (1995), Yoshida et al. (2016)
Rural residence	Holland et al. (1995), Zarnowska et al. (2008)
Ethnicity	Congdon and Lloyd (2011), Walsh and Haseeb (2012)

387 subjects over the age of 15 years (Magnaval et al. 1994). A similarly high seroprevalence of 86% was recorded in children aged 0.5–6 years in St Lucia (Thompson et al. 1986). In European countries, seroprevalence ranges from 1.6% to 33% (Smith and Noordin 2006), with one of the highest rates (31%) being reported in Irish schoolchildren (Holland et al. 1995). In the USA, prevalence was recently reported as 3.6% in children aged 6–17 years and 5.3% in adults, indicating a decreasing trend in *Toxocara* seroprevalence since the 1990s (Farmer et al. 2017).

Risk factors associated with *Toxocara* spp. seropositivity have been reported to include dog ownership (Fan et al. 2005; Jarosz et al. 2010), age (Fan et al. 2004; Holland et al. 1995), geophagia (Won et al. 2008; Negri et al. 2013), rural location (Holland et al. 1995; Zarnowska et al. 2008), consumption of raw/undercooked meat, fruit or vegetables (Yoshikawa et al. 2008; Noh et al. 2012; Klapec and Borecka 2012), poverty (Hotez and Wilkins 2009; Congdon and Lloyd 2011) and ethnicity (Congdon and Lloyd 2011; Walsh and Haseeb 2012) (Table 16.1). Cat ownership is less frequently associated with seropositivity (Woodruff et al. 1982; Jarosz et al. 2010).

In a recent article, the authors conducted a large-scale retrospective study analysing a total of 911 ascarid larva migrans syndrome (LMS) cases having *Toxocara* spp. and/or *Ascaris suum* infections from 2001 to 2015 in Japan (Yoshida et al. 2016), and the results revealed that Japanese patients appear to have a unique characteristic as compared to those in Europe, North American and tropical countries, where ascarid LMS was more common in children. Among Japanese patients, the proportion of toxocariasis cases was estimated somewhere between 85.0 and 91.7%, and the majority of patients was male (male-to-female ratio, 2.37) with a median age of 52.0 years (range, 2–92 years). In addition, 67.8% of them had a dietary history of consuming raw or undercooked meat and/or liver. In a word, toxocariasis in Japan is primarily a disease of adult males with the habit of raw or undercooked meat/liver consumption. Considering the fact that many cultures from all over the globe incorporate raw/undercooked meat into their cuisines, healthcare

specialists should draw attention to the risk of acquiring *Toxocara* infection by eating paratenic host meat.

16.5 Immune Response and Pathogenesis

Like other helminths, *Toxocara* spp. are known to induce a $CD4^+$ T helper-type 2 (Th2) immune response in the infected host, characterised by the production of type 2 cytokines (e.g. IL-4, IL-5, IL-10 and IL-13); increased levels of IgG1, IgM and IgE; and a marked eosinophilia (Kayes 2006; Maizels 2013). In culture, *Toxocara* spp. larvae secrete a range of molecules including large quantities of glycoproteins known as *Toxocara* excretory-secretory (TES) antigens (de Savigny 1975; Maizels et al. 2006), which are known to stimulate a Th2-type response (Del Prete et al. 1991) and form the basis of most diagnostic tests for toxocariasis (see Sect. 16.6).

Serum analysis of *Toxocara* spp.-seropositive patients revealed that IgG1 was the predominant antibody subclass, followed by IgG2, IgG4 and IgG3 (Obwaller et al. 1998). IgG1, IgG2 and IgG4 levels were significantly higher in symptomatic VLM patients compared with asymptomatic seropositive patients, whereas only IgG1 levels were significantly higher in OLM patients compared with asymptomatic patients. These data are consistent with the idea that the infectious dose required for OLM is lower than that for VLM and is not strong enough to stimulate IgG2, IgG3 or IgG4 titres (Glickman and Schantz 1981; Kayes 2006). Indeed, studies in mice have shown that antigen-specific lymphoproliferative responses, antibody titres and eosinophilia all increase in response to increasing numbers of larvae, suggesting an immunological threshold and dose-dependent response (Kayes et al. 1985).

As *Toxocara* spp. larvae migrate through the somatic tissues, they generate a granulomatous inflammatory response, characterised by aggregates of eosinophils, neutrophils and monocytes, resulting in the encapsulation and blocked migration of larvae (Kayes 2006). Some larvae are able to escape the confines of these granulomas, however, and migrate elsewhere explaining why serial sections of an entire granuloma may yield no evidence of the larva which initiated the response (Kayes 2006). Microscopic lesions have been observed in the liver and lungs of *T. canis*-infected mice (Bisseru 1969; Parsons and Grieve 1990) and in the liver (Nelson et al. 1990; Musso et al. 2007), lungs (Anderson et al. 2006), eyes (Taylor 2006; Verallo et al. 2012) and brain (Nelson et al. 1990; Mikhael et al. 1974) of *T. canis*-infected patients. Moreover, the immediate-type and delayed-type hypersensitivity reactions in response to trapped or dying larvae in the viscera or eye are responsible for the clinical manifestations of VLM or OLM (Despommier 2003).

The granulomatous inflammatory response and the raised antibody titres and eosinophilia appear to do little to control or eliminate *Toxocara* spp. larvae, which has given rise to the idea that the parasite is able to evade host immunity (Maizels et al. 2006). Studies have shown that *T. canis* larvae have a mucin-rich, highly labile surface coat which is loosely attached to the parasite epicuticle and is shed when

antibodies and/or eosinophils bind, allowing the parasite to "flee the scene of the crime" (Fattah et al. 1986; Maizels et al. 2006). The lack of a role for eosinophils has been demonstrated in the mouse model, where *T. canis* larvae remain unharmed in mice overexpressing IL-5 (with a resultant hypereosinophilia), while another helminth (*Nippostrongylus brasiliensis*) is eliminated (Dent et al. 1999). Furthermore, when *N. brasiliensis* is introduced to the transgenic IL-5 mice in the presence of TES antigens, their survival is greatly enhanced (Giacomin et al. 2008). Such immune evasion/manipulation, orchestrated by TES, is a likely mechanism by which *Toxocara* spp. facilitate its long-term survival in the host.

Infection with *Toxocara* spp. may also initiate or modulate other immunopathological reactions, in particular asthma (Maizels et al. 2006). Allergic asthma is a chronic inflammatory disorder of the airways characterised by increased serum IgE, eosinophilic inflammation, mucus hypersecretion and bronchial hyper-reactivity (Pinelli et al. 2006). Human infection with *Toxocara* spp. is associated with wheezing, coughing and airflow obstruction (Feldman and Parker 1992), and infection in mice leads to pulmonary inflammation, airway hyper-reactivity and increased IgE (Pinelli et al. 2005, 2008; Hanh et al. 2020). The similar manifestations between *Toxocara* spp. infection and allergic asthma have prompted researchers to investigate whether there is an association between the two (Kanobana et al. 2013; Pinelli et al. 2005). Walsh and colleagues examined the association between *Toxocara* spp. seropositivity and lung function in a nationally representative sample from the US population (11,606 participants; National Health and Nutrition Examination Survey, 1988–1994) (Walsh 2011). The authors demonstrated a significant association between diminished lung function and previous *Toxocara* spp. infection which held true when adjusted for a number of confounding variables including age, education level, smoking status, body mass index and dog ownership. These results highlight the need for awareness of asthma as a potential clinical manifestation of *Toxocara* spp. infection, particularly in children (Kanobana et al. 2013).

MicroRNAs (miRNAs) are a group of endogenous, small, non-coding RNAs with the significant ability to modulate host immune systems, affecting differentiation, development, homeostasis and the function of immune cells (Lodish et al. 2008; Manzano-Roman and Siles-Lucas 2012; Buck et al. 2014). Recently, transcriptomics profiles of *T. canis* miRNAs in adults have been analysed to provide a basis for fundamental investigations of its developmental biology as well as host-parasite interactions (Ma et al. 2016). The findings suggested that miRNA Tc-let-7-5p, Tc-miR-34 and Tc-miR-100 could act as key regulators in host-parasite interactions (Ma et al. 2016). Further research on the characterisation of these regulatory processes might facilitate the understanding of molecular networks at the host-parasite interface and help regulating immune responses against parasites in the future.

16.6 Diagnosis

Humans are considered accidental or dead end hosts of *Toxocara* spp.. Direct diagnosis of *Toxocara* infection through biopsies is not recommended since the larvae continuously migrate and biopsies are usually negative. Searching for eggs in human faeces is not applicable since the larvae do not develop to adult worms in the accidental host. Therefore, diagnosis of toxocariasis is indirect, based on information derived from clinical history and examination, laboratory tests and serodiagnostic assays. The different assays currently used for the diagnosis of human toxocariasis and their limitations are described below.

16.6.1 Clinical Signs and Symptoms

Symptoms of toxocariasis vary depending on the affected organ, the magnitude of infection and the intensity of the host inflammatory response (Pawlowski 2001; Despommier 2003). The broad spectrum of clinical manifestations in toxocariasis (VLM, OLM, NT and CT) varies from asymptomatic to non-specific clinical signs which make it difficult to directly identify clinical cases of toxocariasis. Therefore, patient clinical history regarding risk factors for *Toxocara* spp. infection such as occupation, residence, travel history, contact with soil, pets and consumption of raw vegetables or undercooked meats (Table 16.1) should be gathered as additional information for the diagnosis of toxocariasis.

16.6.2 Medical Imaging Techniques

Medical imaging techniques such as ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI) can be used to detect and localise granulomatous lesions caused by migrating *Toxocara* spp. larvae in tissues and to support a tentative diagnosis of toxocariasis (Dupas et al. 1986; Ishibashi et al. 1992; Baldisserotto et al. 1999; Jabbour et al. 2011). Lesions in the liver appear as small multiple hypoechoic areas with abdominal US (Baldisserotto et al. 1999; Ishibashi et al. 1992) and areas of low density with a CT scan (Dupas et al. 1986). For examination of the CNS, more sensitive MRI can reveal granulomas appearing as hyper-intense areas on T2-weighted images, primarily located cortically or subcortically in brain areas (Ruttinger and Hadidi 1991; Jabbour et al. 2011).

16.6.3 Haematological and Biochemical Assessment

A persistent peripheral blood eosinophilia has been consistently associated with VLM, though not specifically with toxocariasis (Glickman and Schantz 1981). In contrast, OLM patients rarely have eosinophilia due to the low larval burden (Glickman and Schantz 1981). NT patients often show eosinophilia not in the peripheral blood but in the CSF (Jabbour et al. 2011; Finsterer and Auer 2007). In CT patients, blood eosinophilia can be absent in some patients (Taylor et al. 1987). Other laboratory findings include hypergammaglobulinemia and elevated concentrations of total serum IgE (Magnaval et al. 2001). These two findings along with chronic eosinophilia are usually considered as typical laboratory findings of toxocariasis (Magnaval et al. 2001). However, patients with suspected toxocariasis should still be examined using a specific serodiagnostic test for toxocariasis with or without the findings mentioned above.

16.6.4 Serodiagnosis

To confirm suspected toxocariasis, patients should always be examined with serodiagnostic tests using at least two consecutive serum samples taken approximately 2 weeks apart.

16.6.4.1 Antibody Detection

Serological tests based on immunological techniques are recognised as the most effective approach for laboratory diagnosis of toxocariasis (Fillaux and Magnaval 2013). The antigens used in immunoassays include somatic extracts of adult worms, embryonated eggs or intact or sectioned larvae, as well as metabolic products of larvae collected in vitro (Fillaux and Magnaval 2013). Several serodiagnostic kits for toxocariasis are commercially available (Table 16.2). At present, the most commonly used serological tests for confirming toxocariasis are an indirect enzyme linked immunosorbent assay (ELISA) and western blot (WB) based on TES antigens (Smith et al. 2009; Magnaval et al. 2001; Fillaux and Magnaval 2013; de Savigny et al. 1979; Yoshida et al. 2016).

The sensitivity of the TES-based ELISA for the detection of IgG and diagnosis of VLM has been estimated to be 91%, with a specificity of 86% (Jacquier et al. 1991). However, cross-reaction with other parasitic infections occurs particularly in areas where multiple parasites are endemic. False positive results may be observed in patients with ascariasis, anisakidosis, strongyloidiasis, trichinellosis, paragonimiasis and fasciolosis (Gillespie et al. 1993a; Ishida et al. 2003; Romasanta et al. 2003). A WB using TES improves the problem of cross-reactions with other helminth infections because the low-molecular-weight bands (24–32 kDa) are specific for

Table 16.2 Commercially available serodiagnostic kits for the diagnosis of *Toxocara* spp.

Kit	Company/ manufacturer	Antigen	Antibody isotype	Sensitivity (%)	Specificity (%)
ELISA kits					
DRG Toxocara canis IgG (EIA-3518)	DRG Instruments GmbH, USA	TES	IgG	87.5	93.3
EIA Toxocara IgG	TestLine Clinical Diagnostics s.r.o., Czech Republic	TES	IgG	95.5	95.5
Toxocara canis IgG ELISA	Bordier Affinity Products SA, Switzerland	TES	IgG	91	86
RIDASCREEN Toxocara IgG	R-Biopharm AG, Germany	TES	IgG	100	90.7
The NovaLisa™ Toxocara canis IgG ELISA	NovaTec Immundiagnostica GmbH, Germany	Synthetic TES	IgG	>95	>95
Toxocara canis IgG ELISA Kit	IBL International GmbH, Germany	Synthetic TES	IgG	>95	>95
Human anti-Toxocara canis IgG ELISA Kit	Abcam plc, UK	Not stated	IgG	>95	>95
Toxocara IgG CELISA	Cellabs Pty Ltd, Australia	TES	IgG	90	94
AccuDiag™ Toxocara IgG ELISA Kit	Diagnostic Automation/Cortez Diagnostics Inc, USA	TES	IgG	93	88
Toxocara IgG ELISA Kit	Abnova, Taiwan	TES	IgG	Not stated	Not stated
Western blotting kits					
BLOT Toxocara IgG	TestLine Clinical Diagnostics s.r.o., Czech Republic	TES	IgG	95.8	99
Toxocara WB IgG	LDBIO Diagnostics, France	TES	IgG	Not stated	100

Toxocara spp. infection only (Magnaval et al. 1991; Park et al. 2000). However, WB is generally more expensive and labour-intensive than ELISA. Therefore, an effective approach would be first to screen with the indirect TES-based ELISA, followed by confirmation with TES-based WB.

One important aspect to bear in mind during interpretation of serodiagnostic results is that the IgG response elicited after *Toxocara* spp. infection may persist for many years (Cypess et al. 1977), and therefore, a single positive result from an IgG-ELISA does not distinguish between a past and current infection (Roldan and Espinoza 2009). Moreover, the detection of specific *Toxocara*-IgG antibodies by ELISA does not appear to be useful for monitoring therapy due to the high serum

IgG levels after medication (Elefant et al. 2006). Other antibody isotypes, such as IgE, can be more specific but are less sensitive than IgG for the diagnosis of toxocariasis (Elefant et al. 2006; Magnaval et al. 1992). In a follow-up study after chemotherapy, specific serum IgE levels were significantly decreased 1 year after treatment, while specific IgG levels declined 4 years post-treatment (Elefant et al. 2006). Although the specific IgE level is likely to be associated with an active infection, not all patients with elevated total IgE levels have *Toxocara*-IgE antibodies (Magnaval et al. 1992). The detection of specific IgE may therefore be more useful together with the IgG ELISA for the serodiagnosis of toxocariasis. However, *Toxocara*-specific IgE is usually very low and difficult to detect using regular serological assays such as ELISA and WB. More sensitive assays such as radioimmunoassay or fluoroenzyme immunoassay are needed to detect *Toxocara*-specific IgE (Magnaval et al. 1992, 2006). IgM antibodies are not transient in human toxocariasis. Unlike most other infections, IgM levels are present in both the acute and chronic phase of infection and are therefore not useful to distinguish between these two phases (Smith 1993). Measuring the avidity of *Toxocara*-specific IgG antibodies may aid in distinguishing between acute and chronic infections (Dziemian et al. 2008; Rudzinska et al. 2017).

In a study carried out by Rubinsky-Elefant et al., a WB assay based on TES was standardised for monitoring levels of IgG, IgE and IgA after chemotherapy in patients with toxocariasis. Results indicated that bands of >205 kDa for IgG; 29–38, 48–54 and 81–93 kDa for IgA; and 95–121 kDa for IgE were suggested as candidates for monitoring treatment. The authors suggest that further identification of antigen epitopes related to these markers would allow the development of sensitive and specific immunoassays for the diagnosis and therapeutic assessment of toxocariasis (Rubinsky-Elefant et al. 2011).

Among the four human IgG subclasses, detection of IgG2 and IgG3 to TES using ELISA yields a high sensitivity of 98% and 78%, respectively (Watthanakulpanich et al. 2008). The detection of IgG4 specific for TES has a better specificity but a low sensitivity compared to the conventional IgG TES-based ELISA (Noordin et al. 2005).

The cross-reactive antigens in TES limit the use of serodiagnostic methods using TES. Recently, the use of recombinant TES antigens corresponding to the 26 kDa, 30 kDa and 120 kDa proteins in an ELISA has been reported (Mohamad et al. 2009; Yamasaki et al. 2000; Fong and Lau 2004; Santos et al. 2018). The recombinant antigen corresponding to the 26 kDa fraction of TES (rTES-26) in an IgG4 ELISA showed 80% sensitivity and 96% specificity (Mohamad et al. 2009). The rTES-30 appears to be more sensitive (100%) and specific (97.9%) ELISA antigen than the IgG TES-based ELISA (Yamasaki et al. 2000). There were no cross-reactions with sera from ascariasis patients (Yamasaki et al. 2000). Furthermore, there were only minimal cross-reactions with sera from gnathostomiasis, paragonimiasis and spirometriosis patients. In addition to the ELISA technique, Luminex bead-based assay based on rTc-CTL-1 antigen (Loukas et al. 1999), which has 98.6% identity to rTES-30, was developed with high sensitivity (90%) and specificity (99%) (Anderson et al. 2015) and applied to the National Health and Nutrition Examination

Survey in the USA, 2011–2014 (Farmer et al. 2017). rTES-120 was also tested using IgG-ELISA. Fong and Lau reported that rTES-120 reacted with all (8/8) toxocariasis sera tested, but had not reacted with sera from patients with various helminth and protozoan infections (100% sensitivity and 100% specificity) (Fong and Lau 2004). However, the high specificity and sensitivity of the IgG-ELISA when using rTES-30 and rTES-120 may be due to testing a small number of serum samples. Ongoing studies indicate that the use of recombinant TES antigens corresponding to the 26 kDa, 30 kDa and 120 kDa proteins is a valuable tool for improving the sensitivity and specificity of *Toxocara*-specific ELISAs.

Detection of antibodies against *Toxocara* spp. in serum is less sensitive for the diagnosis of OLM compared to VLM, NT and CT, since these patients often have low or undetectable parasite-specific antibodies (Gillespie et al. 1993b). Elevated anti-*Toxocara* antibody titres in intraocular fluids, such as vitreous or aqueous humour, can facilitate the diagnosis of OLM (Benitez del Castillo et al. 1995; de Visser et al. 2008).

16.6.4.2 Antigen Detection

Circulating *Toxocara* spp. antigens in serum have been detected by a sandwich ELISA using monoclonal antibodies (Robertson et al. 1988; Gillespie et al. 1993a). Monoclonal antibodies which recognise species- and genus-specific epitopes of TES can be helpful in the development of more specific assays for the diagnosis of toxocariasis. A monoclonal antibody to the 120 kDa-TES antigen may be useful for determining both the parasite burden in early infection and the efficacy of chemotherapy (Yokoi et al. 2002). Preliminary data indicate that the test was more than 50% sensitive, but there was a false positive rate of 25% in patients with schistosomiasis and filariasis (Gillespie et al. 1993a). Due to low specificity, the test was not recommended as the only test for diagnosis.

16.6.5 Molecular Diagnostic Methods

A definitive diagnosis of human toxocariasis would be possible if larvae could be located in infected tissues by histopathological examination of biopsies. However, due to the continuous migration of larvae through the body, results of biopsy examination are often negative. Furthermore, it remains difficult, or even impossible, to distinguish larvae of different *Toxocara* spp. as well as from larvae of other ascarid nematodes such as that of *Ascaris* spp. based only on their morphology (Nichols 1956). Using molecular approaches, it is possible to distinguish between different helminth species. Each parasite species has unique ribosomal DNA (rDNA) sequences, which can be used as markers to distinguish them from morphologically similar species. The internal transcribed spacer (ITS) regions of rDNA, ITS-1 and ITS-2, have been used as species-specific genetic markers (Hoste et al. 1993;

Campbell et al. 1995; Zhu et al. 2000). Specific amplification of these regions using polymerase chain reaction (PCR) for *Toxocara* spp. identification would provide a useful tool for the diagnosis and molecular epidemiology of toxocariasis (Ishiwata et al. 2004; Rai et al. 1997; Wang et al. 2018). However, extraction of DNA is inconvenient for routine diagnosis of human infection since most patients have a very low worm burden and larvae are often not present in tissue samples. In addition, tissue biopsy is not acceptable for diagnosis from an ethical and technical point of view due to its invasiveness. In an experimental murine model for toxocariasis, *Toxocara* larvae DNA was detected in bronchoalveolar lavage (BAL) of infected animals using NEMO PCR assay (Pinelli et al. 2013). This finding indicated the possibility of using molecular tools and a less invasive method (BAL) for the direct diagnosis of toxocariasis particularly for patients with pulmonary disease. Future studies are needed to evaluate the sensitivity and specificity of these molecular diagnostic methods in human cases.

16.6.6 Diagnosis of T. cati *Infection*

Toxocara cati is also recognised as a causative agent of human toxocariasis. To date, no serological assay allows discrimination between *T. canis* and *T. cati* infections mainly due to the high degree of homology between ES antigens from both species (Kennedy et al. 1987; Poulsen et al. 2015). Although Sakai et al. reported on diagnosis of OLM caused by *T. cati* infection, this is a case report in which only one patient was diagnosed using *T. cati* adult worm somatic antigen ELISA (Sakai et al. 1998). For specific serodiagnosis of *T. cati* infections, additional studies aimed at identifying *T. cati*-specific antigens should be performed. Recently, PCR amplification using species-specific primers allowed the identification and differentiation of *T. cati* and *T. canis* eggs in soil (Borecka and Gawor 2008; Durant et al. 2012; Wang et al. 2018). In addition, very sensitive and specific PCR methods were shown to detect *Toxocara* spp. DNA in liver tissues and BAL of experimentally infected animals (Ishiwata et al. 2004; Pinelli et al. 2013; Rai et al. 1997; Wang et al. 2018). These DNA detection techniques may offer a powerful approach for the identification and discrimination among *Toxocara* spp.

16.7 Need for Standardisation of Diagnostic Tools

16.7.1 Standardisation of Serodiagnosis

Toxocara spp. parasites are unable to complete their life cycle in humans because larval development is arrested at the L3 stage (Magnaval et al. 2001; Smith et al. 2009). Laboratory diagnosis, therefore, depends largely on serodiagnostic techniques. Undoubtedly, the TES-based ELISA has proved to be the most sensitive

and specific serodiagnostic tool for toxocariasis, and it is the assay most extensively used to date. There is, however, a need to standardise this assay in order to compare findings among laboratories throughout the world. This is necessary not only for studies on the epidemiology of toxocariasis but also for clinicians to have consistent interpretations of serodiagnostic results.

16.7.1.1 Standardisation of Antigen for Serodiagnosis

The TES antigen used in the ELISA is made by most laboratories using a modified procedure of de Savigny's original method (de Savigny 1975). TES may be obtained by in vitro culturing of *T. canis* L3 larvae in RPMI 1640 medium supplemented with HEPES and glutamine, as described by Bowman et al. (1987). However, some authors also use the traditional method described by de Savigny, which uses Eagle's minimal essential medium supplemented with HEPES and glutamine (Elefant et al. 2006; Nunes et al. 1997). Culturing larvae in different culture media may result in the presence of different antigenic molecules in TES.

One of the disadvantages of using TES that differ in their molecular composition is that the sensitivity and specificity of the serodiagnostic assay vary between published studies and laboratories worldwide (Glickman et al. 1978; Pollard et al. 1979; Jacquier et al. 1991). In addition to the problem concerning the use of different culture medium, TES may be contaminated with soluble somatic antigens derived from dead or degenerating larvae. Sufficient quality control and quality assurance should be enforced to ensure strict reproducibility among TES batches.

In a study carried out by Speiser and Gottstein, two batches of TES that were prepared independently in two different laboratories were analysed by SDS-PAGE and WB (Speiser and Gottstein 1984). Results from this study revealed at least ten different antigenic components between the two TES preparations. A round-robin testing format was performed and the accordance of serodiagnosis obtained was 80% using 25 sera from patients with suspected toxocariasis. The sera were tested independently with two different ELISAs by two different laboratories, using two different TES batches. The intra- and inter-assay reproducibility was between 85 and 95% for the two ELISA systems using the corresponding TES.

The quality of TES is a key factor for the standardisation of diagnosis; however, matters of quality control and quality assurance for TES have not reached an agreement yet. Since the production of TES depends on culture conditions of the larvae, a recombinant antigen is likely to offer a significant advantage for standardisation. For more than a decade, several studies have reported on the use of recombinant antigens for the serological diagnosis of *Toxocara* spp. infection (Mohamad et al. 2009; Yamasaki et al. 2000; Fong and Lau 2004). Although they are promising, none of the assays based on recombinant antigens has been carried out on a sufficiently large scale to evaluate their potential to replace TES-based ELISA tests. Further work is needed before serodiagnosis with recombinant products will be available for clinical purposes.

16.7.1.2 Evaluation of Commercially Available Kits

In addition to the TES quality, differences in the ELISA conditions, such as the concentration of antigen, the dilution of serum and the definition of cut-off value, might affect the reproducibility of ELISA tests. Commercial diagnostic kits may help the control of TES quality and standardisation of the procedure. Several manufactures of serodiagnostic kits for toxocariasis claim performance levels that are comparable (Table 16.2). However, global evaluation of serodiagnostic methods has not been carried out. The different companies have independently evaluated their assay using their own procedure. In order to validate commercial kits properly, international standardisation of the test procedures and reagents is highly desirable.

16.7.1.3 Representation of Results

In an ELISA, there is a positive association between the intensity of the colour developed and the amount of specific antibody present in the tested sera. A number of qualitative and quantitative ways to represent the results from the ELISA have been used; however, there is no consensus on how to express the results. The different means by which the results are commonly represented are listed below:

- Raw optical density (OD) values: This is the simplest form of data representation, often written as a decimal and multiplied by 1000. Raw OD values are of little diagnostic use without an in-depth knowledge of assay performance, nor are these values useful for intra- or inter-laboratory comparisons.
- End-point titration: The end-point titre is expressed as the reciprocal of the highest serial dilution which shows a minimum of antibody activity. Use of a standard serum allows comparison of data obtained with different ELISA plates on different days. However, it has no diagnostic advantage over single dilution assay, except in cases where more quantitative data are required.
- Signal to noise ratio: Referred to as the positive to negative ratio, by which the OD for the test sample is expressed as a ratio relative to a negative reference standard. This method assumes that the negative sera are truly representative of the normal population.
- Index value: In commercial ELISA kits, the index value is commonly calculated using a formula which is recommended by the manufacturers. Due to varied calculation methods among ELISA kits, the obtained values cannot be compared among the different kits.

16.7.1.4 Cut-off Value Determination

The cut-off for an ELISA is usually calculated using a sufficient number of negative sera from the population. Normally, a cut-off is equal to the mean OD of the negative serum samples plus the standard deviations multiplied by 3. However, the definition

of true negative cases is complicated, and it should be taken into account when calculating the cut-off values. For example, it is difficult to find true negative serum samples in countries with a high prevalence of soil-transmitted helminth infections. Using false-negative samples for the calculation of a cut-off value would considerably undermine the validity of the serodiagnostic test results by ELISA; therefore, internationally standardised negative sera should be used.

16.7.2 Improvement of Alternative Methods for Definitive Diagnosis

The golden standard for diagnosis of human toxocariasis is the detection of the parasite or its components in human tissues. Microscopic examination of biopsies is not recommended since they are often negative due to the continuous migration of the larvae. Improvement of definitive diagnosis could be accomplished using molecular tools. Molecular techniques to detect the parasite, to analyse genetic variation and to evaluate the population genetics of *Toxocara* spp. have been reported (Ishiwata et al. 2004; Rai et al. 1997; Pinelli et al. 2013). Recently, a study using a novel PCR referred to as NEMO-PCR described the detection of *Toxocara* DNA in BAL of *T. canis*-infected mice (Pinelli et al. 2013). The NEMO-PCR has the advantage that, in combination with DNA sequencing, it allows for the detection and identification of *T. canis* and other nematodes in the superfamily Ascaridoidea. PCR methods for *Toxocara* spp. detection and identification in clinical and environmental samples have also been described (Borecka and Gawor 2008; Durant et al. 2012; Fogt-Wyrwas et al. 2007). Although detection of *Toxocara* spp. DNA is a very sensitive approach, it cannot distinguish between an active and past infection since DNA from dead larvae can also be detected. Further studies improving the sensitivity of the NEMO-PCR in addition to its validation using human BAL samples should be performed. In addition to molecular techniques, detection of circulating *Toxocara* spp. antigens can be carried out using a sandwich ELISA (Gillespie et al. 1993a; Robertson et al. 1988; Yokoi et al. 2002). Since most patients have a very low larval burden and larvae can be entrapped and destroyed inside granulomas, the sensitivity of assays detecting *Toxocara* spp. antigen is lower than that in serodiagnosis. However, this test may be a useful tool in confirming the serodiagnosis of human toxocariasis in patients with a high burden, although they are not available as alternative methods of serodiagnosis yet. Further research is needed to standardise and improve the performance of currently available assays.

16.7.3 Problems with Epidemiological Surveys of Human Toxocariasis

Toxocariasis is a public health concern in most countries, and epidemiological surveillance is performed in many areas of the world (Won et al. 2008; Negri et al. 2013; Good et al. 2004; Fan et al. 2005; Farmer et al. 2017). Epidemiological studies on human toxocariasis are based on analysis of data derived from serodiagnosis which has the inherent problems discussed above, producing variation between studies and making comparisons difficult. Standardisation of serodiagnosis will enhance the reliability of epidemiological data, thus improving the determination of risk factors for *Toxocara* spp. infection and appropriate preventive measures against infection.

16.8 Treatment

Treatment of human toxocariasis is a subject of debate due to the self-limiting nature of the disease, the lack of pathognomonic signs and the possible development of allergic responses, particularly in critical sites such as the eye (Othman 2012). However, due to the chronic nature of the disease, treatment is usually recommended especially in the following cases: (1) peripheral blood eosinophilia is prominent, e.g. >1500/μL; (2) inflammation foci in the lungs and/or central nervous system are demonstrated in medical imaging, such as CT, MRI and US; and (3) Active uveitis is present.

Albendazole is the main drug of choice and mebendazole can be used as an alternative (Hossack et al. 2008; Arslan et al. 2019). Generally, albendazole is preferred due to its better tissue distribution than mebendazole and its ability to pass through the blood-brain barrier (Othman 2012). Optimal doses and duration of treatments are largely undefined. Oral administration of albendazole at 10–15 mg/kg of body weight/day in 2–3 divided doses for 5 days is recommended (Pawlowski 2001; Caumes 2003). In neurotoxocariasis, albendazole is used for a period of at least 3 weeks, which often needed to be repeated (Deshayes et al. 2016). According to the review by Kuenzli et al. (2016), in cases with cardiac involvement, various regimens have been employed, such as 800 mg/day for 2 weeks, 50 mg/kg/day for 28 days, 600 mg/day for 14 days or 1000 mg/day for 4 weeks. What appears important is that the standard 5 days regimen of albendazole showed only 32% cure rate (Sturchler et al. 1989).

A study in Japan recommends albendazole for 10~15 mg/kg/day for 4 weeks or even up to 8 weeks (Hombu et al. 2019). They reported an efficacy rate of 78.0% with 15.0% of adverse events, which were well-tolerated. The most frequent adverse events in the long-term treatment were liver dysfunction, which was reversible and well-tolerated in most cases (Hombu et al. 2019). Based on these findings, optimal doses and duration of treatments are 10–15 mg/kg/day (or 800 mg/day) for 4 weeks

or up to 6–8 weeks. Extremely active infections could be successfully treated with this regimen (Kakimoto et al. 2019).

Although ivermectin showed only moderate larvicidal activity in mice (Fok and Kassai 1998) and no significant efficacy was shown in a small-scale clinical study in France (Magnaval 1998), it could be an option when albendazole cannot be used because of the unfavourable conditions, such as drug allergy, pre-existing hepatic dysfunction and adverse events before completing the treatment. It is highly effective against not only gastrointestinal nematodes and filarial worms but scabies and insects as well (Geary 2005; Laing et al. 2017). It also cures cutaneous larva migrans in a single dose (Caumes 2003; Vanhaecke et al. 2014; Del Giudice et al. 2018). Because the synergistic effects of ivermectin have been known on albendazole (Palmeirim et al. 2018; Clarke et al. 2019), the combination of ivermectin and albendazole should be investigated in order to reduce the dose of albendazole.

Treatment may have to be repeated depending on the biological and clinical responses. In a recent longitudinal study of *Toxocara*-seropositive Polish children, treatment with anthelmintic resulted in a decrease in *Toxocara*-specific antibody titters, abdominal pain and enlarged lymph nodes; however, in some cases this was only achieved after three rounds of treatment (Wisniewska-Ligier et al. 2012). Corticosteroids such as prednisolone (1 mg/kg daily for 1 month), given topically or systemically, are also recommended for the treatment of OLM to decrease inflammation and prevent retinal detachment (Othman 2012).

16.9 Prevention and Control of Infection

Toxocara spp. can be infective to a very wide range of accidental and paratenic hosts such as pigs, cattle, sheep, chickens and humans. Human toxocariasis is presumed to be acquired after the accidental ingestion of embryonated eggs or infective larvae. Risk factors for *Toxocara* spp. infection are shown in Table 16.1. Since there are no vaccines available, this zoonotic disease can be prevented by following a series of straight forward measures:

- Control *Toxocara* spp. infection in dogs and cats. Regular anthelmintic treatment, particularly in puppies and kittens, will reduce the number of infectious eggs in the environment (Overgaauw and van Knapen 2013).
- Reduce contact with contaminated soil. When working with soil (through gardening or other activities), it is important to wear gloves (Negri et al. 2013). If gloves are not worn, thoroughly washing your hands is recommended.
- Do not allow children, particularly toddlers, to play in soil contaminated with dog or cat faeces. Geophagia is common among young children and may result in the ingestion of soil containing infectious *Toxocara* spp. eggs. Excluding pet animals from playgrounds and sandpits may be effective to avoid contamination of the environment. Also, placing a vinyl cover over sandpits at night has been shown to reduce egg contamination in sandpits (Fan et al. 2005; Uga and Kataoka 1995).

- Wash vegetables and fruit before eating. Soil that could be contaminated with infectious *Toxocara* spp. eggs should be washed from vegetables and fruits in order to interrupt transmission to humans (Avcioglu et al. 2011; Klapec and Borecka 2012).
- Avoid consumption of undercooked meat. Ingestion not only of infectious *Toxocara* spp. eggs but also of larvae present in paratenic hosts could result in human infections (Nagakura et al. 1989; Salem and Schantz 1992; Yoshikawa et al. 2008).

In order to increase awareness of the potential zoonotic hazards, veterinary practitioners, general practitioners and public health agencies should provide sufficient information and advice for minimising the risk of infection. Continuous education with emphasis on zoonotic risks is strongly recommended.

Toxocara spp. eggs are very resistant to adverse environmental conditions and remain infective for years (Parsons 1987). Since no practical methods exist for reducing environmental egg burdens, prevention of initial contamination of the environment is the most important tool. Periodic anthelmintic treatment of puppies, kittens, nursing bitches and queens is of great value for the control of *Toxocara* spp. infection in dogs and cats. Uniform guidelines for the control and treatment of parasites in dogs and cats were developed and published by CAPC in the USA (CAPC 2012) and ESCCAP in Europe (ESCAAP 2020). However, even strict compliance by dog and cat owners will not reduce the environmental contamination with *T. canis* eggs originating from foxes (Deplazes et al. 2004). Co-ordinated control programmes aimed at minimising infection pressure from zoonotic parasites attributable to the considerable European fox and stray cat populations have so far not been implemented.

Although not currently available, vaccines could eventually prove useful in controlling *Toxocara* spp. infection in dogs and cats and would therefore have a major impact in controlling human toxocariasis. Studies carried out by Abo-Shehada et al. (1991) showed that acquired immunity develops after *T. canis* infection in mice and that vaccination using ultraviolet irradiated embryonated *T. canis* eggs showed the best protection after reinfection. When these authors used TES-antigen, it rendered less protection, and either whole adult worm or L2 somatic vaccines elicited no protection. In another study, immunisation of mice with soluble extracts from embryonated *T. canis* eggs induced 37% resistance to challenge infection when the extract was administered alone and 76% resistance when administered with *Escherichia coli* lipopolysaccharide (LPS) (Barriga 1988). However, when particulate fractions of the embryonated eggs were administered together with complete Freund's adjuvant (CFA), it increased larval number by 60% in comparison with non-immunised mice. These results indicate that insoluble parasite antigens might suppress the host's immunity. Taken together, these findings indicate that candidate vaccine molecules are present in embryonated eggs, larval TES and larval somatic antigens; however, they remained to be identified, purified, separated from molecules with immunosuppressive properties and tested in vaccination trials (Munn 1997). To identify novel vaccine antigens, genomic, transcriptomic, proteomic and

in silico analyses were recently conducted to identify antigens derived from the somatic or ES *Toxocara* proteins as potential vaccine candidates (Salazar Garces et al. 2020; da Silva et al. 2018; Soleyman et al. 2020). In addition to identifying the protective antigen(s), the dose of these antigens and the adjuvant used are important factors to take into consideration for inducing efficient protective immunity and immunological memory.

16.10 Conclusions

Toxocariasis is a neglected zoonosis which affects millions of people around the world. Clinical manifestations in toxocariasis (VLM, OLM, NT and CT) vary, and most patients are asymptomatic. Therefore, the disease may be overlooked, as clinical investigations and/or diagnostic tests are conducted less. There is currently no vaccine available against *Toxocara* parasites. The periodic anthelminthic treatment used in the elimination of adult worms from definitive hosts (e.g. dogs and cats) is recommended to effectively reduce and prevent the environmental contamination with *Toxocara* eggs, which pose a risk of infection to humans as well as paratenic hosts. A collaboration between veterinary and public health professionals within the "One Health" concept will continue to play a significant role in the control of toxocariasis.

References

Abo-Shehada MN, al-Zubaidy BA, Herbert IV (1991) Acquired immunity to *Toxocara canis* infection in mice. Vet Parasitol 38(4):289–298

Anderson A, Fordham LA, Bula ML, Blatt J (2006) Visceral larval migrans masquerading as metastatic disease in a toddler with Wilms tumor. Pediatr Radiol 36(3):265–267. https://doi.org/10.1007/s00247-005-0061-6

Anderson JP, Rascoe LN, Levert K, Chastain HM, Reed MS, Rivera HN, McAuliffe I, Zhan B, Wiegand RE, Hotez PJ, Wilkins PP, Pohl J, Handali S (2015) Development of a luminex bead based assay for diagnosis of toxocariasis using recombinant antigens Tc-CTL-1 and Tc-TES-26. PLoS Negl Trop Dis 9(10):e0004168. https://doi.org/10.1371/journal.pntd.0004168

Arslan F, Baysal NB, Aslan A, Simsek BC, Vahaboglu H (2019) *Toxocara* related peritonitis: a case report and review of literature. Parasitol Int 73:101950. https://doi.org/10.1016/j.parint.2019.101950

Avcioglu H, Soykan E, Tarakci U (2011) Control of helminth contamination of raw vegetables by washing. Vector Borne Zoonotic Dis 11(2):189–191. https://doi.org/10.1089/vbz.2009.0243

Baldisserotto M, Conchin CF, Soares MG, Araujo MA, Kramer B (1999) Ultrasound findings in children with toxocariasis: report on 18 cases. Pediatr Radiol 29(5):316–319

Barriga OO (1988) A critical look at the importance, prevalence and control of toxocariasis and the possibilities of immunological control. Vet Parasitol 29(2–3):195–234

Bartelink AK, Kortbeek LM, Huidekoper HJ, Meulenbelt J, van Knapen F (1993) Acute respiratory failure due to *Toxocara* infection. Lancet 342(8881):1234

Bass JL, Mehta KA, Glickman LT, Eppes BM (1983) Clinically inapparent *Toxocara* infection in children. N Engl J Med 308(12):723–724

Beaver PC, Snyder CH, Carrera GM, Dent JH, Lafferty JW (1952) Chronic eosinophilia due to visceral larva migrans; report of three cases. Pediatrics 9(1):7–19

Benitez del Castillo JM, Herreros G, Guillen JL, Fenoy S, Banares A, Garcia J (1995) Bilateral ocular toxocariasis demonstrated by aqueous humor enzyme-linked immunosorbent assay. Am J Ophthalmol 119(4):514–516

Bisseru B (1969) Studies on the liver, lung, brain and blood of experimental animals infected with *Toxocara canis*. J Helminthol 43(3):267–272

Borecka A, Gawor J (2008) Modification of gDNA extraction from soil for PCR designed for the routine examination of soil samples contaminated with *Toxocara* spp. eggs. J Helminthol 82(2): 119–122. https://doi.org/10.1017/s0022149x07877522

Bowman DD, Mika-Grieve M, Grieve RB (1987) Circulating excretory-secretory antigen levels and specific antibody responses in mice infected with *Toxocara canis*. Am J Trop Med Hyg 36(1):75–82

Buck AH, Coakley G, Simbari F, McSorley HJ, Quintana JF, Le Bihan T, Kumar S, Abreu-Goodger C, Lear M, Harcus Y, Ceroni A, Babayan SA, Blaxter M, Ivens A, Maizels RM (2014) Exosomes secreted by nematode parasites transfer small RNAs to mammalian cells and modulate innate immunity. Nat Commun 5:5488. https://doi.org/10.1038/ncomms6488

Burren CH (1971) The distribution of *Toxocara* larvae in the central nervous system of the mouse. Trans R Soc Trop Med Hyg 65(4):450–453

Campbell AJ, Gasser RB, Chilton NB (1995) Differences in a ribosomal DNA sequence of strongylus species allows identification of single eggs. Int J Parasitol 25(3):359–365. https://doi.org/10.1016/0020-7519(94)00116-6

CAPC (2012) Current advice on parasite control: intestinal parasites–ascarid. http://www.capcvet.org/capc-recommendations/ascarid-roundworm

Caumes E (2003) Treatment of cutaneous larva migrans and *Toxocara* infection. Fundam Clin Pharmacol 17(2):213–216

Clarke NE, Doi SAR, Wangdi K, Chen Y, Clements ACA, Nery SV (2019) Efficacy of anthelminthic drugs and drug combinations against soil-transmitted helminths: a systematic review and network meta-analysis. Clin Infect Dis 68(1):96–105. https://doi.org/10.1093/cid/ciy423

Coati N, Schnieder T, Epe C (2004) Vertical transmission of *Toxocara cati* Schrank 1788 (Anisakidae) in the cat. Parasitol Res 92(2):142–146. https://doi.org/10.1007/s00436-003-1019-y

Congdon P, Lloyd P (2011) *Toxocara* infection in the United States: the relevance of poverty, geography and demography as risk factors, and implications for estimating county prevalence. Int J Public Health 56(1):15–24. https://doi.org/10.1007/s00038-010-0143-6

Cox DM, Holland CV (2001) Relationship between three intensity levels of *Toxocara canis* larvae in the brain and effects on exploration, anxiety, learning and memory in the murine host. J Helminthol 75(1):33–41

Cypess RH, Karol MH, Zidian JL, Glickman LT, Gitlin D (1977) Larva-specific antibodies in patients with visceral larva migrans. J Infect Dis 135(4):633–640

da Silva MB, Urrego AJ, Oviedo Y, Cooper PJ, Pacheco LGC, Pinheiro CS, Ferreira F, Briza P, Alcantara-Neves NM (2018) The somatic proteins of *Toxocara canis* larvae and excretory-secretory products revealed by proteomics. Vet Parasitol 259:25–34. https://doi.org/10.1016/j.vetpar.2018.06.015

de Savigny DH (1975) *In vitro* maintenance of *Toxocara canis* larvae and a simple method for the production of *Toxocara* ES antigen for use in serodiagnosis test for visceral larva migrans. J Parasitol 61:781–782

de Savigny DH, Voller A, Woodruff AW (1979) Toxocariasis: serological diagnosis by enzyme immunoassay. J Clin Pathol 32(3):284–288

de Visser L, Rothova A, de Boer JH, van Loon AM, Kerkhoff FT, Canninga-van Dijk MR, Weersink AY, de Groot-Mijnes JD (2008) Diagnosis of ocular toxocariasis by establishing intraocular antibody production. Am J Ophthalmol 145(2):369–374. https://doi.org/10.1016/j.ajo.2007.09.020

Del Giudice P, Hubiche T, Marie Roger P (2018) Extensive cutaneous larva migrans. Am J Trop Med Hyg 99(2):246. https://doi.org/10.4269/ajtmh.18-0101

Del Prete GF, De Carli M, Mastromauro C, Biagiotta R, Macchia D, Falagiani P, Ricci M, Romagnani S (1991) Purified protein derivative of Mycobacterium tuberculosis and excretory/secretory antigen(s) of *Toxocara canis* expand in vitro human T cells with stable and opposite (type 1 T helper or type 2 T helper) profile of cytokine production. J Clin Invest 88: 346–350

Dent JH, Nichols RL, Beaver PC, Carrera GM, Staggers RJ (1956) Visceral larva migrans; with a case report. Am J Pathol 32(4):777–803

Dent LA, Daly CM, Mayrhofer G, Zimmerman T, Hallett A, Bignold LP, Creaney J, Parsons JC (1999) Interleukin-5 transgenic mice show enhanced resistance to primary infections with *Nippostrongylus brasiliensis* but not primary infections with *Toxocara canis*. Infect Immun 67(2):989–993

Deplazes P, Hegglin D, Gloor S, Romig T (2004) Wilderness in the city: the urbanization of Echinococcus multilocularis. Trends Parasitol 20(2):77–84

Deshayes S, Bonhomme J, de La Blanchardiere A (2016) Neurotoxocariasis: a systematic literature review. Infection 44(5):565–574. https://doi.org/10.1007/s15010-016-0889-8

Despommier D (2003) Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. Clin Microbiol Rev 16(2):265–272

Docu Axelerad A, Stroe AZ, Gogu AE, Pusztai A, Jianu DC, Daniel D, Docu Axelerad D (2021) Clinical spectrum of symptoms in cerebral toxocariasis (review). Exp Ther Med 21(5):521. https://doi.org/10.3892/etm.2021.9953

Dubinsky P, Havasiova-Reiterova K, Petko B, Hovorka I, Tomasovicova O (1995) Role of small mammals in the epidemiology of toxocariasis. Parasitology 110(Pt 2):187–193

Dupas B, Barrier J, Barre P (1986) Detection of *Toxocara* by computed tomography. Br J Radiol 59(701):518–519

Durant JF, Irenge LM, Fogt-Wyrwas R, Dumont C, Doucet JP, Mignon B, Losson B, Gala JL (2012) Duplex quantitative real-time PCR assay for the detection and discrimination of the eggs of *Toxocara canis* and *Toxocara cati* (Nematoda, Ascaridoidea) in soil and fecal samples. Parasit Vectors 5:288. https://doi.org/10.1186/1756-3305-5-288

Dziemian E, Zarnowska H, Kolodziej-Sobocinska M, Machnicka B (2008) Determination of the relative avidity of the specific IgG antibodies in human toxocariasis. Parasite Immunol 30(3): 187–190. https://doi.org/10.1111/j.1365-3024.2007.01010.x

Eberhard ML, Alfano E (1998) Adult *Toxocara cati* infections in U.S. children: report of four cases. Am J Trop Med Hyg 59(3):404–406

Elefant GR, Shimizu SH, Sanchez MC, Jacob CM, Ferreira AW (2006) A serological follow-up of toxocariasis patients after chemotherapy based on the detection of IgG, IgA, and IgE antibodies by enzyme-linked immunosorbent assay. J Clin Lab Anal 20(4):164–172. https://doi.org/10.1002/jcla.20126

Epe C (2006) Current and future options for the prevention and treatment of canids. In: Holland CV, Smith H (eds) Toxocara: the enigmatic parasite. CABI Publishing, Wallingford, UK, pp 239–252

ESCAAP (2020) Worm control in cats and dogs. www.esccap.org. ESCCAP

Fahrion AS, Staebler S, Deplazes P (2008) Patent *Toxocara canis* infections in previously exposed and in helminth-free dogs after infection with low numbers of embryonated eggs. Vet Parasitol 152(1–2):108–115. https://doi.org/10.1016/j.vetpar.2007.11.022

Fan CK, Hung CC, Du WY, Liao CW, Su KE (2004) Seroepidemiology of *Toxocara canis* infection among mountain aboriginal schoolchildren living in contaminated districts in eastern

Taiwan. Tropical Med Int Health 9(12):1312–1318. https://doi.org/10.1111/j.1365-3156.2004.01332.x

Fan CK, Liao CW, Kao TC, Li MH, Du WY, Su KE (2005) Sero-epidemiology of *Toxocara canis* infection among aboriginal schoolchildren in the mountainous areas of north-eastern Taiwan. Ann Trop Med Parasitol 99(6):593–600. https://doi.org/10.1179/136485905x51373

Fan CK, Liao CW, Cheng YC (2013) Factors affecting disease manifestation of toxocarosis in humans: genetics and environment. Vet Parasitol 193(4):342–352. https://doi.org/10.1016/j.vetpar.2012.12.030

Farmer A, Beltran T, Choi YS (2017) Prevalence of *Toxocara* species infection in the U.S.: results from the national health and nutrition examination survey, 2011–2014. PLoS Negl Trop Dis 11(7):e0005818. https://doi.org/10.1371/journal.pntd.0005818

Fattah DI, Maizels RM, McLaren DJ, Spry CJ (1986) *Toxocara canis*: interaction of human blood eosinophils with the infective larvae. Exp Parasitol 61(3):421–431

Feldman GJ, Parker HW (1992) Visceral larva migrans associated with the hypereosinophilic syndrome and the onset of severe asthma. Ann Intern Med 116(10):838–840

Fillaux J, Magnaval JF (2013) Laboratory diagnosis of human toxocariasis. Vet Parasitol 193(4):327–336. https://doi.org/10.1016/j.vetpar.2012.12.028

Finsterer J, Auer H (2007) Neurotoxocarosis. Rev Inst Med Trop Sao Paulo 49(5):279–287

Fisher M (2003) *Toxocara cati*: an underestimated zoonotic agent. Trends Parasitol 19(4):167–170

Fogt-Wyrwas R, Jarosz W, Mizgajska-Wiktor H (2007) Utilizing a polymerase chain reaction method for the detection of *Toxocara canis* and *T. cati* eggs in soil. J Helminthol 81(1):75–78. https://doi.org/10.1017/s0022149x07241872

Fok E, Kassai T (1998) *Toxocara canis* infection in the paratenic host: a study on the chemosusceptibility of the somatic larvae in mice. Vet Parasitol 74(2–4):243–259

Fong MY, Lau YL (2004) Recombinant expression of the larval excretory-secretory antigen TES-120 of *Toxocara canis* in the methylotrophic yeast Pichia pastoris. Parasitol Res 92(2):173–176. https://doi.org/10.1007/s00436-003-1020-5

Fortenberry JD, Kenney RD, Younger J (1991) Visceral larval migrans producing static encephalopathy in an infant. Pediatr Infect Dis J 10(5):403–406

Gavignet B, Piarroux R, Aubin F, Millon L, Humbert P (2008) Cutaneous manifestations of human toxocariasis. J Am Acad Dermatol 59(6):1031–1042. https://doi.org/10.1016/j.jaad.2008.06.031

Geary TG (2005) Ivermectin 20 years on: maturation of a wonder drug. Trends Parasitol 21(11):530–532. https://doi.org/10.1016/j.pt.2005.08.014

Giacomin PR, Cava M, Tumes DJ, Gauld AD, Iddawela DR, McColl SR, Parsons JC, Gordon DL, Dent LA (2008) *Toxocara canis* larval excretory/secretory proteins impair eosinophil-dependent resistance of mice to *Nippostrongylus brasiliensis*. Parasite Immunol 30(8):435–445. https://doi.org/10.1111/j.1365-3024.2008.01040.x

Gillespie SH, Bidwell D, Voller A, Robertson BD, Maizels RM (1993a) Diagnosis of human toxocariasis by antigen capture enzyme linked immunosorbent assay. J Clin Pathol 46(6):551–554

Gillespie SH, Dinning WJ, Voller A, Crowcroft NS (1993b) The spectrum of ocular toxocariasis. Eye (Lond) 7(Pt 3):415–418. https://doi.org/10.1038/eye.1993.82

Glickman LT, Schantz PM (1981) Epidemiology and pathogenesis of zoonotic toxocariasis. Epidemiol Rev 3:230–250

Glickman LT, Shofer FS (1987) Zoonotic visceral and ocular larva migrans. Vet Clin North Am Small Anim Pract 17(1):39–53

Glickman L, Schantz P, Dombroske R, Cypess R (1978) Evaluation of serodiagnostic tests for visceral larva migrans. Am J Trop Med Hyg 27(3):492–498

Glickman LT, Magnaval JF, Domanski LM, Shofer FS, Lauria SS, Gottstein B, Brochier B (1987) Visceral larva migrans in French adults: a new disease syndrome? Am J Epidemiol 125(6):1019–1034

Good B, Holland CV, Stafford P (2001) The influence of inoculum size and time post-infection on the number and position of *Toxocara canis* larvae recovered from the brains of outbred CD1 mice. J Helminthol 75(2):175–181

Good B, Holland CV, Taylor MR, Larragy J, Moriarty P, O'Regan M (2004) Ocular toxocariasis in schoolchildren. Clin Infect Dis 39(2):173–178. https://doi.org/10.1086/421492

Greve JH (1971) Age resistance to *Toxocara canis* in ascarid-free dogs. Am J Vet Res 32(8): 1185–1192

Hamilton CM, Stafford P, Pinelli E, Holland CV (2006) A murine model for cerebral toxocariasis: characterization of host susceptibility and behaviour. Parasitology 132(Pt 6):791–801. https://doi.org/10.1017/s0031182006009887

Hamilton CM, Brandes S, Holland CV, Pinelli E (2008) Cytokine expression in the brains of *Toxocara canis*-infected mice. Parasite Immunol 30(3):181–185. https://doi.org/10.1111/j.1365-3024.2007.01002.x

Hanh NTL, Lee YL, Lin CL, Chou CM, Cheng PC, Quang HH, Fan CK (2020) Evidence for asthma in the lungs of mice inoculated with different doses of *Toxocara canis*. Am J Trop Med Hyg 103(6):2305–2314. https://doi.org/10.4269/ajtmh.20-0484

Hill IR, Denham DA, Scholtz CL (1985) *Toxocara canis* larvae in the brain of a British child. Trans R Soc Trop Med Hyg 79(3):351–354

Holland CV, Hamilton CM (2006) The significance of cerebral toxocariasis. In: Holland CV, Smith H (eds) Toxocara: the enigmatic parasite. CABI Publishing, Wallingford, UK, pp 58–73

Holland CV, Hamilton CM (2013) The significance of cerebral toxocariasis: a model system for exploring the link between brain involvement, behaviour and the immune response. J Exp Biol 216(Pt 1):78–83. https://doi.org/10.1242/jeb.074120

Holland CV, O'Lorcain P, Taylor MR, Kelly A (1995) Sero-epidemiology of toxocariasis in school children. Parasitology 110(Pt 5):535–545

Hombu A, Yoshida A, Kikuchi T, Nagayasu E, Kuroki M, Maruyama H (2019) Treatment of larva migrans syndrome with long-term administration of albendazole. J Microbiol Immunol Infect 52(1):100–105. https://doi.org/10.1016/j.jmii.2017.07.002

Hossack J, Ricketts P, Te HS, Hart J (2008) A case of adult hepatic toxocariasis. Nat Clin Pract Gastroenterol Hepatol 5(6):344–348. https://doi.org/10.1038/ncpgasthep1140

Hoste H, Gasser RB, Chilton NB, Mallet S, Beveridge I (1993) Lack of intraspecific variation in the second internal transcribed spacer (ITS-2) of *Trichostrongylus colubriformis* ribosomal DNA. Int J Parasitol 23(8):1069–1071. https://doi.org/10.1016/0020-7519(93)90128-l

Hotez PJ, Wilkins PP (2009) Toxocariasis: America's most common neglected infection of poverty and a helminthiasis of global importance? PLoS Negl Trop Dis 3(3):e400. https://doi.org/10.1371/journal.pntd.0000400

Humbert P, Niezborala M, Salembier R, Aubin F, Piarroux R, Buchet S, Barale T (2000) Skin manifestations associated with toxocariasis: a case-control study. Dermatology 201(3):230–234. https://doi.org/10.1159/000018493

Iddawela DR, Kumarasiri PV, de Wijesundera MS (2003) A seroepidemiological study of toxocariasis and risk factors for infection in children in Sri Lanka. Southeast Asian J Trop Med Public Health 34(1):7–15

Ishibashi H, Shimamura R, Hirata Y, Kudo J, Onizuka H (1992) Hepatic granuloma in toxocaral infection: role of ultrasonography in hypereosinophilia. J Clin Ultrasound 20(3):204–210

Ishida MM, Rubinsky-Elefant G, Ferreira AW, Hoshino-Shimizu S, Vaz AJ (2003) Helminth antigens (*Taenia solium, Taenia crassiceps, Toxocara canis, Schistosoma mansoni* and *Echinococcus granulosus*) and cross-reactivities in human infections and immunized animals. Acta Trop 89(1):73–84

Ishiwata K, Shinohara A, Yagi K, Horii Y, Tsuchiya K, Nawa Y (2004) Identification of tissue-embedded ascarid larvae by ribosomal DNA sequencing. Parasitol Res 92(1):50–52. https://doi.org/10.1007/s00436-003-1010-7

Ismail MA, Khalafallah O (2005) *Toxocara canis* and chronic urticaria in Egyptian patients. J Egypt Soc Parasitol 35(3):833–840

Jabbour RA, Kanj SS, Sawaya RA, Awar GN, Hourani MH, Atweh SF (2011) *Toxocara canis* myelitis: clinical features, magnetic resonance imaging (MRI) findings, and treatment outcome in 17 patients. Medicine (Baltimore) 90(5):337–343. https://doi.org/10.1097/MD.0b013e31822f63fb

Jacquier P, Gottstein B, Stingelin Y, Eckert J (1991) Immunodiagnosis of toxocarosis in humans: evaluation of a new enzyme-linked immunosorbent assay kit. J Clin Microbiol 29(9): 1831–1835

Jarosz W, Mizgajska-Wiktor H, Kirwan P, Konarski J, Rychlicki W, Wawrzyniak G (2010) Developmental age, physical fitness and *Toxocara* seroprevalence amongst lower-secondary students living in rural areas contaminated with *Toxocara* eggs. Parasitology 137(1):53–63. https://doi.org/10.1017/s0031182009990874

Kakimoto MMM, Mitsumoto-Kaseida F, Ogawa E, Matsumoto Y, Kusaga A, Toyoda K, Hayashi T, Ura K, Kanno K, Furusyo N, Tazuma S (2019) Toxocariasis suspected of having infiltrated directly from the liver to the lung through the diaphragm. Intern Med 58(18): 2737–2741. https://doi.org/10.2169/internalmedicine.2716-19

Kanobana K, Vereecken K, Junco Diaz R, Sariego I, Rojas L, Bonet Gorbea M, Polman K (2013) *Toxocara* seropositivity, atopy and asthma: a study in Cuban schoolchildren. Tropical Med Int Health 18(4):403–406. https://doi.org/10.1111/tmi.12073

Kayes SG (2006) Inflammatory and Immunological Responses to *Toxocara canis*. In: Holland CV, Smith H (eds) Toxocara: the enigmatic parasite. CABI Publishing, Wallingford, UK, pp 158–173

Kayes SG, Omholt PE, Grieve RB (1985) Immune responses of CBA/J mice to graded infections with *Toxocara canis*. Infect Immun 48(3):697–703

Keegan JD, Holland CV (2010) Contamination of the hair of owned dogs with the eggs of *Toxocara* spp. Vet Parasitol 173(1–2):161–164. https://doi.org/10.1016/j.vetpar.2010.06.010

Keegan JD, Holland CV (2013) A comparison of *Toxocara canis* embryonation under controlled conditions in soil and hair. J Helminthol 87(1):78–84. https://doi.org/10.1017/s0022149x12000065

Kennedy MW, Maizels RM, Meghji M, Young L, Qureshi F, Smith HV (1987) Species-specific and common epitopes on the secreted and surface antigens of *Toxocara cati* and *Toxocara canis* infective larvae. Parasite Immunol 9(4):407–420

Kim JH, Chung WB, Chang KY, Ko SY, Park MH, Sa YK, Choi YS, Park CS, Lee MY (2012) Eosinophilic myocarditis associated with visceral larva migrans caused by *Toxocara canis* infection. J Cardiovasc Ultrasound 20(3):150–153. https://doi.org/10.4250/jcu.2012.20.3.150

Klapec T, Borecka A (2012) Contamination of vegetables, fruits and soil with geohelmints eggs on organic farms in Poland. Ann Agric Environ Med 19(3):421–425

Kuenzli E, Neumayr A, Chaney M, Blum J (2016) Toxocariasis-associated cardiac diseases–a systematic review of the literature. Acta Trop 154:107–120. https://doi.org/10.1016/j.actatropica.2015.11.003

Laing R, Gillan V, Devaney E (2017) Ivermectin–old drug, new tricks? Trends Parasitol 33(6): 463–472. https://doi.org/10.1016/j.pt.2017.02.004

Lee AC, Schantz PM, Kazacos KR, Montgomery SP, Bowman DD (2010) Epidemiologic and zoonotic aspects of ascarid infections in dogs and cats. Trends Parasitol 26(4):155–161. https://doi.org/10.1016/j.pt.2010.01.002

Lewis JW (2006) Epidemiological Surveillance of *Toxocara* and *Toxocariasis*. In: Holland CV, Smith H (eds) Toxocara: the enigmatic parasite. CABI Publishing, Wallingford, UK, pp 195–210

Liao CW, Fan CK, Kao TC, Ji DD, Su KE, Lin YH, Cho WL (2008) Brain injury-associated biomarkers of TGF-beta1, S100B, GFAP, NF-L, tTG, AbetaPP, and tau were concomitantly enhanced and the UPS was impaired during acute brain injury caused by *Toxocara canis* in mice. BMC Infect Dis 8:84. https://doi.org/10.1186/1471-2334-8-84

Lightner L, Christensen BM, Beran GW (1978) Epidemiologic findings on canine and feline intestinal nematode infections from records of the Iowa State University veterinary clinic. J Am Vet Med Assoc 172:564–567

Lodish HF, Zhou B, Liu G, Chen CZ (2008) Micromanagement of the immune system by microRNAs. Nat Rev Immunol 8(2):120–130. https://doi.org/10.1038/nri2252

Loukas A, Mullin NP, Tetteh KK, Moens L, Maizels RM (1999) A novel C-type lectin secreted by a tissue-dwelling parasitic nematode. Curr Biol 9(15):825–828. https://doi.org/10.1016/s0960-9822(99)80366-2

Ma G, Luo Y, Zhu H, Luo Y, Korhonen PK, Young ND, Gasser RB, Zhou R (2016) MicroRNAs of *Toxocara canis* and their predicted functional roles. Parasit Vectors 9:229. https://doi.org/10.1186/s13071-016-1508-3

Macuhova K, Akao N, Fujinami Y, Kumagai T, Ohta N (2012) Contamination, distribution and pathogenicity of *Toxocara canis* and *T. cati* eggs from sandpits in Tokyo, Japan. J Helminthol:1–6. https://doi.org/10.1017/s0022149x12000314

Magnaval JF (1998) Apparent weak efficacy of ivermectin for treatment of human toxocariasis. Antimicrob Agents Chemother 42(10):2770. https://doi.org/10.1128/AAC.42.10.2770

Magnaval JF, Fabre R, Maurieres P, Charlet JP, de Larrard B (1991) Application of the western blotting procedure for the immunodiagnosis of human toxocariasis. Parasitol Res 77(8):697–702

Magnaval JF, Fabre R, Maurieres P, Charlet JP, de Larrard B (1992) Evaluation of an immunoenzymatic assay detecting specific anti-*Toxocara* immunoglobulin E for diagnosis and posttreatment follow-up of human toxocariasis. J Clin Microbiol 30(9):2269–2274

Magnaval JF, Michault A, Calon N, Charlet JP (1994) Epidemiology of human toxocariasis in La Reunion. Trans R Soc Trop Med Hyg 88(5):531–533

Magnaval JF, Glickman LT, Dorchies P, Morassin B (2001) Highlights of human toxocariasis. Korean J Parasitol 39(1):1–11

Magnaval JF, Faufingue JH, Morassin B, Fabre R (2006) Eosinophil cationic protein, specific IgE and IgG4 in human toxocariasis. J Helminthol 80(4):417–423

Maizels RM (2013) Toxocara canis: molecular basis of immune recognition and evasion. Vet Parasitol 193(4):365–374. https://doi.org/10.1016/j.vetpar.2012.12.032

Maizels R, Schabussova I, Callister DM, Nicoll G (2006) Molecular biology and immunology of *Toxocara canis*. In: Holland CV, Smith H (eds) Toxocara: the enigmatic parasite. CABI Publishing, Wallingford, UK, pp 3–17

Manzano-Roman R, Siles-Lucas M (2012) MicroRNAs in parasitic diseases: potential for diagnosis and targeting. Mol Biochem Parasitol 186(2):81–86. https://doi.org/10.1016/j.molbiopara.2012.10.001

Mikhael NZ, Montpetit VJ, Orizaga M, Rowsell HC, Richard MT (1974) *Toxocara canis* infestation with encephalitis. Can J Neurol Sci 1(2):114–120

Mizgajska-Wiktor H, Uga S (2006) Exposure and environmental contamination. In: Holland CV, Smith H (eds) Toxocara: the enigmatic parasite. CABI Publishing, Wallingford, UK, pp 211–227

Mohamad S, Azmi NC, Noordin R (2009) Development and evaluation of a sensitive and specific assay for diagnosis of human toxocariasis by use of three recombinant antigens (TES-26, TES-30USM, and TES-120). J Clin Microbiol 47(6):1712–1717. https://doi.org/10.1128/jcm.00001-09

Moore TA, McCarthy JS (2006) Toxocariasis and larva migrans syndromes. In: Guerrant RL, Walker DH, Weller PF (eds) Tropical infectious diseases: principles, pathogens and practice. Elsevier Churchill Livingstone, Philadelphia, PA, pp 1209–1216

Moorhouse DE (1982) Toxocariasis. A possible cause of the Palm Island mystery disease. Med J Aust 1(4):172–173

Morgan ER, Azam D, Pegler K (2013) Quantifying sources of environmental contamination with *Toxocara* spp. eggs. Vet Parasitol 193(4):390–397. https://doi.org/10.1016/j.vetpar.2012.12.034

Munn EA (1997) Rational design of nematode vaccines: hidden antigens. Int J Parasitol 27(4): 359–366

Musso C, Castelo JS, Tsanaclis AM, Pereira FE (2007) Prevalence of *Toxocara*-induced liver granulomas, detected by immunohistochemistry, in a series of autopsies at a Children's Reference Hospital in Vitoria, ES, Brazil. Virchows Arch 450(4):411–417. https://doi.org/10.1007/s00428-007-0388-5

Nagakura K, Tachibana H, Kaneda Y, Kato Y (1989) Toxocariasis possibly caused by ingesting raw chicken. J Infect Dis 160(4):735–736

Negri EC, Santarém V, Rubinsky-Elefant G, Giuffrida R (2013) Anti-*Toxocara* spp. antibodies in an adult healthy population: serosurvey and risk factors in Southeast Brazil. Asian Pac J Trop Biomed 3(3):211–216

Nelson J, Frost JL, Schochet SS Jr (1990) Unsuspected cerebral *Toxocara* infection in a fire victim. Clin Neuropathol 9(2):106–108

Nichols RL (1956) The etiology of visceral larva migrans. I. Diagnostic morphology of infective second-stage *Toxocara* larvae. J Parasitol 42(4 Section 1):349–362

Noh Y, Hong ST, Yun JY, Park HK, Oh JH, Kim YE, Jeon BS (2012) Meningitis by *Toxocara canis* after ingestion of raw ostrich liver. J Korean Med Sci 27(9):1105–1108. https://doi.org/10.3346/jkms.2012.27.9.1105

Noordin R, Smith HV, Mohamad S, Maizels RM, Fong MY (2005) Comparison of IgG-ELISA and IgG4-ELISA for *Toxocara* serodiagnosis. Acta Trop 93(1):57–62. https://doi.org/10.1016/j.actatropica.2004.09.009

Nunes CM, Tundisi RN, Garcia JF, Heinemann MB, Ogassawara S, Richtzenhain LJ (1997) Cross-reactions between *Toxocara canis* and *Ascaris suum* in the diagnosis of visceral larva migrans by western blotting technique. Rev Inst Med Trop Sao Paulo 39(5):253–256

Obwaller A, Jensen-Jarolim E, Auer H, Huber A, Kraft D, Aspock H (1998) *Toxocara* infestations in humans: symptomatic course of toxocarosis correlates significantly with levels of IgE/anti-IgE immune complexes. Parasite Immunol 20(7):311–317

O'Lorcain P (1994) Epidemiology of *Toxocara* spp. in stray dogs and cats in Dublin, Ireland. J Helminthol 68(4):331–336

Ota KV, Dimaras H, Heon E, Babyn PS, Yau YC, Read S, Budning A, Gallie BL, Chan HS (2009) Toxocariasis mimicking liver, lung, and spinal cord metastases from retinoblastoma. Pediatr Infect Dis J 28(3):252–254. https://doi.org/10.1097/INF.0b013e31818a896d

Othman AA (2012) Therapeutic battle against larval toxocariasis: are we still far behind? Acta Trop 124(3):171–178. https://doi.org/10.1016/j.actatropica.2012.08.003

Overgaauw PA (1997a) Aspects of *Toxocara* epidemiology: human toxocarosis. Crit Rev Microbiol 23(3):215–231. https://doi.org/10.3109/10408419709115137

Overgaauw PA (1997b) Aspects of *Toxocara* epidemiology: toxocarosis in dogs and cats. Crit Rev Microbiol 23(3):233–251. https://doi.org/10.3109/10408419709115138

Overgaauw PA, van Knapen F (2013) Veterinary and public health aspects of Toxocara spp. Vet Parasitol 193(4):398–403. https://doi.org/10.1016/j.vetpar.2012.12.035

Palmeirim MS, Hurlimann E, Knopp S, Speich B, Belizario V Jr, Joseph SA, Vaillant M, Olliaro P, Keiser J (2018) Efficacy and safety of co-administered ivermectin plus albendazole for treating soil-transmitted helminths: a systematic review, meta-analysis and individual patient data analysis. PLoS Negl Trop Dis 12(4):e0006458. https://doi.org/10.1371/journal.pntd.0006458

Park SP, Park I, Park HY, Lee SU, Huh S, Magnaval JF (2000) Five cases of ocular toxocariasis confirmed by serology. Korean J Parasitol 38(4):267–273

Parsons JC (1987) Ascarid infections of cats and dogs. Vet Clin North Am Small Anim Pract 17(6): 1307–1339

Parsons JC, Grieve RB (1990) Kinetics of liver trapping of infective larvae in murine toxocariasis. J Parasitol 76(4):529–536

Pawlowski Z (2001) Toxocariasis in humans: clinical expression and treatment dilemma. J Helminthol 75(4):299–305

Piarroux R, Gavignet B, Hierso S, Humbert P (2006) Toxocariasis and the skin. In: Holland CV, Smith H (eds) Toxocara: the enigmatic parasite. CABI Publishing, Wallingford, UK, pp 145–173

Pinelli E, Withagen C, Fonville M, Verlaan A, Dormans J, van Loveren H, Nicoll G, Maizels RM, van der Giessen J (2005) Persistent airway hyper-responsiveness and inflammation in *Toxocara canis*-infected BALB/c mice. Clin Exp Allergy 35(6):826–832. https://doi.org/10.1111/j.1365-2222.2005.02250.x

Pinelli E, Dormans J, Van Die I (2006) *Toxocara* and asthma. In: Holland CV, Smith H (eds) Toxocara: the enigmatic parasite. CABI Publishing, Wallingford, UK, pp 42–57

Pinelli E, Brandes S, Dormans J, Gremmer E, van Loveren H (2008) Infection with the roundworm *Toxocara canis* leads to exacerbation of experimental allergic airway inflammation. Clin Exp Allergy 38(4):649–658. https://doi.org/10.1111/j.1365-2222.2007.02908.x

Pinelli E, Roelfsema JH, Brandes S, Kortbeek T (2013) Detection and identification of *Toxocara canis* DNA in bronchoalveolar lavage of infected mice using a novel real-time PCR. Vet Parasitol 193(4):337–341. https://doi.org/10.1016/j.vetpar.2012.12.029

Pivetti-Pezzi P (2009) Ocular toxocariasis. Int J Med Sci 6(3):129–130

Pollard ZF, Jarrett WH, Hagler WS, Allain DS, Schantz PM (1979) ELISA for diagnosis of ocular toxocariasis. Ophthalmology 86(5):743–752

Poulsen CS, Skov S, Yoshida A, Skallerup P, Maruyama H, Thamsborg SM, Nejsum P (2015) Differential serodiagnostics of *Toxocara canis* and *Toxocara cati*–is it possible? Parasite Immunol 37(4):204–207. https://doi.org/10.1111/pim.12181

Rai SK, Uga S, Wu Z, Takahashi Y, Matsumura T (1997) Use of polymerase chain reaction in the diagnosis of toxocariasis: an experimental study. Southeast Asian J Trop Med Public Health 28(3):541–544

Raistrick ER, Hart JC (1976) Ocular toxocariasis in adults. Br J Ophthalmol 60(5):365–370

Richards DT, Harris S, Lewis JW (1993) Epidemiology of *Toxocara canis* in red foxes (Vulpes vulpes) from urban areas of Bristol. Parasitology 107(Pt 2):167–173

Richartz E, Buchkremer G (2002) Cerebral toxocariasis: a rare cause of cognitive disorders. A contribution to differential dementia diagnosis. Nervenarzt 73(5):458–462

Robertson BD, Burkot TR, Gillespie SH, Kennedy MW, Wambai Z, Maizels RM (1988) Detection of circulating parasite antigen and specific antibody in *Toxocara canis* infections. Clin Exp Immunol 74(2):236–241

Roddie G, Stafford P, Holland C, Wolfe A (2008) Contamination of dog hair with eggs of *Toxocara canis*. Vet Parasitol 152(1–2):85–93. https://doi.org/10.1016/j.vetpar.2007.12.008

Roldan WH, Espinoza YA (2009) Evaluation of an enzyme-linked immunoelectrotransfer blot test for the confirmatory serodiagnosis of human toxocariasis. Mem Inst Oswaldo Cruz 104(3):411–418

Romasanta A, Romero JL, Arias M, Sanchez-Andrade R, Lopez C, Suarez JL, Diaz P, Diez-Banos P, Morrondo P, Paz-Silva A (2003) Diagnosis of parasitic zoonoses by immunoenzymatic assays--analysis of cross-reactivity among the excretory/secretory antigens of *Fasciola hepatica, Toxocara canis,* and *Ascaris suum*. Immunol Investig 32(3):131–142

Rostami A, Riahi SM, Holland CV, Taghipour A, Khalili-Fomeshi M, Fakhri Y, Omrani VF, Hotez PJ, Gasser RB (2019) Seroprevalence estimates for toxocariasis in people worldwide: a systematic review and meta-analysis. PLoS Negl Trop Dis 13(12):e0007809. https://doi.org/10.1371/journal.pntd.0007809

Rubinsky-Elefant G, Hirata CE, Yamamoto JH, Ferreira MU (2010) Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic and ocular forms. Ann Trop Med Parasitol 104(1):3–23. https://doi.org/10.1179/136485910x12607012373957

Rubinsky-Elefant G, Hoshino-Shimizu S, Jacob CM, Sanchez MC, Ferreira AW (2011) Potential immunological markers for diagnosis and therapeutic assessment of toxocariasis. Rev Inst Med Trop Sao Paulo 53(2):61–65

Rudzinska M, Kowalewska B, Sikorska K (2017) Clinical usefulness of Western blotting and ELISA avidity for the diagnosis of human toxocariasis. Parasite Immunol 39(1). https://doi.org/10.1111/pim.12400

Ruttinger P, Hadidi H (1991) MRI in cerebral toxocaral disease. J Neurol Neurosurg Psychiat 54(4): 361–362

Sakai R, Kawashima H, Shibui H, Kamata K, Kambara C, Matsuoka H (1998) *Toxocara cati*-induced ocular toxocariasis. Arch Ophthalmol 116(12):1686–1687

Salazar Garces LF, Santiago LF, Santos SPO, Jaramillo Hernandez DA, da Silva MB, Alves VDS, Silveira EF, Barrouin-Melo SM, Cooper PJ, Pacheco LGC, Pinheiro CDS, Alcantara-Neves NM (2020) Immunogenicity and protection induced by recombinant *Toxocara canis* proteins in a murine model of toxocariasis. Vaccine 38(30):4762–4772. https://doi.org/10.1016/j.vaccine.2020.04.072

Salem G, Schantz P (1992) Toxocaral visceral larva migrans after ingestion of raw lamb liver. Clin Infect Dis 15(4):743–744

Salvador S, Ribeiro R, Winckler MI, Ohlweiler L, Riesgo R (2010) Pediatric neurotoxocariasis with concomitant cerebral, cerebellar, and peripheral nervous system involvement: case report and review of the literature. J Pediatr 86(6):531–534. https://doi.org/10.2223/JPED.2037

Santarem VA, Leli FN, Rubinsky-Elefant G, Giuffrida R (2011) Protective and risk factors for toxocariasis in children from two different social classes of Brazil. Rev Inst Med Trop Sao Paulo 53(2):66–72

Santos LMD, Magalhaes CG, Telmo PL, Cerqueira MP, Donassolo RA, Leite FPL, Elefant GR, Avila L, Scaini CJ, Moreira AN, Conceicao FR (2018) Sensitivity and specificity of recombinant proteins in Toxocara spp. for serodiagnosis in humans: differences in adult and child populations. PLoS One 13(12):e0208991. https://doi.org/10.1371/journal.pone.0208991

Schantz PM (1989) *Toxocara* larva migrans now. Am J Trop Med Hyg 41(3 Suppl):21–34

Scheid R, Tina Jentzsch R, Schroeter ML (2008) Cognitive dysfunction, urinary retention, and a lesion in the thalamus–beware of possible toxocariasis of the central nervous system. Clin Neurol Neurosurg 110(10):1054–1057. https://doi.org/10.1016/j.clineuro.2008.06.014

Schnieder T, Laabs EM, Welz C (2011) Larval development of *Toxocara canis* in dogs. Vet Parasitol 175(3–4):193–206. https://doi.org/10.1016/j.vetpar.2010.10.027

Smith HV (1993) Antibody reactivity in human toxocariasis. In: Lewis JW, Maizels RM (eds) Toxocara and toxocariasis: clinical, epidemiological, and molecular perspectives. Institute of Biology and the British Society for Parasitology, London, UK, pp 91–109

Smith H, Noordin R (2006) Diagnostic limitations and future trends in the serodiagnosis of human toxocariasis. In: Holland CV, Smith H (eds) Toxocara: the enigmatic parasite. CABI Publishing, Wallingford, UK, pp 89–112

Smith H, Holland C, Taylor M, Magnaval JF, Schantz P, Maizels R (2009) How common is human toxocariasis? Towards standardizing our knowledge. Trends Parasitol 25(4):182–188. https://doi.org/10.1016/j.pt.2009.01.006

Soleyman NM, Darnhofer B, Gruenberger RB, Abnous K, Borji H (2020) Proteomic analysis of soluble protein extract of adult To*xocara cati*. Comp Immunol Microbiol Infect Dis 73:101528. https://doi.org/10.1016/j.cimid.2020.101528

Speiser F, Gottstein B (1984) A collaborative study on larval excretory/secretory antigens of *Toxocara canis* for the immunodiagnosis of human toxocariasis with ELISA. Acta Trop 41(4):361–372

Sturchler D, Schubarth P, Gualzata M, Gottstein B, Oettli A (1989) Thiabendazole vs. albendazole in treatment of toxocariasis: a clinical trial. Ann Trop Med Parasitol 83(5):473–478. https://doi.org/10.1080/00034983.1989.11812374

Taira K, Saitoh Y, Kapel CM (2011) *Toxocara cati* larvae persist and retain high infectivity in muscles of experimentally infected chickens. Vet Parasitol 180(3–4):287–291. https://doi.org/10.1016/j.vetpar.2011.03.020

Taylor MR (2001) The epidemiology of ocular toxocariasis. J Helminthol 75(2):109–118

Taylor MR (2006) Ocular toxocariasis. In: Holland CV, Smith H (eds) Toxocara: the enigmatic parasite. CABI Publishing, Wallingford, UK, pp 127–144

Taylor MR, Keane CT, O'Connor P, Girdwood RW, Smith H (1987) Clinical features of covert toxocariasis. Scand J Infect Dis 19(6):693–696. https://doi.org/10.3109/00365548709117206

Taylor MR, Keane CT, O'Connor P, Mulvihill E, Holland C (1988) The expanded spectrum of toxocaral disease. Lancet 1(8587):692–695

Thompson DE, Bundy DA, Cooper ES, Schantz PM (1986) Epidemiological characteristics of *Toxocara canis* zoonotic infection of children in a Caribbean community. Bull World Health Organ 64(2):283–290

Uga S, Kataoka N (1995) Measures to control *Toxocara* egg contamination in sandpits of public parks. Am J Trop Med Hyg 52(1):21–24

Uga S, Hoa NT, Noda S, Moji K, Cong L, Aoki Y, Rai SK, Fujimaki Y (2009) Parasite egg contamination of vegetables from a suburban market in Hanoi, Vietnam. Nepal Med Coll J 11(2):75–78

Vanhaecke C, Perignon A, Monsel G, Regnier S, Bricaire F, Caumes E (2014) The efficacy of single dose ivermectin in the treatment of hookworm related cutaneous larva migrans varies depending on the clinical presentation. J Eur Acad Dermatol Venereol 28(5):655–657. https://doi.org/10.1111/jdv.12097

Verallo O, Fragiotta S, Verboschi F, Vingolo EM (2012) Diagnostic aspects and retinal imaging in ocular toxocariasis: a case report from Italy. Case Rep Med 2012:984512. https://doi.org/10.1155/2012/984512

Walsh MG (2011) *Toxocara* infection and diminished lung function in a nationally representative sample from the United States population. Int J Parasitol 41(2):243–247. https://doi.org/10.1016/j.ijpara.2010.09.006

Walsh MG, Haseeb MA (2012) Reduced cognitive function in children with toxocariasis in a nationally representative sample of the United States. Int J Parasitol 42(13–14):1159–1163. https://doi.org/10.1016/j.ijpara.2012.10.002

Wang Z, Shibata M, Nguyen YTH, Hayata Y, Nonaka N, Maruyama H, Yoshida A (2018) Development of nested multiplex polymerase chain reaction (PCR) assay for the detection of *Toxocara canis, Toxocara cati* and *Ascaris suum* contamination in meat and organ meats. Parasitol Int 67(5):622–626. https://doi.org/10.1016/j.parint.2018.06.006

Warren EG (1969) Infections of *Toxocara canis* in dogs fed infected mouse tissues. Parasitology 59(4):837–841

Watthanakulpanich D, Smith HV, Hobbs G, Whalley AJ, Billington D (2008) Application of *Toxocara canis* excretory-secretory antigens and IgG subclass antibodies (IgG1-4) in serodiagnostic assays of human toxocariasis. Acta Trop 106(2):90–95. https://doi.org/10.1016/j.actatropica.2008.01.008

Webster GA (1958) A report on *Toxocara Canis* Werner, 1782. Can J Comp Med Vet Sci 22(8):272–279

Wells DL (2007) Public understanding of toxocariasis. Public Health 121(3):187–188. https://doi.org/10.1016/j.puhe.2006.10.016

Wilder HC (1950) Nematode endophthalmitis. Trans Am Acad Ophthalmol Otolaryngol 55:99–109

Wisniewska-Ligier M, Wozniakowska-Gesicka T, Sobolewska-Dryjanska J, Markiewicz-Jozwiak-A, Wieczorek M (2012) Analysis of the course and treatment of toxocariasis in children-a long-term observation. Parasitol Res 110(6):2363–2371. https://doi.org/10.1007/s00436-011-2772-y

Wolfe A, Wright IP (2003) Human toxocariasis and direct contact with dogs. Vet Rec 152(14):419–422

Won KY, Kruszon-Moran D, Schantz PM, Jones JL (2008) National seroprevalence and risk factors for Zoonotic *Toxocara* spp. infection. Am J Trop Med Hyg 79(4):552–557

Woodhall D, Starr MC, Montgomery SP, Jones JL, Lum F, Read RW, Moorthy RS (2012) Ocular toxocariasis: epidemiologic, anatomic, and therapeutic variations based on a survey of ophthalmic subspecialists. Ophthalmology 119(6):1211–1217. https://doi.org/10.1016/j.ophtha.2011.12.013

Woodruff AW, de Savigny DH, Hendy-Ibbs PM (1982) Toxocaral and toxoplasmal antibodies in cat breeders and Icelanders exposed to cats but not to dogs. Br Med J 284:309–310

Yamasaki H, Araki K, Lim PK, Zasmy N, Mak JW, Taib R, Aoki T (2000) Development of a highly specific recombinant *Toxocara canis* second-stage larva excretory-secretory antigen for immunodiagnosis of human toxocariasis. J Clin Microbiol 38(4):1409–1413

Yokoi K, Kobayashi F, Sakai J, Usui M, Tsuji M (2002) Sandwich ELISA detection of excretory-secretory antigens of *Toxocara canis* larvae using a specific monoclonal antibody. Southeast Asian J Trop Med Public Health 33(1):33–37

Yoshida A, Hombu A, Wang Z, Maruyama H (2016) Larva migrans syndrome caused by *Toxocara* and *Ascaris* roundworm infections in Japanese patients. Eur J Clin Microbiol Infect Dis 35(9): 1521–1529. https://doi.org/10.1007/s10096-016-2693-x

Yoshikawa M, Nishiofuku M, Moriya K, Ouji Y, Ishizaka S, Kasahara K, Mikasa K, Hirai T, Mizuno Y, Ogawa S, Nakamura T, Maruyama H, Akao N (2008) A familial case of visceral toxocariasis due to consumption of raw bovine liver. Parasitol Int 57(4):525–529. https://doi.org/10.1016/j.parint.2008.08.002

Zarnowska H, Borecka A, Gawor J, Marczynska M, Dobosz S, Basiak W (2008) A serological and epidemiological evaluation of risk factors for toxocariasis in children in central Poland. J Helminthol 82(2):123–127. https://doi.org/10.1017/s0022149x08912372

Zhu X, Gasser RB, Jacobs DE, Hung GC, Chilton NB (2000) Relationships among some ascaridoid nematodes based on ribosomal DNA sequence data. Parasitol Res 86(9):738–744. https://doi.org/10.1007/pl00008561

Chapter 17
Can the Study of Parasitic Helminths Be Fruitful for Human Diseases?

Justyna Rzepecka and William Harnett

Abstract Parasitic helminths are recognised as master modulators of the host immune system. This has led to them being considered as a novel therapy for human diseases associated with unwanted immune responses, in particular, due to their ability to block inflammation, for conditions driven by aberrant inflammatory responses such as allergy and autoimmunity. In this article we discuss, with a particular focus on mouse models, recent data relating to the use of a range of helminths, their extracts, excretory-secretory products, and defined molecules, in ameliorating six of the most commonly studied conditions, asthma, rheumatoid arthritis, multiple sclerosis, type 1 diabetes, inflammatory bowel disease and metabolic syndrome. We describe in detail the range of mechanisms of immunomodulation which the helminths employ, incorporating the array of cell types which may be targeted, a role for new helminth components such as extracellular vesicles, and the increasing awareness of the interplay between helminth immunomodulation and the gut microbiome.

17.1 Introduction

Parasitic helminths, incorporating nematodes, flukes and tapeworms, are large, multicellular organisms that are generally very well adapted to their hosts. One of the consequences of this has been development of a distinct immunological environment that provides a range of benefits to the hosts. This is manifested by an apparent lack of pathology in most humans infected with helminths (Hayes et al. 2004) and, more importantly from the point of view of this article, dampened-down immune responses to third-party antigens such as allergens and autoantigens (Elliott

J. Rzepecka
Concept Life Sciences, Edinburgh, UK

W. Harnett (✉)
Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK
e-mail: w.harnett@strath.ac.uk

F. Bruschi (ed.), *Helminth Infections and their Impact on Global Public Health*,
https://doi.org/10.1007/978-3-031-00303-5_17

and Weinstock 2012). Due to this development, helminths have attracted a substantial attention in the research community as a potential source of novel therapies against diseases associated with aberrant immune/inflammatory responses, e.g. allergies and autoimmune conditions (Harnett and Harnett 2010; Lothstein and Gause 2021). Further support for this idea is provided by epidemiological data in which researchers surveyed human cohorts exposed to helminths to determine if protection against inflammatory disorders was present. Indeed, observational studies of natural helminth infections in patients with multiple sclerosis that spanned over almost 5 years revealed a remarkable beneficial effect of worms on the course of the disease (Correale and Farez 2007; Correale and Farez 2011). In addition, a protective association of prior hookworm infection with Crohn's disease was revealed in studies published by Kabeerdoss et al. (2011). In a similar manner, a negative association between worms and inflammatory diseases was reported by Panda et al. (2013), in that they showed that rheumatoid arthritis patients were free of filarial nematode infection in an area in India where filariasis was endemic. Also, hookworm infection was found to be a protective factor against atopy as patients harbouring worms had lowered reactivity to house dust mite in a skin prick test (Hamid et al. 2013). It should be noted however that other reports rejected the idea that worm presence can indeed limit the burden of different types of inflammatory disease (Bager et al. 2012; van der Werff et al. 2013). In the face of sometimes contradictory data derived from the field studies, researchers thus started focusing on studying the beneficial impact of helminths on inflammatory disorders using well-controlled laboratory models. In consequence, there is an abundance of experimental data investigating the phenomenon, as will be shown in the course of this book chapter.

Infection with helminths does not go unnoticed by the host immune system. Indeed, a strong response is launched shortly after the parasite has entered the host's body and relies largely on helminth sensing by stromal cells such as mucosal epithelial cells and keratinocytes which then through release of alarmins, e.g. IL-25, thymic stromal lymphopoietin (TSLP) and IL-33, drive type 2 immune responses (Wiedemann and Voehringer 2020). In addition, antigen-presenting cells such as dendritic cells (DCs) respond to molecules released by worms and facilitate priming of a specific T helper cell response with the subsequent occurrence of Th2 cells that produce IL-4, IL-5, IL-9 and IL-13 cytokines. These cytokines then increase numbers of eosinophils, basophils, mast cells and alternatively activated macrophages, both in affected tissue and systemically (Allen and Maizels 2011). The immune responses triggered during worm invasion are sufficient to attenuate the infection and potentially lead to worm expulsion (Anthony et al. 2007); however, this is rarely the case, and the parasite very often establishes a chronic infection within the host (Hayes et al. 2004; Grencis 2015). Thus, worms are able to counteract host immune responses, and this helminth-driven immune regulation can be extrapolated to unrelated antigens, as mentioned earlier. In practical terms, deciphering the way parasites modulate immune responses of the host can in theory be used to create novel drugs to combat allergies and autoimmune disorders. This involves model studies on how live infections with parasites limit pathologies;

characterization of parasitic products with anti-inflammatory activities, both in native and recombinant form; and finally design of drugs based on their structure that can be commercialized.

17.2 Worms and Asthma

Numerous helminth species have been reported to attenuate symptoms of experimentally induced allergic airway inflammation in mice (Table 17.1). Eosinophil influx into the lungs, especially eosinophil numbers in the bronchoalveolar lavage, is a useful cellular marker in determining the intensity of allergic inflammation in the laboratory setting. Decreased number of these cells in helminth-treated diseased mice has been therefore often reported in the scientific literature. Thus, for example, infection with *Schistosoma mansoni* was shown to reduce eosinophil numbers in the lungs of mice in which asthma-like symptoms were provoked by a combination of systemic immunization with ovalbumin (OVA) in aluminium hydroxide adjuvant (Alum) and a series of intranasal OVA challenges (Pacifico et al. 2009). In addition, production of Th2 cytokines, IL-4 and IL-5 as well as IgE antibodies was inhibited in mice infected with the parasite. Interestingly, protection against asthma was also achieved when schistosome eggs were injected into the sick mice (Pacifico et al. 2009). In this case, mice that received the eggs showed elevated numbers of CD4+-CD25+Foxp3+ T cells and levels of IL-10. Subsequent neutralization studies, using anti-CD25 and anti-IL-10R antibodies, concluded that regulatory T cells (Tregs) but not IL-10 are responsible for the ability of worm eggs to suppress asthma. This therapeutic effect of *S. mansoni* eggs was confirmed by Obieglo et al. (2018). In an attempt to further dissect the mechanism by which schistosomes can ameliorate asthma, three *S. mansoni* antigens were tested in the OVA-induced airway inflammation model (Cardoso et al. 2010). All three proteins were able to ameliorate the total cell counts and eosinophil numbers in the bronchoalveolar lavage and decrease the levels of IgE antibody. In addition, two proteins—PIII and Sm22.6—lowered levels of IL-4 and IL-5. The frequencies of Tregs, on the other hand, was increased in the groups of mice that received the proteins; however, only Sm22.6 could upregulate IL-10. In conclusion, it could be said in these studies that induction of Tregs might be an important mechanism contributing to the suppression of asthma by helminth products, whereas IL-10 seems to play no or a minor role in this process. It was reported that infection of asthmatic mice with another species of fluke, *S. japonicum* also resulted in suppressed lung eosinophilia, lowered IL-4 and IL-5 levels and reduced concentration of allergen-specific IgE antibodies (Liu et al. 2010; Mo et al. 2008). Mechanistically, the authors showed that the transfer of DCs isolated from *S. japonicum*-infected mice greatly contributed to the protective effect of the parasite on asthma in the studied model (Liu et al. 2010). Similar to *S. mansoni* eggs, injection of egg antigens of *S. japonicum* into asthmatic mice also reversed the disease parameters (Yang et al. 2007). The proposed mechanism of action was studied and showed to be CD4+CD25+ T cell-dependent. Consistent with the ability

Table 17.1 Helminth species and their products displaying beneficial effects on the course of experimental asthma

Helminth species	Infection/antigen/cells	Disease model	Reference
Schistosoma mansoni	Antigens: PIII, Sm22.6, Sm29	OVA-induced airway inflammation	Cardoso et al. (2010)
	Infection	OVA-induced airway inflammation	Pacífico et al. (2009)
	Injection of eggs	OVA-induced airway inflammation	Pacífico et al. (2009), Obieglo et al. (2018)
Schistosoma japonicum	Infection	OVA-induced airway inflammation	Liu et al. (2010), Mo et al. (2008), Qiu et al. (2017)
	Egg antigens	OVA-induced airway inflammation	Yang et al. (2007)
Trichinella spiralis	Infection	OVA-induced airway inflammation	Aranzamendi et al. (2013), Park et al. (2011)
	Extract	OVA-induced airway inflammation	Sun et al. (2019)
Acanthocheilonema viteae	Product: ES-62	OVA-induced airway inflammation	Rzepecka et al. (2013), Melendez et al. (2007)
	Recombinant product rAv-17	OVA-induced airway inflammation	Schnoeller et al. (2008)
		Grass pollen-specific allergic responses	Daniłowicz-Luebert et al. (2013)
Heligmosomoides polygyrus	Excretory-secretory products	OVA-induced airway inflammation	McSorley et al. (2012), McSorley et al. (2014)
	Recombinant product: HpARI	Alternaria allergen administration	Osbourn et al. (2017)
	B cells from helminth-infected mice	OVA-induced airway inflammation	Wilson et al. (2010)
	Infection	OVA-induced airway inflammation	Hartmann et al. (2009), Rzepecka et al. (2007), Kitagaki et al. (2006), Gao et al. (2019)
		HDM sensitization and challenge	Zaiss et al. (2015)

(continued)

Table 17.1 (continued)

Helminth species	Infection/antigen/cells	Disease model	Reference
Anisakis simplex	Recombinant product: macrophage migration inhibitory factor-like protein	OVA-induced airway inflammation	Park et al. (2009)
Ascaris suum	Product: PAS-1	OVA-induced airway inflammation	Araújo et al. (2008)
		APAS-3-induced airway inflammation	Itami et al. (2005)
	Pseudocoelomic fluid	Sensitization with ragweed	McConchie et al. (2006)
	Adult worm extract	OVA-induced airway inflammation	Lima et al. (2002)
Toxascaris leonina	Excretory-secretory products	OVA-induced airway inflammation	Lee et al. (2008)
	Total protein	OVA-induced airway inflammation	Lee et al. (2008)
Litomosoides sigmodontis	Infection	OVA-induced airway inflammation	Dittrich et al. (2008)
Nippostrongylus brasiliensis	Excretory-secretory products	OVA-induced airway inflammation	Trujillo-Vargas et al. (2007)
	Infection	OVA-induced airway inflammation	Wohlleben et al. (2004)
Angiostrongylus cantonensis	Extract	OVA-induced airway inflammation	Pascoal et al. (2020)
Angiostrongylus costaricensis	Extract	OVA-induced airway inflammation	Pinto et al. (2006)
	Infection	OVA-induced airway inflammation	Pinto et al. (2004)
Strongyloides stercoralis	Infection	OVA-induced airway inflammation	Wang et al. (2001)

of *S. japonicum* egg antigens to dampen down allergic immune responses, active *S. japonicum* infection was also demonstrated to provide protection from house dust mite sensitization (Qiu et al. 2017).

Information Box 1: Asthma

- Common chronic inflammatory disorder of the airways
- Patients experience recurrent episodes of airway obstruction and wheezing
- According to the World Health Organization statistics, it is estimated that 300 million people have asthma
- Westernized countries experienced a sharp increase in the prevalence, morbidity and mortality associated with asthma between 1960 and 1970, and since then the disease has plateaued in the high-income countries (WAO White Book on Allergy 2011–2012: Executive Summary)
- Modern asthma management is achieved mostly with anti-inflammatory drugs, such as inhaled corticosteroids
- A considerable fraction of asthmatic patients responds poorly to the mainstay therapies
- T helper type 2 disease, characterized by increased levels of type 2 cytokines, i.e. IL-4, IL-5 and IL-13, presence of allergen-specific IgE antibodies, influx of inflammatory cell such as eosinophils, neutrophils, basophils, monocytes and T cells into the airways

Several species of nematode were also demonstrated to protect mice from experimentally induced asthma. *Heligmosomoides polygyrus* is one of the most intensely studied nematodes in the context of asthma and has been shown to have beneficial effects on the course of disease in many publications. Regarding the mechanisms of action, it was demonstrated that protection is IL-10-dependent and that adoptive transfer of cells from helminth-infected/OVA-exposed mice suppressed OVA-induced eosinophilic inflammation, suggesting a role for regulatory cells (Kitagaki et al. 2006). Elevated numbers of Foxp3 Tregs in helminth-infected mice that were subjected to OVA-induced airway inflammation were also reported in the study published by Hartmann et al. (2009). Tregs are not the only cell population that can play a role in the suppression of allergy by *H. polygyrus*, as shown by Wilson et al. (2010). In this paper, suppression of airway eosinophilia, IL-5 secretion and pathology following allergen challenge was also achieved upon transfer of CD4-CD19+ B cells isolated from lymph nodes of infected mice. Transferred B cells from IL-10 knockout mice could also mediate the therapeutic effect on asthma suggesting the presence of a yet to be identified mechanism that allows this regulatory cell population to suppress lung pathology. It is worth mentioning at this point that even though *H. polygyrus*-mediated suppression of asthma might involve a regulatory B cell (Breg) population that does not require IL-10 to exert its beneficial effect in the course of allergic lung inflammation, Bregs expressing IL-10 have been shown to be induced by infection of mice with

S. mansoni and contribute to the protection against allergic inflammation afforded by the fluke (Amu et al. 2010; van der Vlugt et al. 2012; Haeberlein et al. 2017). Indeed, more recently, Gao et al. (2019), reported induction of IL-10+ Breg, IL-10+ Treg and FoxP3+ Treg populations in mesenteric lymph node and spleen of mice infected with *H. polygyrus* and sensitized with OVA. They demonstrated that the adoptive transfer of IL-10+ Bregs and IL-10+ Tregs prevented the lung immunopathology in the allergic mice.

Continuing on *H. polygyrus*-induced downregulation of asthma in mice, excretory-secretory products of the worm (HES) were tested for their potential to prevent and treat asthma in the OVA/Alum model (McSorley et al. 2012). In both cases reduction in eosinophil numbers was noted in the nematode product-treated mice; however, only application of HES at the sensitization stage decreased pathogenic T-cell responses. HES had previously been shown to induce differentiation of Tregs in vitro, and transfer of these cells into asthmatic mice suppressed allergic airway inflammation (Grainger et al. 2010). In addition, HES was shown to suppress allergic immune responses through interference with the IL-33 pathway (McSorley et al. 2014). Osbourn et al. (2017), subsequently identified *H. polygyrus* Alarmin Release Inhibitor (HpARI), an IL-33-suppressive 26-kDa protein which when administered in vivo abrogated IL-33, group 2 innate lymphoid cell (ILC2) and eosinophilic responses to *Alternaria* allergen administration and diminished eosinophilic responses to *Nippostrongylus brasiliensis*, thereby increasing parasite burden.

As demonstrated above, *H. polygyrus*, either through active infection or release of immunomodulatory molecules, exerts a potent anti-inflammatory effect in experimental allergy models. Moreover, Zaiss et al. (2015), explored an interesting idea relating to whether the afforded protection was helminth-intrinsic or whether it was also due to engagement of the local microbiota. They reported that the parasite altered the intestinal habitat coinciding with increased production of short-chain fatty acids (SCFAs) and that the transfer of parasite-modified microbiota alone led to diminished allergic symptoms.

Similar to HES, excretory-secretory products released by *Trichinella spiralis* were found to induce expansion of CD4+CD25+Foxp3 Tregs in an in vitro assay (Aranzamendi et al. 2012). Expansion of Tregs in vivo during the chronic stage of *T. spiralis* infection is very significant, and transfer of splenic CD4+ T cells from helminth-infected mice could afford protection against experimental allergic airway inflammation (Aranzamendi et al. 2013). Consistent with this, infection of mice with *T. spiralis* protected them from asthma development, and this beneficial effect coincided with increased recruitment of Tregs into the lungs and elevated levels of IL-10 and TGF-β (Park et al. 2011). Furthermore, Sun et al. (2019), demonstrated that soluble products derived from *T. spiralis*, especially from adult worms, were able to ameliorate OVA-induced airway inflammatory responses, an effect associated with reduced eosinophil infiltration, OVA-specific IgE, Th2 cytokine IL-4 and increased IL-10 and TGF-β, and the authors hypothesized that stimulation of the Treg response may contribute to the alleviated allergic inflammation. Worm extracts from the porcine parasite *Ascaris suum* were also shown to suppress accumulation of

eosinophils in the airways and decrease levels of IL-4, IL-5 and eotaxin in a model of lung inflammation (Lima et al. 2002). Subsequent studies revealed that *A. suum* adult worms contain an anti-allergenic protein PAS-1 that could inhibit eosinophilic airway inflammation and hyper-responsiveness induced by a pro-allergenic molecule APAS-3 (also found in the *A. suum* extract) (Itami et al. 2005). The suppressive effects of PAS-1 were also demonstrated in the OVA model of asthma and shown to be dependent on IL-10 and IFN-γ (Araújo et al. 2008). McConchie et al. (2006), worked with a distinct fraction of molecules originating from *A. suum*. They showed that the pseudocoelomic fluid of the parasite can effectively decrease immunological parameters of asthma in mice sensitized with ragweed and that this protection is IL-10-independent. Extracts from another nematode, *Angiostrongylus costaricensis*, were reported to protect mice from asthma (Pinto et al. 2006), in agreement with the finding that infection with the same parasite could also mediate beneficial effects on the course of disease (Pinto et al. 2004).

ES-62 is a native molecule purified from excretory-secretory products of the filarial nematode *Acanthocheilonema viteae*. This tetrameric protein with complex, immunologically active post-translational modifications, in particular attachment of multiple phosphorylcholine (PC) moieties to *N*-type glycans, was shown to inactivate mast cells via a TLR-4-dependent mechanism and to suppress eosinophil recruitment in mice with experimentally induced asthma (Melendez et al. 2007). Subsequent work confirmed the ability of ES-62 to subvert eosinophil influx into the lungs, and in addition it was revealed that the molecule also attenuated infiltration of neutrophils, inflammatory cells that are usually associated with severe, steroid-resistant asthma (Rzepecka et al. 2013). Mechanistically, ES-62 was shown to protect from asthma via IFN-γ-mediated suppression of pathogenic Th2/Th17 responses.

A considerable amount of data has been obtained when applying a recombinant filarial cystatin in two different asthma models. This work showed that the molecule could inhibit eosinophil recruitment, reduce levels of OVA-specific and total IgE and downregulate IL-4 production in the OVA-induced airway inflammation model (Schnoeller et al. 2008). Depletion of macrophages by clodronate-containing liposomes and blocking of IL-10R signalling restored the reduced number of infiltrating cells and the levels of OVA-specific IgE in the cystatin-treated asthmatic mice. Administration of the filarial immunomodulator into mice with grass pollen-induced asthma, suppressed allergen-specific Th2 responses and airway inflammation, inhibited local recruitment of eosinophils, reduced levels of allergen-specific IgE and downregulated IL-5 and IL-13 in the bronchoalveolar lavage (Daniłowicz-Luebert et al. 2013). Interestingly, incubation of human peripheral blood mononuclear cells isolated from timothy grass pollen allergic patients, with cystatin suppressed allergen-specific IL-13 and increased IFN-γ suggesting that this cytokine, as was reported earlier with ES-62, could promote helminth-induced regulation of asthma.

17.3 Worms and Arthritis

Several species of helminth have been shown to be able to attenuate symptoms of arthritis in a mouse model (Table 17.2). In particular, two species of fluke *S. japonicum* and *S. mansoni* significantly reduced the severity and/or the incidence of experimental autoimmune collagen-induced arthritis (CIA) (Song et al. 2011; He et al. 2010; Osada et al. 2009, 2019). Such protection was mostly associated with reduction in production of the pro-inflammatory cytokines TNF-α, IL-6, IL-1β, IFN-γ and IL-17, which appeared with concomitant induction of the Th2 cytokine, IL-4 and the anti-inflammatory cytokine, IL-10. Collectively, it could be proposed that the Th2/regulatory cytokine milieu stimulated by infection with *Schistosome* species counteracts pro-arthritic Th1/Th17 cell activation. DCs represent an important cell type in arthritis due to their ability to sense the immunogens, e.g. collagen, presented to them in the inflammatory context. Such priming induces collagen-specific T helper cell responses and production of collagen-specific autoantibodies, which in turn causes joint swelling and inflammation. There are reports that helminth molecules can subvert these initial processes that lead to pro-arthritic responses in mice. A total extract from *Fasciola hepatica* was shown to induce tolerogenic properties in CpG-ODN-maturated DCs, which when transferred into DBA/J1 mice with CIA diminished the severity and incidence of symptoms (Carranza et al. 2012). The therapeutic effect correlated with significantly lower levels of IL-17 and IFN-γ but enhanced production of TGF-β and IL-10 from draining lymph node cells. The authors showed that the improvement of the disease upon the transfer of helminth extract-stimulated DCs could be due to the action of Tregs and TGF-β. Interestingly, when a synthetic peptide corresponding to 34 amino acids of C-terminal sequence of *Fasciola* helminth defence molecule-1 (C-FhHDM-1) was tested for its impact on CIA, it was found to have joint-specific effects protecting against cartilage destruction. It was reported in particular to maintain bone mass and bone architecture of joints, while suppressing the expression of TNF, IL-17 and IFN-γ in joints but not their serum levels and without impacting on systemic inflammation (Khan et al. 2020).

Information Box 2: Rheumatoid arthritis

- Chronic inflammatory autoimmune disease of the joints
- Characterized by a persistent inflammation of the joint synovium that can lead to long-term joint damage, resulting in chronic pain, loss of function and disability
- Affects 1–2% of the population worldwide
- Conventional treatments are mainly immune suppressants which have a variety of adverse effects and do not inhibit the inflammatory process in a specific manner

(continued)

Table 17.2 Helminth species and their products displaying beneficial effect on the course of experimental arthritis

Helminth species	Infection/antigen	Disease model	Reference
Schistosoma mansoni	Infection	Collagen-induced arthritis	Osada et al. (2009, 2019)
Schistosoma japonicum	Infection	Collagen-induced arthritis	He et al. (2010), Song et al. (2011)
	Recombinant antigen: rSj16	CFA-induced arthritis	Sun et al. (2010)
Hymenolepis diminuta	Infection	CFA-induced arthritis	Shi et al. (2011)
Fasciola hepatica	Extract	Collagen-induced arthritis	Carranza et al. (2012)
	Recombinant product C-FhHDM-1		Khan et al. (2020)
Trichinella spiralis	Infection	Collagen-induced arthritis	Osada et al. (2020)
Heligmosomoides polygyrus	Infection	MRL/lpr model	Salinas-Carmona et al. (2009)
Nippostrongylus brasiliensis	Infection	MRL/lpr model	Salinas-Carmona et al. (2009)
		Serum-induced arthritis	Chen et al. (2016)
Ascaris suum	Extract	Zymosan-induced arthritis; collagen-induced arthritis	Rocha et al. (2008)
Acanthocheilonema viteae	Product: ES-62; structural moiety within ES-62 (PC)	Collagen-induced arthritis	Pineda et al. (2012), McInnes et al. (2003), Harnett et al. (2008), Doonan et al. (2019)

Information Box 2 (continued)

- The disease is characterized by infiltration of the joint tissue with Th1 and Th17 cells that upon local reactivation with autoantigen release cytokines such as IFN-γ and IL-17 and various chemokines that promote accumulation of macrophages and neutrophils

Apart from flukes, a tapeworm species *Hymenolepis diminuta* exerted antiarthritic effects in CFA-injected mice. This required a viable infection and was found to be dependent on adaptive immunity, as infection with *H. diminuta* did not protect mice lacking T cells and B cells and in addition, the IL-4 receptor α chain (Shi et al. 2011).

In addition to platyhelminths that have been shown to reduce arthritis, two nematode species, namely, *H. polygyrus* and *N. brasiliensis*, were reported for their beneficial effects in protecting MRL/lpr mice from spontaneously developing an autoimmune disease affecting joints (Salinas-Carmona et al. 2009). Moreover, *H. polygyrus* and also its excretory-secretory products attenuated inflammatory

arthritis and bone destruction as demonstrated by Sarter et al. (2017). Also, *T. spiralis* infection was shown to protect DBA/1 mice from CIA through a mechanism independent from induction of STAT-6 responses but involving IL-10 (Osada et al. 2020). Triggering of Th2-type immunity and eosinophil responses was shown to contribute to suppression of inflammatory arthritis during *N. brasiliensis* infection as shown by Chen et al. (2016).

The *A. viteae*-derived molecule, ES-62, was found to inhibit priming and polarization of IL-17 responses in CIA by targeting a complex IL-17-producing network, involving signalling between DCs and γ/δ or CD4+ T cells (Pineda et al. 2012). This paper confirms and expands the initial observation of the protective effects of ES-62 in the CIA model that, at that time, was mostly shown to correlate with inhibition of collagen-specific pro-inflammatory/Th1 cytokine (TNF-α, IL-6, and IFN-γ) release (McInnes et al. 2003). In the more recent study, bone marrow-derived DCs from healthy DBA/1 mice and mice with CIA pretreated with ES-62 before being matured with LPS showed significant downregulation of the pro-inflammatory cytokine TNF-α and two other cytokines that are involved in the polarization and maintenance of Th17 cells, IL-6 and IL-23, respectively. Consistent with these findings, ES-62-treated DCs showed a reduced ability to skew naive OVA-specific T cells towards a Th17 phenotype in vitro (Pineda et al. 2012). Interestingly and in addition to the effects of ES-62 on DCs, this molecule could also directly target in vitro-differentiated Th17 cells to produce lower levels of IL-17. The anti-inflammatory actions of ES-62 in CIA appear to be dependent on the PC moiety as indicated by the reduction in severity of disease and also suppression of collagen-specific T helper 1 cytokine production observed when testing PC conjugated to the carrier protein ovalbumin (Harnett et al. 2008).

As alluded to in the section on asthma, helminth-triggered protection from inflammatory conditions can also be mediated by exploiting the host microbiota. Consistent with this, Doonan et al. (2019), showed that subcutaneous injection of ES-62 protected against joint disease in CIA, which was associated with normalization of gut microbiota and prevention of loss of intestinal barrier integrity.

In another set of studies, an extract from *A. suum* given orally protected from arthritis severity in CIA and also zymosan-induced arthritis (ZYA) (Rocha et al. 2008).

17.4 Worms and Multiple Sclerosis

Similar to the studies discussed in the previous paragraphs, different *Schistosome* species and their products have been used intensively to study the impact of helminths on multiple sclerosis (MS) (Table 17.3). In the early studies performed by La Flamme et al. (2003), it was shown that *S. mansoni* significantly reduced the incidence and delayed the onset of experimental autoimmune encephalomyelitis (EAE) in C57BL/6J mice immunized with myelin oligodendrocyte glycoprotein (MOG) (35–55) peptide. Analysis of cytokine production revealed lowered levels

of IFN-γ and TNF-α, as well as nitric oxide, in the helminth-treated groups of mice. In a subsequent study, it was shown that immunization with *S. mansoni* eggs decreased the severity of EAE as measured by decreased clinical scores and CNS cellular infiltrates (Sewell et al. 2003). Disease suppression in this case was associated with decreased IFN-γ and increased IL-4, TGF-β and IL-10 in the periphery and an enhanced percentage of IL-4-producing autoantigen-specific T cells in the brain. Importantly, the authors also showed that the helminth-induced protection from EAE could only be achieved in STAT-6 sufficient mice, which points towards the involvement of the Th2 environment in this process.

Information Box 3: Multiple sclerosis

- Chronic autoimmune, inflammatory, demyelinating neurological disease of the central nervous system that causes severe disability
- More than 2,000,000 people in the world have multiple sclerosis
- Current treatments are only partially effective, and no cure is available
- Immunopathology thought to be mediated by excessive Th1 and Th17 responses

More recently, SEA from *S. japonicum* was shown to prevent EAE while downregulating IFN-γ and/or increasing IL-4 levels (Zheng et al. 2008). Subsequent studies by Correale and Farez (2009), shed light on how SEA modulates phenotype and effector functions of DCs and B cells isolated from patients with MS. Namely, SEA suppressed the LPS-induced DCs' production of pro-inflammatory cytokines and enhanced TGF-β and IL-10 production. In addition, it also diminished LPS-induced expression of co-stimulatory molecules. The effect of SEA was mediated via regulation of TLR2 and ERK1/2 MAP kinase signalling.

Another trematode, *F. hepatica*, attenuated the clinical signs of EAE, and this effect correlated with the suppression of Th1 and Th17 responses (Walsh et al. 2009). The beneficial effect of *F. hepatica* infection was also present when IL-10-deficient mice were infected with the parasite. The effect however was reversed when EAE-suffering mice infected with the helminth were treated with neutralizing anti-TGF-β antibodies, providing strong evidence for the involvement of TGF-β rather than IL-10 in the modulation of the autoimmune disease. Furthermore, total extract from *F. hepatica* also protected against EAE, and this protection has been shown to be associated with a direct effect on T cells, especially IL-17-secreting γδ T cells that play a key pathogenic role in CNS autoimmune disease (Quinn et al. 2019).

Some *cestodes* have also been shown to improve the course of EAE. For example, infection with *T. crassiceps* reduced the severity of EAE with concomitant downregulation of IL-17 and TNF-α and upregulation of IL-4 and IL-10 (Reyes et al. 2011).

The immunomodulatory effect of nematode products on DC function and its importance in ameliorating EAE was studied by Safronic-Milosavljevic et al. (2013). In particular, they showed that DCs stimulated with excretory-secretory products

Table 17.3 Helminth species and their products displaying beneficial effect on the course of experimental multiple sclerosis

Helminth species	Infection/antigen/cells	Disease model	Reference
Schistosoma mansoni	Infection	Experimental autoimmune encephalomyelitis	La Flamme et al. (2003)
	Injection of eggs	Experimental autoimmune encephalomyelitis	Sewell et al. (2003)
Schistosoma japonicum	Egg antigens	Experimental autoimmune encephalomyelitis	Zheng et al. (2008)
Fasciola hepatica	Infection	Experimental autoimmune encephalomyelitis	Walsh et al. (2009)
	Extract	Experimental autoimmune encephalomyelitis	Quinn et al. (2019)
Taenia crassiceps	Infection	Experimental autoimmune encephalomyelitis	Reyes et al. (2011)
Trichinella spiralis	Infection	Experimental autoimmune encephalomyelitis	Gruden-Movsesijan et al. (2008)
	Soluble products	Experimental autoimmune encephalomyelitis	Kuijk et al. (2012)
	Excretory-secretory products	Experimental autoimmune encephalomyelitis	Sofronic-Milosavljenic et al. (2013)
Trichinella pseudospiralis	Infection	Experimental autoimmune encephalomyelitis	Wu et al. (2010)
Strongyloides venezuelensis	Infection	Experimental autoimmune encephalomyelitis	Chiuso-Minicucci et al. (2011)
Heligmosomoides polygyrus	Infection	Experimental autoimmune encephalomyelitis	Donskow-Lysoniewska et al. (2012a, b, 2018)
	B cells from helminth-infected mice	Experimental autoimmune encephalomyelitis	Wilson et al. (2010)

released from encysted muscle larvae of *T. spiralis* (ES L1) and transferred into rats with EAE ameliorated the disease symptoms. Increased production of IL-4, IL-10 and TGF-β and decreased production of IFN-γ and IL-17 were observed. This study is a follow-up on the initial observation performed by the group that infection with *T. spiralis* L1 stage muscle larvae (TSL1) reduced the severity of the autoimmune disease as judged by lower maximal clinical score, cumulative index, duration of illness and degree of mononuclear cell infiltration in *T. spiralis*-infected animals compared to the control, EAE-induced group (Gruden-Movsesijan et al. 2010). A close relative of *T. spiralis*, *T. pseudospiralis*, was also shown to be able to suppress EAE by reducing the inflammatory infiltration in CNS, and this is likely associated with inhibition of Th17 and Th1 responses by the infection (Wu et al. 2010). Interestingly, also in this case, the beneficial effects of the parasite correlated with enhanced Th2 responses in the EAE-suffering mice.

In agreement with the above-described studies is the publication by Kuijk et al. (2012). Similar to the previous observations, treatment of mice with EAE with

soluble products from *T. spiralis* resulted in significant suppression of disease symptoms. The same effects could be achieved when the mice were injected with *Trichuris suis* soluble extract.

H. polygyrus is another nematode species that has been shown to reduce the symptoms of EAE (Donskow-Łysoniewska et al. 2012a, b, 2018). Attenuated disease correlated with an inhibited proliferative response of encephalitogenic T cells, expansion of Tregs and elevated levels of nerve growth factor and TGF-β (Donskow-Łysoniewska et al. 2018). A potential mechanism for the therapeutic effects of *H. polygyrus* in EAE as well as in asthma might involve induction of a functionally distinct, to naïve mice, population of B cells that when transferred into the EAE-suffering mice reduced the disease symptoms in an IL-10-independent manner (Wilson et al. 2010).

17.5 Worms and Type 1 Diabetes

As early as 1999, Anne Cooke and colleagues showed that infection with *S. mansoni* or application of parasite eggs alone significantly decreased the spontaneous incidence of insulin-dependent diabetes mellitus in NOD mice (Table 17.4). Later on, it was shown that soluble extracts of *S. mansoni* worms or eggs completely prevented the onset of type 1 diabetes in these mice (Zaccone et al. 2003). SEA in this model acted to induce functional changes in antigen-presenting cells and expanded Th2 cells and T regulatory cells (Zaccone et al. 2009, 2010). Subsequently, one of the major glycoproteins present in SEA known as ω-1 was shown to condition DCs to drive Th2 responses and induce Foxp3 T cells from NOD mouse naïve T cells (Zaccone et al. 2011). This raises the possibility that a single helminth molecule acting by multiple mechanisms can inhibit onset of diabetes in NOD mice and as such might be a strong candidate for therapeutic modulation of autoimmunity.

Information Box 4: Type 1 diabetes

- Autoimmune disease in which the pancreatic insulin-producing β cells are selectively destroyed by the immune system
- Remains an important health problem, particularly in Western countries, where the incidence has been increasing in younger children
- CD4 Th1 and Th17 T cells have important roles in this process

Another SEA-derived molecule, lacto-N-fucopentaose III (LNFPIII), a Lewis(X)-containing immunomodulatory glycan was subsequently reported to improve glucose tolerance and insulin sensitivity in diet-induced obese mice (Bhargava et al. 2012). More recently two further schistosome molecules, recombinant *S. japonicum*-derived cystatin and fructose-1,6-bisphosphate aldolase, were each shown to reduce diabetes incidence and severity in the NOD mouse and again by switching cytokine

Table 17.4 Helminth species and their products displaying beneficial effect on the course of experimental type 1 diabetes

Helminth species	Infection/antigen/cells	Disease model	Reference
Schistosoma mansoni	Infection	Non-obese diabetic mice	Cooke et al. (1999)
	Soluble products	Non-obese diabetic mice	Zaccone et al. (2003)
	Egg antigens	Non-obese diabetic mice	Zaccone et al. (2009, 2010)
	Antigen: ω-1	Non-obese diabetic mice	Zaccone et al. (2011)
Schistosoma japonicum	Antigen: recombinant fructose-1,6-bisphosphate aldolase and cystatin	Non-obese diabetic mice	Yan et al. (2020)
	Antigen: LNFPIII	Diet-induced obese mice	Bhargava et al. (2012)
Taenia crassiceps	Infection	Multiple low dose streptozotocin-induced diabetes	Espinoza-Jiménez et al. (2010)
Heligmosomoides polygyrus	Infection	Non-obese diabetic mice	Saunders et al. (2007), Liu et al. (2009), Mishra et al. (2013)
		Multiple low dose streptozotocin-induced diabetes	Shimokawa et al. (2020)
Trichinella spiralis	Infection	Non-obese diabetic mice	Saunders et al. (2007)
Litomosoides sigmodontis	Infection	Non-obese diabetic mice	Hübner et al. (2009, 2012)
Strongyloides venezuelensis	Infection	Streptozotocin-induced diabetes	Peres et al. (2013)

production from Th1- to Th2-associated and by increasing levels of Tregs and associated anti-inflammatory cytokines IL-10 and TGF-β (Yan et al. 2020).

In a model of multiple low-dose streptozotocin-induced diabetes, *T. crassiceps*-infected mice demonstrated lower blood glucose levels throughout the study, no insulitis and normal insulin content in the pancreas. In terms of immunological parameters, helminth infection induced greater numbers of alternatively activated macrophages and increased IL-4 levels relative to uninfected mice, with no expansion of Tregs (Espinoza-Jiménez et al. 2010). In the same model of type 1 diabetes, immunization with soluble *Strongyloides venezuelensis* antigen in complete Freund's adjuvant followed by infection with the parasite protected mice from developing disease (Peres et al. 2013).

Interestingly, ES-62, which as discussed earlier prevents disease development in mouse models of rheumatoid arthritis and airway hyperreactivity fails to protect NOD mice from developing diabetes (Doonan et al. 2018). Staying with filarial nematodes, however, infection with live *L. sigmodontis* protected NOD mice against

diabetes development, and the protection correlated with upregulated IL-4, IL-5 and insulin-specific IgG1 antibodies as well as increased numbers of splenic CD4+-CD25+Foxp3+ T cells suggesting a shift towards Th2/Treg type immune responses (Hübner et al. 2009). These findings were further explored using IL-4-deficient NOD mice (Hübner et al. 2012). These mice failed to generate the shift towards Th2 immunity after infection but were also protected from the disease, arguing that the presence of type 2 cytokines and antibodies might be an epiphenomenon and does not play an active role in dampening diabetes in this model. Both strains of NOD mouse, IL-4-deficient and non-manipulated, when infected with the helminth upregulated frequencies and numbers of Tregs, and continuous depletion of TGF-β but not IL-10 prevented the beneficial effect of *L. sigmodontis* in the model. In a similar fashion, neither the in vivo depletion of CD4+CD25+ T cells nor blocking of IL-10 signalling affected *H. polygyrus*-induced protection against type 1 diabetes (Liu et al. 2009). Using the same model system, Mishra et al. (2013), showed that *H. polygyrus* inoculation of NOD and NOD-IL4^{-}/$^{-}$ mice markedly downregulated development of type 1 diabetes, pancreatic β-cell destruction and components of the Th1-type inflammatory immune response. This once again shows that there is no absolute requirement for IL-4 in the helminth-mediated amelioration of diabetes in NOD mice. However, contrary to the previous publication, which dismissed the role of IL-10 in the process, IL-10 blockade in NOD-IL-4-deficient mice inhibited *H. polygyrus*-induced prevention of type 1 diabetes, but not in the NOD-IL-4 sufficient strain (Mishra et al. 2013). This suggests that in the absence of a Th2-type response, IL-10 can still be induced and have potent inhibitory effects on pancreatic β-cell destruction and type 1 diabetes development.

Another cell type targeted by parasitic worms is the CD8+ Treg. In particular, using the streptozotocin C57BL/6 mouse model, it was found that protection against disease development afforded by *H. polygyrus* was dependent on induction of this cell type (Shimokawa et al. 2020). Induction of the CD8+ regulatory T cells was in turn dependent on the production and release of trehalose by *H. polygyrus.* A role for the microbiome was shown by the failure to induce this cell type in mice treated with antibiotics, and it was noted that elevations in CD8+ Treg numbers correlated with increases in the genus *Ruminococcus* and also that administration of trehalose alone was able to induce these. Furthermore, administration of a single *Ruminococcus* species to mice was able to both induce the regulatory cells and suppress increased blood glucose. Interestingly and with respect to clinical significance, type 1 diabetes patients were found to have reduced CD8+ Tregs, bloodstream trehalose and lower ratio of *Ruminococcus* species in faecal microbiota (Shimokawa et al. 2020).

Finally, an interesting recent observation is that as with parasitic worm antigens, an extract of the free-living *Caenorhabditis elegans* can also protect against type 1 diabetes development in the NOD mouse (Jackson-Thompson et al. 2020). Like parasitic worms, the extract induced a Th2 response although evidence of a regulatory response was not apparent. In any case, the use of free-living helminths like *C. elegans* might present a therapeutic opportunity due to easier availability of worm material relative to parasitic organisms.

17.6 Worms and Inflammatory Bowel Disease

Reardon et al. (2001) published one of the first reports describing beneficial effects of helminths on colitis (Table 17.5). In this study, mice were infected with *H. diminuta*, and colitis was provoked by administration of DSS in drinking water. Infected mice had reduced colitis-induced abnormalities in epithelial ion transport, which suggested that helminths indeed might confer protection in the colitic mice. The same parasite was later used prophylactically and therapeutically in mice with DNBS-induced colitis and was shown to protect from the disease in both models as measured by reduced clinical disease, histological damage score and myeloperoxidase levels (Hunter et al. 2005). Mechanistically, it was shown that the protective effect in this model depended on IL-10 and did not predispose to enhanced enteric sensitivity to a third-party antigen, suggested perhaps that there is minimal risk of side effects associated with potential application of helminths to colitic patients. In addition, *H. diminuta* has been shown to be superior to dexamethasone in preventing DNBS-induced colitis and did not result in additional side effects (i.e. collagen deposition) (Melon et al. 2010). Either a high molecular mass fraction of adult *H. diminuta* or excretory-secretory products reduced macrophage production of proinflammatory cytokines such as IL-1β, IL-6 and TNF-α after LPS challenge, and injection of the fraction into the colitic mice caused less inflammatory disease (Johnston et al. 2010).

Information Box 5: Inflammatory bowel disease

- Chronic inflammatory disorder that manifests in an unbalanced immune response towards intestinal microbiota
- The disease manifests by phases of remissions and relapses during which symptoms including abdominal pain, severe diarrhoea, dehydration and weight loss reoccur
- Current therapies help to induce and maintain remission, but not all patients respond to such drug treatments; there is no long-term cure for this disease at present
- Pathogenic immune response is initiated by innate cells such as dendritic cells and macrophages that respond to signals derived from bacterial cells and produce cytokines such as IL-12, IL-23, TNF-α and IL-6 that lead to disruption of the mucosal barrier in the intestine; activation of Th1 and Th17 T cells is a marker of chronic inflammation

Schistosome species have been intensely tested in colitis models. For example, *S. mansoni* egg exposure attenuated TNBS-induced colitis and protected mice from lethal inflammation (Elliott et al. 2003). Protected mice showed reduced production of colonic IFN-γ, but increased levels of IL-4 and IL-10 and the therapeutic effect were dependent on STAT-6 signalling. In a similar manner, injection of TNBS-treated mice with *S. japonicum* eggs reduced the inflammation in the colon and

Table 17.5 Helminth species and their products displaying beneficial effect on the course of experimental inflammatory bowel disease

Helminth species	Infection/antigen/cells	Disease model	Reference
Schistosoma mansoni	Injection of eggs	TNBS colitis	Elliott et al. (2003)
	Infection	DSS-induced colitis	Smith et al. (2007), Bodammer et al. (2011)
	Soluble products	TNBS colitis	Ruyssers et al. (2009, 2010)
Schistosoma japonicum	Injection of eggs	TNBS colitis	Mo et al. (2007), Zhao et al. (2009), Xia et al. (2011)
Hymenolepis diminuta	Infection	DSS-induced colitis	Reardon et al. (2001)
	Infection	DNBS colitis	Hunter et al. (2005), Melon et al. (2010)
	Soluble products	DNBS colitis	Johnston et al. (2010)
Trichinella spiralis	Infection	DSS-induced colitis	Khan et al. (2002)
	Soluble products	DNBS colitis	Motomura et al. (2009)
	Recombinant product: rTsP3	TNBS colitis	Du et al. (2011)
	Excretory-secretory products	TNBS colitis	Jin et al. (2019)
	Extracellular vesicles	TNBS colitis	Yang et al. (2020)
	Recombinant serine protease	TNBS colitis	Pang et al. (2020)
Heligmosomoides polygyrus	Infection	Piroxicam-induced colitis	Elliott et al. (2004)
	CD8+ T cell from infected mice	Piroxicam-induced colitis	Metwali et al. (2006)
	Infection	TNBS colitis	Setiawan et al. (2007), Sutton et al. (2008)
		Colitic IL-10-deficient mice	Elliott et al. (2008)
	Infection	Rag IL-10−/− transfer model of colitis	Hang et al. (2010), Blum et al. (2012)
	Infection	Pan-enterocolitis triggered by feeding with ovalbumin	Leung et al. (2012)
	Infection	DSS-induced colitis	Donskow-Łysoniewska et al. (2012a, b)

(continued)

Table 17.5 (continued)

Helminth species	Infection/antigen/cells	Disease model	Reference
Acanthocheilonema viteae	Recombinant product: rAv-17	DSS-induced colitis	Schnoeller et al. (2008)
Ancylostoma caninum	Soluble products	TNBS colitis	Ruyssers et al. (2009)
	Excretory-secretory products	DSS-induced colitis	Ferreira et al. (2013)
Ancylostoma ceylanicum	Soluble products	DSS-induced colitis	Cançado et al. (2011)
	Excretory-secretory products	DSS-induced colitis	Cançado et al. (2011)
Anisakis simplex	Recombinant product: macrophage migration inhibitory factor-like protein	DSS-induced colitis	Cho et al. (2011)

suppressed IFN-γ levels, while IL-4, IL-5 and IL-10 cytokines were increased (Mo et al. 2007). In this report, the percentage of Tregs was shown to increase in the colitis-protected mice; however, their involvement in the parasite-induced protection from colitis was not assessed. Zhao et al. (2009), reported similar findings indicating that *S. japonicum* eggs could prevent TNBS colitis. In addition to decreased IFN-γ and increased IL-4 and IL-10, helminth egg-treated mice showed decreased expression of TLR4 and reduced intestinal bacterial translocation. These data are in tune with a later publication that showed that *S. japonicum* eggs maintained epithelial barrier function through increasing tight junction proteins, thus causing less exposure of NOD2 (an intracellular pattern recognition receptor, which recognizes a peptidoglycan constituent of bacteria) to the luminal antigens which may activate a series of inflammatory factors and induce colitis (Xia et al. 2011). Active infection with *S. mansoni* has also been shown to render DSS-exposed mice refractory to colitis via a novel mechanism dependent on macrophages, rather than by simple modulation of Th2 responses, or via induction of regulatory CD4+ or CD25+ cells, IL-10 or TGF-β (Smith et al. 2007). Soluble proteins from *S. mansoni* can also reverse intestinal inflammation as shown in mice with TNBS-induced colitis. This positive effect driven by helminth proteins correlated with decreased pro-inflammatory cytokine production (IFN-γ and IL-17) and increased anti-inflammatory cytokines (IL-10 and TGF-β) (Ruyssers et al. 2009). In addition, there was evidence that *S. mansoni* proteins also ameliorated gut motility disturbances during murine colitis (Ruyssers et al. 2010).

Prior infection with *T. spiralis* also reduced the severity of colitis together with decreasing mortality in mice and was correlated with a downregulation of myeloperoxidase activity, Th1-type cytokine expression in colonic tissue and emergence of a Th2-type immune response (Khan et al. 2002). In a subsequent study, *T. spiralis* antigens were assessed for their ability to modify intestinal inflammation in mice and were shown to reduce the severity of the disease (Motomura et al. 2009). One of the *T. spiralis* proteins in a recombinant form known as rTsP53 was shown to

ameliorate TNBS-induced colitis in mice (Du et al. 2011). Another recombinant *T. spiralis* molecule, adult serine protease-like protein rTs-ADSp-7, was additionally recently found to be protective in this model and by a mechanism incorporating downregulation of Th1/Th17-inducing cytokines and upregulating Th2 and Treg-related cytokines (Pang et al. 2020). A similar effect was attributed to a secreted protease inhibitor of filarial nematodes that modulated macrophage-mediated inflammation in a murine model of DSS-induced colitis (Schnoeller et al. 2008). Also, a recombinant protein type 2 MIF (As-MIF) from *Anisakis simplex* third stage larvae was found to ameliorate DSS-induced colitis (Cho et al. 2011). Interestingly, ES-62 is not protective in the DSS model of colitis (Doonan et al. 2018) although another phosphorylcholine (ES-62's anti-inflammatory moiety)-containing molecule, tuftsin-phosphorylcholine, shows efficacy (Ben-Ami Shor et al. 2019).

Therapeutic potential of adult hookworm, *Ancylostoma ceylanicum*, and also crude and excretory-secretory products was shown in DSS colitis (Cançado et al. 2011). Similar to previous observations, treatment with the helminth decreased production of Th1 and Th17 cytokines in the inflamed colon. This protective effect of hookworms was confirmed in a subsequent publication that showed a beneficial role of excretory-secretory products of *A. caninum* (Ferreira et al. 2013). Interestingly, diminishing protein activity within this antigenic mixture resulted in loss of anti-colitic effect and reversed helminth product-induced upregulation of a CD4+IL-4+IL-10+ cell population. Using the same model, it has also been shown that the protective effects of excretory-secretory products of *T. spiralis* are dependent on upregulation of the negative signal-delivering PD-1 on M2 macrophages (Wang et al. 2020).

H. polygyrus has been widely used in the studies investigating the potential of helminths to influence experimental colitis. A paper by Elliott et al. (2004), showed that this nematode inhibited ongoing piroxicam-induced colitis in IL-10-deficient mice in part through blocking mucosal Th1 cytokine production and that resolution of inflammation was associated with increased IL-13 production and could be adoptively transferred by MLN T cells. In addition to dampening down the Th1 arm of immunity, colonization of colitic IL-10-deficient mice with *H. polygyrus* suppressed lamina propria mononuclear cell-derived production of IL-17, another pathogenic cytokine in this model (Elliott et al. 2008).

In a similar model of colitis, it was shown that *H. polygyrus* could reverse piroxicam-induced gut inflammation in Rag KO mice (T- and B-cell-deficient) reconstituted with IL-10-deficient T cells (Metwali et al. 2006). It appears that in this model, *H. polygyrus* induces regulatory CD8+ lamina propria T cells that are potent suppressors of T-cell proliferation. Interestingly, these regulatory cells were shown to act independently of IL-10 and TGF-β signalling; however, their alternative mechanism of action has not been addressed so far. Also, in the Rag IL-$10^{-/-}$ T-cell transfer model of colitis, *H. polygyrus* prevented and reversed intestinal inflammation with concomitant downregulation of IFN-γ and IL-17 responses (Hang et al. 2010; Leung et al. 2012). Here, the worm infection changed the phenotype of lamina propria DCs from Rag mice such that the cells displayed lower expression levels of CD80 and CD86, heightened levels of plasmocytoid

DC marker Ag-1 and CD40 and impaired ability to present antigen to antigen-specific T cells. This impact of *H. polygyrus* on DC functions was further investigated in a paper published by Blum et al. (2012). These authors showed that intestinal DCs isolated from *H. polygyrus*-infected Rag KO mice blocked antigen-specific production of IFN-γ/IL-17 from lamina propria mononuclear cells in vitro. More importantly, transfer of the worm-primed DCs into Rag mice reconstituted with IL-10-deficient T cells protected animals from colitis.

H. polygyrus also blocked colitis in TNBS-treated mice by decreasing Th1 and increasing Th2 cytokines (Setiawan et al. 2007). Blocking of IL-10 signalling in vitro restored Th1 cytokine secretion from lamina propria mononuclear cells, whereas in vivo intervention worsened colitis in *H. polygyrus*-infected mice. Staying with the TNBS model, DCs pulsed with *T. spiralis* muscle larva excretory-secretory products were also shown to transfer protection against disease (Jin et al. 2019).

More recently, there has been interest in whether parasitic worm-derived extracellular vesicles (EV)—structures currently of much interest in biology and biomedicine—might offer protection against inflammatory bowel disease. Thus, for example, this question was addressed with respect to EV derived from *T. spiralis* muscle larvae in the TNBS mouse model of colitis, and a significant amelioration of disease was observed (Yang et al. 2020). This protective effect was associated with improved intestinal epithelial barrier integrity, decreased gut neutrophil infiltration and a reduction in pro-inflammatory cytokine production but increased immunoregulatory cytokines. In addition, a polarization from the T-cell response away from Th1/Th17 towards Th2/Treg was observed. Mechanistically, the potential importance of EV microRNAs was highlighted (Yang et al. 2020).

17.7 Worms and Metabolic Syndrome

There has been developing interest in the idea that parasitic worms might protect against the increasing global problem of metabolic syndrome and the associated obesity, type 2 diabetes and cardiovascular disease (Table 17.6). Certainly, that parasitic worms might safeguard against the latter has been suspected for many years, and indeed a decrease in blood vessel wall plaque size was observed following infection of mice with *S. mansoni*, almost 20 years ago (Doenhoff et al. 2002). Such a protective effect was subsequently also shown with a soluble extract of schistosome eggs (Stanley et al. 2009) and most recently at the individual helminth molecule level, when ES-62 was shown to reduce by ~60%, aortic plaques in Gld. $ApoE^{-/-}$ mice, a model for the accelerated atherosclerosis that may occur in human lupus patients (Aprahamian et al. 2015).

Turning to type 2 diabetes, there have been an increasing number of studies in recent years showing that parasitic worms or their products can improve insulin dependence and glucose homeostasis in mice, and this is often associated with Th2 polarization in metabolic organs (Berbudi et al. 2016). Emerging data from human epidemiological studies also supports such a protective role for parasitic worms

Table 17.6 Helminth species and their products displaying beneficial effect on the course of experimental metabolic syndrome and developing comorbidities

Helminth species	Infection/antigen/cells	Disease model	Reference
Schistosoma mansoni	Infection	Atherosclerosis in ApoE$^{-/-}$ mice	Doenhoff et al. (2002)
	Injection of eggs	Atherosclerosis in ApoE$^{-/-}$ mice	Stanley et al. (2009)
	Infection; soluble egg antigen	Diet-induced obese mice	Hussaarts et al. (2015)
	Antigen: ω-1	Diet-induced obese mice	van der Zande et al. (2021)
Acanthocheilonema viteae	Product: ES-62	Atherosclerosis in Gld. ApoE$^{-/-}$ mice	Aprahamian et al. (2015)
		Diet-induced aged obese mice	Crowe et al. (2020)
	ES-62 small molecule analogues	Diet-induced obese mice	Lumb et al. (2019)
Litomosoides sigmodontis	Infection; soluble antigen	Diet-induced obese mice	Berbudi et al. (2016)
Heligmosomoides polygyrus	Infection	Diet-induced obese mice	Su et al. (2020)
Strongyloides venezuelensis	Infection	Diet-induced obese mice	Pace et al. (2018)

(Rajamanickam et al. 2019). Interestingly, a key feature of helminth mechanism of action appears to be their universal ability to drive polarization of macrophages to an M2 state, as the associated metabolic reprogramming of these cells results in improvements in whole body metabolism (Hussaarts et al. 2015). Furthermore, recent work employing *H. polygyrus* infection of mice fed a high fat diet (Su et al. 2020), showed that adoptive transfer of M2 macrophages generated in helminth-infected mice was sufficient to prevent obesity and that this was associated with changes in the gut microbiota of the recipient mice and at the same time increases in SCFAs like acetate and propionate, molecules which are known to be able to play a role in glucose and lipid homeostasis. STAT6$^{-/-}$ mice were employed to show a Th2 dependence for the observed changes in the microbiome and SCFAs, and microbiome transfer was sufficient to protect against obesity. Essentially the same findings—protection against obesity associated with Th2/Treg/M2 macrophage polarization and changes in the microbiome—were observed with *T. spiralis*, and of interest, the protective effects persisted after removal of the parasites showing the potential for a long-term therapeutic effect (Kang et al. 2021).

Nevertheless, not all protective effects of parasitic worms on metabolic homeostasis may be dependent on inducing a Th2 response. For example, drug-like small molecule analogues of ES-62 promote metabolic homeostasis in a high-calorie diet mouse model, but this could not be correlated with polarization of the immune system in a Th2/anti-inflammatory direction (Lumb et al. 2019). In addition, the schistosome protein ω-1 both improves glucose homeostasis and induces Th2

responses in white adipose tissue in obese mice, but the protective effects on metabolic homeostasis are still observed in STAT6$^{-/-}$ mice (van der Zande et al. 2021). The absence of a need for STAT6 and in addition IL-10 was also observed with respect to *S. mansoni*-induced protection in the mouse streptozotocin-induced diabetes model (Osada et al. 2017), although this is usually considered more of a type 1 diabetes model.

Information Box 6: Metabolic syndrome

- Metabolic syndrome refers to several of a group of conditions including abdominal obesity, high blood sugar, high serum triglycerides and hypertension
- Metabolic syndrome increases the risk of developing type 2 diabetes and cardiovascular disease
- Risk factors include ageing, diet and low physical activity
- Increased inflammatory markers such as C-reactive protein and pro-inflammatory cytokines may be detected in the bloodstream
- Metabolic syndrome is increasing at an alarming rate, e.g. more than one third of all adults in the USA suffer from it.

Finally, weekly administration of ES-62 throughout life is able to increase both healthspan (the period of life before diseases associated with ageing arise) and lifespan of mice fed a high-calorie diet (Crowe et al. 2020). Interestingly, however, whereas the protective effect on healthspan is noted in both sexes, increased longevity is restricted to male animals. Mechanistic analysis suggests that this differential effect may be more dependent on improved gut health and microbiome normalization than on ES-62's well-known anti-inflammatory effects. Related to this there is a pattern emerging of helminths and helminth products being beneficial for gut health, e.g. *S. venezuelensis* increases the expression of tight junction proteins in intestinal cells thereby maintaining barrier permeability and decreasing LPS leakage to the bloodstream in mice (Pace et al. 2018), and ES-62 administration to mice alters the microbiome to increase SCFA-producing species even in the absence of inflammation (Doonan et al. 2019).

17.8 Conclusions

As can be seen in this updated book chapter, there continues to be an increasing abundance of experimental data confirming the potential of parasitic helminths to treat asthma, rheumatoid arthritis, multiple sclerosis, type 1 diabetes, inflammatory bowel disease and, now, the comorbidities of metabolic syndrome. Interestingly, some species of helminths or their products can be effective against more than one disease. ES-62 is effective for example against both asthma and rheumatoid arthritis,

two types of inflammatory disorders whose pathologies are shaped by different arms of immunity (Fig. 17.1). This implies that there might be one mechanism, with regulatory B cells being a candidate that can bring the right balance to the deregulated immune responses in different types of inflammatory disorders. Alternatively, worms or their molecules can exert multiple mechanisms simultaneously or depending on the disease context that ultimately protects from a range of inflammatory disorders. Increasingly we are understanding and appreciating the myriad of immunomodulatory mechanisms, which parasitic worms employ, but nevertheless, further work is needed in this area. However, based on the literature review as presented in this book chapter, the following patterns of helminth-induced immunoregulation emerge with respect to the different disease conditions:

Asthma: helminths improve asthma-like disease in mice by decreasing the Th2 type of immune response via employment of regulatory T- and B-cell populations; in addition some helminth molecules such as PAS-1 from *A. suum*, ES-62 from *A. viteae* and filarial cystatin can counteract allergic immune responses by increasing the IFN-γ axis and thus resetting the Th1/Th2 balance.

Arthritis: improvement of arthritis by helminths is mostly associated with a decreased ability of DCs to prime for pathogenic Th1/Th17 responses.

Multiple sclerosis: therapeutic potential of helminths in the murine model of multiple sclerosis correlates with decreased Th1/Th17 and upregulated Th2/Tregs axis.

Type 1 diabetes: symptoms of type 1 diabetes and appearance of pathogenic Th1 cells are reduced by helminths via induction of Tregs.

IBD: helminths protect mice from experimentally induced IBD by decreasing the potential of DCs to prime Th1/Th17 responses and by inducing Tregs.

Metabolic syndrome: helminths drive Th2/Treg/M2 macrophage polarization but not in all cases.

In addition, helminths seem to alter the composition of gut microbiota and shield the immune system from being exposed to bacterial products by improving mucosal barrier functions. Perhaps somewhat surprisingly, this can have implications for diseases other than those associated with the gut, e.g. rheumatoid arthritis (Doonan et al. 2019).

Considerable progress has thus been made in elucidating the beneficial effects of different helminth species and helminth product(s) on the course of inflammatory disorders. In the near future, we should find out if helminth-based therapies have found their way into the clinic. The need for new solutions to treat inflammatory diseases is so great that some UK patients with Crohn's disease are sourcing helminths in an attempt to relief the disease symptoms that could not be treated by commercially available drugs (Flowers and Hopkins 2013). Certainly, initial trials conducted by Summers et al. (2005a, b), showed that ingestion of live eggs from *Trichuris suis* reduced symptoms of Crohn's disease and ulcerative colitis in the studied group of patients, and it was shown that such treatment is well tolerated and did not result in short- or long-term treatment-related side effects (Sandborn et al. 2013). However, further more recent clinical trials have questioned the efficacy of *T. suis* for treatment of Crohn's disease (Scholmerich et al. 2017) and also a number

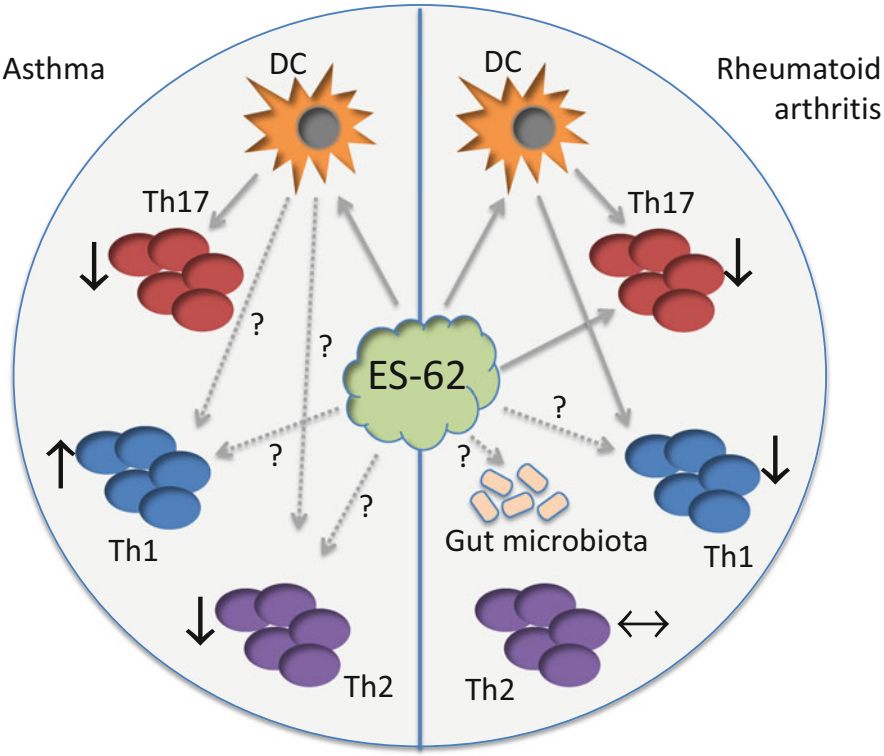

Fig. 17.1 ES-62-mediated immune regulation that confers protection of mice from experimentally induced asthma and rheumatoid arthritis. ES-62 alters the potential of dendritic cells (DCs) to prime naïve CD4+ T cells to differentiate into Th17 cells that are pathogenic in both disease models (IL-17 released by Th17 cells is associated with increased numbers of neutrophils in afflicted tissue; application of ES-62 therefore leads to decreased numbers of these cells in the asthmatic lungs and arthritic joints). ES-62 can also act directly on Th17 cells via TLR4 to attenuate IL-17 release in vitro. Interestingly, ES-62 has opposing effects on Th1 cells depending on the immunological context—Th1 cell are pathogenic in RA and in this model ES-62 down-regulates IFNγ-producing cells; in asthma, on the other hand, Th1 cells might counterbalance pathogenic Th2 responses and ES-62 in this model leads to enhanced numbers of IFNγ-positive cells. Neutralization of IFNγ in the asthma model reversed the protection afforded by ES-62 and increased frequencies of Th2 and Th17 cells. Th2 cells are not increased in the RA model upon injection with ES-62, whereas, as suggested earlier, ES-62 reduces numbers of Th2 cells in the model of asthma. Protection against joint disease in CIA afforded by ES-62 was shown to be associated with normalisation of gut microbiota and prevention of loss of intestinal barrier integrity

of other inflammatory conditions (reviewed by Elliott and Weinstock 2017). For example, with respect to the latter, several clinical phase 1 and phase 2 trials have been conducted evaluating effects of helminths in patients suffering from MS. Studies conducted in such patients using oral inoculation of *T. suis* eggs and intradermal application of *Necator americanus* larvae demonstrated that despite both treatments being well tolerated, they failed to show strong therapeutic efficacy

(Yordanova et al. 2021). Hence it is possible that a more profitable therapeutic route when considering helminths for the treatment of inflammatory conditions may be to focus on defined individual helminth products or drugs derived from them.

References

Allen JE, Maizels RM (2011) Diversity and dialogue in immunity to helminths. Nat Rev Immunol 11:375–388

Amu S, Saunders SP, Kronenberg M, Mangan NE, Atzberger A, Fallon PG (2010) Regulatory B cells prevent and reverse allergic airway inflammation via FoxP3-positive T regulatory cells in a murine model. J Allergy Clin Immunol 125:1114–1124

Anthony RM, Rutitzky LI, Urban JF Jr, Stadecker MJ, Gause WC (2007) Protective immune mechanisms in helminth infection. Nat Rev Immunol 7:975–987

Aprahamian TR, Zhong X, Amir S, Binder CJ, Chiang LK, Al-Rayami L et al (2015) The immunomodulatory parasitic worm product ES-62 reduces lupus-associated atherosclerosis in a mouse model. Int J Parasitol 45:203–207

Aranzamendi C, Fransen F, Langelaar M, Franssen F, van der Ley P, van Putten JP et al (2012) Trichinella spiralis-secreted products modulate DC functionality and expand regulatory T cells in vitro. Parasite Immunol 34:210–223

Aranzamendi C, de Bruin A, Kuiper R, Boog CJ, van Eden W, Rutten V et al (2013) Protection against allergic airway inflammation during the chronic and acute phases of Trichinella spiralis infection. Clin Exp Allergy 43:103–115

Araújo CA, Perini A, Martins MA, Macedo MS, Macedo-Soares MF (2008) PAS-1, a protein from Ascaris suum, modulates allergic inflammation via IL-10 and IFN-gamma, but not IL-12. Cytokine 44:335–341

Bager P, Vinkel Hansen A, Wohlfahrt J, Melbye M (2012) Helminth infection does not reduce risk for chronic inflammatory disease in a population-based cohort study. Gastroenterology 142:55–62

Ben-Ami Shor D, Lachnish J, Bashi T, Dahan S, Shemer A, Segal Y et al (2019) Immunomodulation of murine chronic DSS-induced colitis by tuftsin-phosphorylcholine. J Clin Med 9:65

Berbudi A, Surendar J, Ajendra J, Gondorf F, Schmidt H, Neumann A-L, APF WA et al (2016) J Innate Immun 8:601–616

Bhargava P, Li C, Stanya KJ, Jacobi D, Dai L, Liu S et al (2012) Immunomodulatory glycan LNFPIII alleviates hepatosteatosis and insulin resistance through direct and indirect control of metabolic pathways. Nat Med 18:1665–1672

Blum AM, Hang L, Setiawan T, Urban JP Jr, Stoyanoff KM, Leung J et al (2012) Heligmosomoides polygyrus bakeri induces tolerogenic dendritic cells that block colitis and prevent antigen-specific gut T cell responses. J Immunol 189:2512–2520

Bodammer P, Waitz G, Loebermann M, Holtfreter MC, Maletzki C, Krueger MR et al (2011) Schistosoma mansoni infection but not egg antigen promotes recovery from colitis in outbred NMRI mice. Dig Dis Sci 56:70–78

Cançado GG, Fiuza JA, de Paiva NC, Lemos Lde C, Ricci ND, Gazzinelli-Guimarães PH et al (2011) Hookworm products ameliorate dextran sodium sulfate-induced colitis in BALB/c mice. Inflamm Bowel Dis 17:2275–2286

Cardoso LS, Oliveira SC, Góes AM, Oliveira RR, Pacífico LG, Marinho FV et al (2010) Schistosoma mansoni antigens modulate the allergic response in a murine model of ovalbumin-induced airway inflammation. Clin Exp Immunol 160:266–274

Carranza F, Falcón CR, Nuñez N, Knubel C, Correa SG, Bianco I et al (2012) Helminth antigens enable CpG-activated dendritic cells to inhibit the symptoms of collagen-induced arthritis through Foxp3+ regulatory T cells. PLoS One 7:e40356

Chen Z, Andreev D, Oeser K et al (2016) Th2 and eosinophil responses suppress inflammatory arthritis. Nat Commun 7:11596

Chiuso-Minicucci F, Van DB, Zorzella-Pezavento SF, Peres RS, Ishikawa LL, Rosa LC et al (2011) Experimental autoimmune encephalomyelitis evolution was not modified by multiple infections with Strongyloides venezuelensis. Parasite Immunol 33(5):303–308

Cho MK, Lee CH, Yu HS (2011) Amelioration of intestinal colitis by macrophage migration inhibitory factor isolated from intestinal parasites through toll-like receptor 2. Parasite Immunol 33:265–275

Cooke A, Tonks P, Jones FM, O'Shea H, Hutchings P, Fulford AJ et al (1999) Infection with Schistosoma mansoni prevents insulin dependent diabetes mellitus in non-obese diabetic mice. Parasite Immunol 21:169–176

Correale J, Farez M (2007) Association between parasite infection and immune responses in multiple sclerosis. Ann Neurol 61:97–108

Correale J, Farez M (2009) Helminth antigens modulate immune responses in cells from multiple sclerosis patients through TLR2-dependent mechanisms. J Immunol 183:5999–6012

Correale J, Farez MF (2011) The impact of parasite infections on the course of multiple sclerosis. J Neuroimmunol 233:6–11

Crowe J, Lumb FE, Doonan J, Broussard M, Tarafdar A, Pineda MA, Landabaso C et al (2020) The parasitic worm product ES-62 promotes health- and life-span in a high-calorie diet-accelerated mouse model of ageing. PLoS Pathog 16:e1008391

Daniłowicz-Luebert E, Steinfelder S, Kühl AA, Drozdenko G, Lucius R, Worm M et al (2013) A nematode immunomodulator suppresses grass pollen-specific allergic responses by controlling excessive Th2 inflammation. Int J Parasitol 43:201–210

Dittrich AM, Erbacher A, Specht S, Diesner F, Krokowski M, Avagyan A et al (2008) Helminth infection with Litomosoides sigmodontis induces regulatory T cells and inhibits allergic sensitization, airway inflammation, and hyperreactivity in a murine asthma model. J Immunol 1(180): 1792–1799

Doenhoff MJ, Stanley RG, Griffiths K, Jackson CL (2002) An anti-atherogenic effect of *Schistosoma mansoni* infections in mice associated with a parasite-induced lowering of total blood cholesterol. Parasitology 125:415–421

Donskow-Łysoniewska K, Krawczak K, Doligalska M (2012a) Heligmosomoides polygyrus: EAE remission is correlated with different systemic cytokine profiles provoked by L4 and adult nematodes. Exp Parasitol 132:243–248

Donskow-Łysoniewska K, Majewski P, Brodaczewska K, Jóźwicka K, Doligalska M (2012b) Heligmosomoides polygyrus fourth stages induce protection against DSS-induced colitis and change opioid expression in the intestine. Parasite Immunol 34:536–546

Donskow-Łysoniewska K, Krawczak K, Bocian K, Doligalska M (2018) The effects of intestinal nematode L4 stage on mouse experimental autoimmune encephalomyelitis. Arch Immunol Ther Exp 66:231–243

Doonan J, Thomas D, Wong MH, Ramage HJ, Al-riyami L, Lum FE et al (2018) Failure of the anti-inflammatory parasitic worm product ES-62 to provide protection in mouse models of type 1 diabetes, multiple sclerosis, and inflammatory bowel disease. Molecules 23:2669

Doonan J, Tarafdar A, Pineda MA, Lumb FE, Crowe J, Khan AM et al (2019) The parasitic worm product ES-62 normalises the gut microbiota bone marrow axis in inflammatory arthritis. Nat Commun 10:1554

Du L, Tang H, Ma Z, Xu J, Gao W, Chen J et al (2011) The protective effect of the recombinant 53-kDa protein of Trichinella spiralis on experimental colitis in mice. Dig Dis Sci 56:2810–2817

Elliott DE, Weinstock JV (2012) Helminth-host immunological interactions: prevention and control of immune-mediated diseases. Ann N Y Acad Sci 1247:83–96

Elliott DE, Weinstock JV (2017) Nematodes and human therapeutic trials for inflammatory diseases. Parasite Immunol 39:14027

Elliott DE, Li J, Blum A, Metwali A, Qadir K, Urban JF Jr et al (2003) Exposure to schistosome eggs protects mice from TNBS-induced colitis. Am J Physiol Gastrointest Liver Physiol 284: G385–G391

Elliott DE, Setiawan T, Metwali A, Blum A, Urban JF Jr, Weinstock JV (2004) Heligmosomoides polygyrus inhibits established colitis in IL-10-deficient mice. Eur J Immunol 34:2690–2698

Elliott DE, Metwali A, Leung J, Setiawan T, Blum AM, Ince MN et al (2008) Colonization with Heligmosomoides polygyrus suppresses mucosal IL-17 production. J Immunol 181:2414–2419

Espinoza-Jiménez A, Rivera-Montoya I, Cárdenas-Arreola R, Morán L, Terrazas LI (2010) Taenia crassiceps infection attenuates multiple low-dose streptozotocin-induced diabetes. J Biomed Biotechnol 2010:850541

Ferreira I, Smyth D, Gaze S, Aziz A, Giacomin P, Ruyssers N et al (2013) Hookworm excretory/secretory products induce interleukin-4 (IL-4)+ IL-10+ CD4+ T cell responses and suppress pathology in a mouse model of colitis. Infect Immun 81:2104–2111

Flowers S, Hopkins M (2013) Autoimmune disease: patients self-treat with parasitic worms. Nature 493:163

Gao X, Ren X, Wang Q, Yang Z, Li Y, Su Z, Li J (2019) Critical roles of regulatory B and T cells in helminth parasite-induced protection against allergic airway inflammation. Clin Exp Immunol 198:390–402

Grainger JR, Smith KA, Hewitson JP, McSorley HJ, Harcus Y, Filbey KJ et al (2010) Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-β pathway. J Exp Med 207:2331–2341

Grencis RK (2015) Immunity to helminths: resistance, regulation, and susceptibility to gastrointestinal nematodes. Annu Rev Immunol 33:201–225

Gruden-Movsesijan A, Ilic N, Mostarica-Stokjovic M, Stosic-Grujicic S, Milic M, Sofronic-Miosaljevic LJ (2008) Trichinella spiralis: modulation of experimental autoimmune encephalomyelitis in DA rats. Exp Parasitol 118:641–647

Gruden-Movsesijan A, Ilic N, Mostarica-Stojkovic M, Stosic-Grujicic S, Milic M, Sofronic-Milosavljevic L (2010) Mechanisms of modulation of experimental autoimmune encephalomyelitis by chronic Trichinella spiralis infection in Dark Agouti rats. Parasite Immunol 32:450–459

Haeberlein S, Obieglo K, Ozir-Fazalalikhan A, Chayé MAM, Veninga H, van der Vlugt LEPM, Voskamp A, Boon L, den Haan JMM, Westerhof LB, Wilbers RHP, Schots A, Schramm G, Hokke CH, Smits HH (2017) Schistosome egg antigens, including the glycoprotein IPSE/alpha-1, trigger the development of regulatory B cells. PLoS Pathog 28:13

Hamid F, Wiria AE, Wammes LJ, Kaisar MM, Djuardi Y, Versteeg SA, Wahyuni S, van Ree R, Sartono E, Supali T, Yazdanbakhsh M (2013) Risk factors associated with the development of atopic sensitization in Indonesia. PLoS One 8:e67064

Hang L, Setiawan T, Blum AM, Urban J, Stoyanoff K, Arihiro S et al (2010) Heligmosomoides polygyrus infection can inhibit colitis through direct interaction with innate immunity. J Immunol 185:3184–3189

Harnett W, Harnett MM (2010) Helminth-derived immunomodulators: can understanding the worm produce the pill? Nat Rev Immunol 10:278–284

Harnett MM, Kean DE, Boitelle A, McGuiness S, Thalhamer T, Steiger CN et al (2008) The phosphorycholine moiety of the filarial nematode immunomodulator ES-62 is responsible for its anti-inflammatory action in arthritis. Ann Rheum Dis 67:518–523

Hartmann S, Schnoeller C, Dahten A, Avagyan A, Rausch S, Lendner M et al (2009) Gastrointestinal nematode infection interferes with experimental allergic airway inflammation but not atopic dermatitis. Clin Exp Allergy 39:1585–1596

Hayes KS, Bancroft AJ, Grencis RK (2004) Immune-mediated regulation of chronic intestinal nematode infection. Immunol Rev 201:75–88

He Y, Li J, Zhuang W, Yin L, Chen C, Li J et al (2010) The inhibitory effect against collagen-induced arthritis by Schistosoma japonicum infection is infection stage-dependent. BMC Immunol 11:28

Hübner MP, Stocker JT, Mitre E (2009) Inhibition of type 1 diabetes in filaria-infected non-obese diabetic mice is associated with a T helper type 2 shift and induction of FoxP3+ regulatory T cells. Immunology 127:512–522

Hübner MP, Shi Y, Torrero MN, Mueller E, Larson D, Soloviova K et al (2012) Helminth protection against autoimmune diabetes in nonobese diabetic mice is independent of a type 2 immune shift and requires TGF-β. J Immunol 188:559–568

Hunter MM, Wang A, Hirota CL, McKay DM (2005) Neutralizing anti-IL-10 antibody blocks the protective effect of tapeworm infection in a murine model of chemically induced colitis. J Immunol 174:7368–7375

Hussaarts L, Garcia-Tardon N, van Beek L, Heemskerk MM, Haeberlein S, van der Zon GC et al (2015) Chronic helminth infection and helminth-derived egg antigens promote adipose tissue M2 macrophages and improve insulin sensitivity in obese mice. FASEB J 29:3027–3039

Itami DM, Oshiro TM, Araujo CA, Perini A, Martins MA, Macedo MS et al (2005) Modulation of murine experimental asthma by Ascaris suum components. Clin Exp Allergy 35:873–879

Jackson-Thompson BM, Torrero M, Mitre BK, Long J, Packiam M, Mitre E (2020) Axenic Caenorhabditis elegans antigen protects against development of type 1 diabetes in NOD mice. J Trans Autoimmun 3:100065

Jin X, Yang Y, Bai X, Shi H, Zhang W, Zhang Z et al (2019) Dendritic cells treated by *Trichinella spiralis* muscle larval excretory-secretory products alleviate TNBS-induced colitis in mice. Int Immunopharmacol 70:378–386

Johnston MJ, Wang A, Catarino ME, Ball L, Phan VC, MacDonald JA et al (2010) Extracts of the rat tapeworm, Hymenolepis diminuta, suppress macrophage activation in vitro and alleviate chemically induced colitis in mice. Infect Immun 78:1364–1375

Kabeerdoss J, Pugazhendhi S, Subramanian V, Binder HJ, Ramakrishna BS (2011) Exposure to hookworms in patients with Crohn's disease: a case-control study. Aliment Pharmacol Ther 34: 923–930

Kang SA, Choi JH, Baek K-W, Lee DI, Jeong M-J, Yu HS (2021) *Trichinella spiralis* infection ameliorated diet-induced obesity in mice. Int J Parasitol 51:63–71

Khan WI, Blennerhasset PA, Varghese AK, Chowdhury SK, Omsted P, Deng Y et al (2002) Intestinal nematode infection ameliorates experimental colitis in mice. Infect Immun 70:5931–5937

Khan YA, Maurya SK, Kulkarni C, Tiwari MC, Nagar GK, Chattopadhyay N (2020) Fasciola helminth defense molecule-1 protects against experimental arthritis by inhibiting osteoclast formation and function without modulating the systemic immune response. FASEB J 34: 1091–1106

Kitagaki K, Businga TR, Racila D, Elliott DE, Weinstock JV, Kline JN (2006) Intestinal helminths protect in a murine model of asthma. J Immunol 177:1628–1635

Kuijk LM, Klaver EJ, Kooij G, van der Pol SM, Heijnen P, Bruijns SC et al (2012) Soluble helminth products suppress clinical signs in murine experimental autoimmune encephalomyelitis and differentially modulate human dendritic cell activation. Mol Immunol 51:210–218

La Flamme AC, Ruddenklau K, Bäckström BT (2003) Schistosomiasis decreases central nervous system inflammation and alters the progression of experimental autoimmune encephalomyelitis. Infect Immun 71:4996–5004

Lee KH, Park HK, Jeong HJ, Park SK, Lee SJ, Choi SH et al (2008) Immunization of proteins from Toxascaris leonina adult worm inhibits allergic specific Th2 response. Vet Parasitol 156:216–225

Leung J, Hang L, Blum A, Setiawan T, Stoyanoff K, Weinstock J (2012) Heligmosomoides polygyrus abrogates antigen-specific gut injury in a murine model of inflammatory bowel disease. Inflamm Bowel Dis 18:1447–1455

Lima C, Perini A, Garcia ML, Martins MA, Teixeira MM, Macedo MS (2002) Eosinophilic inflammation and airway hyper-responsiveness are profoundly inhibited by a helminth (Ascaris suum) extract in a murine model of asthma. Clin Exp Allergy 32:1659–1666

Liu Q, Sundar K, Mishra PK, Mousavi G, Liu Z, Gaydo A et al (2009) Helminth infection can reduce insulitis and type 1 diabetes through CD25- and IL-10-independent mechanisms. Infect Immun 77:5347–5358

Liu P, Li J, Yang X, Shen Y, Zhu Y, Wang S et al (2010) Helminth infection inhibits airway allergic reaction and dendritic cells are involved in the modulation process. Parasite Immunol 32:57–66

Lothstein KE, Gause WC (2021) Mining helminths for novel therapeutics. Trends Mol Med 27: 345–364

Lumb FE, Crowe J, Doonan J, Suckling CJ, Selman C, Harnett MM et al (2019) Synthetic small molecule analogues of the immunomodulatory *Acanthocheilonema viteae* product ES-62 promote metabolic homeostasis during obesity in a mouse model. Mol Biochem Parasitol 234: 111232

McConchie BW, Norris HH, Bundoc VG, Trivedi S, Boesen A, Urban JF Jr et al (2006) Ascaris suum-derived products suppress mucosal allergic inflammation in an interleukin-10-independent manner via interference with dendritic cell function. Infect Immun 74:6632–6641

McInnes IB, Leung BP, Harnett M, Gracie JA, Liew FY, Harnett W (2003) A novel therapeutic approach targeting articular inflammation using the filarial nematode-derived phosphorylcholine-containing glycoprotein ES-62. J Immunol 171:2127–2133

McSorley HJ, O'Gorman MT, Blair N, Sutherland TE, Filbey KJ, Maizels RM (2012) Suppression of type 2 immunity and allergic airway inflammation by secreted products of the helminth Heligmosomoides polygyrus. Eur J Immunol 42:2667–2682

McSorley HJ, Blair NF, Smith KA, McKenzie AN, Maizels RM (2014) Blockade of IL-33 release and suppression of type 2 innate lymphoid cell responses by helminth secreted products in airway allergy. Mucosal Immunol 7:1068–1078

Melendez AJ, Harnett MM, Pushparaj PN, Wong WS, Tay HK, McSharry CP et al (2007) Inhibition of Fc epsilon RI-mediated mast cell responses by ES-62, a product of parasitic filarial nematodes. Nat Med 13:1375–1381

Melon A, Wang A, Phan V, McKay DM (2010) Infection with Hymenolepis diminuta is more effective than daily corticosteroids in blocking chemically induced colitis in mice. J Biomed Biotechnol 2010:384523

Metwali A, Setiawan T, Blum AM, Urban J, Elliott DE, Hang L et al (2006) Induction of CD8+ regulatory T cells in the intestine by Heligmosomoides polygyrus infection. Am J Physiol Gastrointest Liver Physiol 291:G253–G259

Mishra PK, Patel N, Wu W, Bleich D, Gause WC (2013) Prevention of type 1 diabetes through infection with an intestinal nematode parasite requires IL-10 in the absence of a Th2-type response. Mucosal Immunol 6:297–308

Mo HM, Liu WQ, Lei JH, Cheng YL, Wang CZ, Li YL (2007) Schistosoma japonicum eggs modulate the activity of CD4+ CD25+ Tregs and prevent development of colitis in mice. Exp Parasitol 116:385–389

Mo HM, Lei JH, Jiang ZW, Wang CZ, Cheng YL, Li YL, Liu WQ (2008) Schistosoma japonicum infection modulates the development of allergen-induced airway inflammation in mice. Parasitol Res 103:1183–1189

Motomura Y, Wang H, Deng Y, El-Sharkawy RT, Verdu EF, Khan WI (2009) Helminth antigen-based strategy to ameliorate inflammation in an experimental model of colitis. Clin Exp Immunol 155:88–95

Obieglo K, Schuijs MJ, Ozir-Fazalalikhan A, Otto F, van Wijck Y, Boon L, Lambrecht BN, Taube C, Smits H (2018) Isolated Schistosoma mansoni eggs prevent allergic airway inflammation. Parasite Immunol 40(10):e12579. https://doi.org/10.1111/pim.12579

Osada Y, Shimizu S, Kumagai T, Yamada S, Kanazawa T (2009) Schistosoma mansoni infection reduces severity of collagen-induced arthritis via down-regulation of pro-inflammatory mediators. Int J Parasitol 39:457–464

Osada Y, Fujiyama T, Kamimura N, Kaji T, Nakae S, Sudo K et al (2017) Dual absence of STAT6 and IL-10 does not abrogate anti-hyperglycemic effects of *Schistosoma mansoni* in streptozotocin-treated diabetic mice. Exp Parasitol 177:1–12

Osada Y, Horie Y, Nakae S, Sudo K, Kanazawa T (2019) STAT6 and IL-10 are required for the anti-arthritic effects of Schistosoma mansoni via different mechanisms. Clin Exp Immunol 195: 109–120

Osada Y, Morita K, Tahara S, Ishihara T, Wu Z, Nagano I, Maekawa Y, Nakae S, Sudo K (2020) Kanazawa T Th2 signals are not essential for the anti-arthritic effects of Trichinella spiralis in mice. Parasite Immunol 42:e12677

Osbourn M, Soares DC, Vacca F, Cohen ES, Scott IC, Gregory WF, Smyth DJ, Toivakka M, Kemter AM, le Bihan T, Wear M, Hoving D, Filbey KJ, Hewitson JP, Henderson H, Gonzàlez-Cìscar A, Errington C, Vermeren S, Astier AL, Wallace WA, Schwarze J, Ivens AC, Maizels RM, McSorley HJ (2017) HpARI protein secreted by a helminth parasite suppresses interleukin-33. Immunity 47(4):739–751

Pace F, Carvalho BM, Zanotto TM, Santos A, Guadagnini D, Silva KLC et al (2018) Helminth infection in mice improces insulin sensitivity via modulation of gut microbiota and fatty acid metabolism. Pharmacol Res 132:33–46

Pacífico LG, Marinho FA, Fonseca CT, Barsante MM, Pinho V, Sales-Junior PA et al (2009) Schistosoma mansoni antigens modulate experimental allergic asthma in a murine model: a major role for CD4+ CD25+ Foxp3+ T cells independent of interleukin-10. Infect Immun 77: 98–107

Panda AK, Ravindran B, Das BK (2013) Rheumatoid arthritis patients are free of filarial infection in an area where filariasis is endemic: comment on the article by Pineda et al. Arthritis Rheum 65: 1402–1403

Pang J, Ding J, Zhang L, Zhang Y, Yang Y, Bai X et al (2020) Effect or recombinant serine protease from adult stage of Trichinella spiralis on TNBS-induced experimental colitis in mice. Int Immunopharmacol 86:106699

Park SK, Cho MK, Park HK et al (2009) Macrophage migration inhibitory factor homologs of anisakis simplex suppress Th2 response in allergic airway inflammation model via CD4+-CD25+Foxp3+ T cell recruitment. J Immunol 182:6907–6914

Park HK, Cho MK, Choi SH, Kim YS, Yu HS (2011) Trichinella spiralis: infection reduces airway allergic inflammation in mice. Exp Parasitol 127:539–544

Pascoal VF, da Cunha AA, Morassutti AL, Antunes GL, da Silveira KA, Silveira JS, Nuñez NK, de Souza RG, Graeff-Teixeira C, Pitrez PM (2020) Immunomodulatory effect of different extracts from Angiostrongylus cantonensis on airway inflammation in an allergic asthma model. Parasitol Res 119:3719–3728

Peres RS, Chiuso-Minicucci F, da Rosa LC, Domingues A, Zorzella-Pezavento SF, França TG et al (2013) Previous contact with Strongyloides venezuelensis contributed to prevent insulitis in MLD-STZ diabetes. Exp Parasitol 134:183–189

Pineda MA, McGrath MA, Smith PC, Al-Riyami L, Rzepecka J, Gracie JA et al (2012) The parasitic helminth product ES-62 suppresses pathogenesis in collagen-induced arthritis by targeting the interleukin-17-producing cellular network at multiple sites. Arthritis Rheum 64: 3168–3178

Pinto LA, Pitrez PM, Fontoura GR, Machado DC, Jones MH, Graeff-Teixeira C et al (2004) Infection of BALB/c mice with Angiostrongylus costaricensis decreases pulmonary inflammatory response to ovalbumin. Parasite Immunol 26:151–155

Pinto LA, Dias AC, Rymer BL, Fernandes FF, Barbosa GL, Machado DC et al (2006) Effect of Angiostrongylus costaricensis extract on eosinophilic pulmonary response in BALB/c mice. Parasitol Res 98:295–298

Qiu S, Fan X, Yang Y, Dong P, Zhou W, Xu Y, Zhou Y, Guo F, Zheng Y, Yang JQ (2017) Schistosoma japonicum infection downregulates house dust mite-induced allergic airway inflammation in mice. PLoS One 12(6):e0179565

Quinn SM, Raverdeau M, McGinley AM, Mills KHG (2019) Helminths products directly modulate T cells that mediate experimental autoimmune encephalomyelitis. Eur J Immunol 49:1291–1294

Rajamanickam A, Munisankar S, Bhootra Y, Dolla C, Thiruvengadam K, Nutman TB et al (2019) Metabolic consequences of concomitant Strongyloides stercoralis infection in patients with type 2 diabetes mellitus. Clin Infect Dis 69:697–704

Reardon C, Sanchez A, Hogaboam CM, McKay DM (2001) Tapeworm infection reduces epithelial ion transport abnormalities in murine dextran sulfate sodium-induced colitis. Infect Immun 69: 4417–4423

Reyes JL, Espinoza-Jiménez AF, González MI, Verdin L, Terrazas LI (2011) Taenia crassiceps infection abrogates experimental autoimmune encephalomyelitis. Cell Immunol 267:77–87

Rocha FA, Leite AK, Pompeu MM, Cunha TM, Verri WA Jr, Soares FM et al (2008) Protective effect of an extract from Ascaris suum in experimental arthritis models. Infect Immun 76:2736–2745

Ruyssers NE, De Winter BY, De Man JG, Loukas A, Pearson MS, Weinstock JV et al (2009) Therapeutic potential of helminth soluble proteins in TNBS-induced colitis in mice. Inflamm Bowel Dis 15:491–500

Ruyssers NE, De Winter BY, De Man JG, Ruyssers ND, Van Gils AJ, Loukas A et al (2010) Schistosoma mansoni proteins attenuate gastrointestinal motility disturbances during experimental colitis in mice. World J Gastroenterol 16:703–712

Rzepecka J, Donskow-Schmelter K, Doligalska M (2007) Heligmosomoides polygyrus infection down-regulates eotaxin concentration and CCR3 expression on lung eosinophils in murine allergic pulmonary inflammation. Parasite Immunol 29:405–413

Rzepecka J, Siebeke I, Coltherd JC, Kean DE, Steiger CN, Al-Riyami L et al (2013) The helminth product, ES-62, protects against airway inflammation by resetting the Th cell phenotype. Int J Parasitol 43:211–223

Salinas-Carmona MC, de la Cruz-Galicia G, Pérez-Rivera I, Solís-Soto JM, Segoviano-Ramirez JC, Vázquez AV, Garza MA (2009) Spontaneous arthritis in MRL/lpr mice is aggravated by Staphylococcus aureus and ameliorated by Nippostrongylus brasiliensis infections. Autoimmunity 42:25–32

Sandborn WJ, Elliott DE, Weinstock J, Summers RW, Landry-Wheeler A, Silver N et al (2013) Randomised clinical trial: the safety and tolerability of Trichuris suis ova in patients with Crohn's disease. Aliment Pharmacol Ther 38:255–263

Sarter K, Kulagin M, Schett G, Harris NL, Zaiss MM (2017) Inflammatory arthritis and systemic bone loss are attenuated by gastrointestinal helminth parasites. Autoimmunity 50:151–157

Saunders KA, Raine T, Cooke A, Lawrence CE (2007) Inhibition of autoimmune type 1 diabetes by gastrointestinal helminth infection. Infect Immun 75:397–407

Schnoeller C, Rausch S, Pillai S, Avagyan A, Wittig BM, Loddenkemper C et al (2008) A helminth immunomodulator reduces allergic and inflammatory responses by induction of IL-10-producing macrophages. J Immunol 180:4265–4272

Scholmerich J, Fellermann K, Siebold FW, Rogler G, Langhorst J, Howaldt S et al (2017) A randomized, double-blind, placebo-controlled trial of *Trichuris suis* ova in active Crohn's disease. J Crohns Colitis 11:390–399

Setiawan T, Metwali A, Blum AM, Ince MN, Urban JF Jr, Elliott DE et al (2007) Heligmosomoides polygyrus promotes regulatory T-cell cytokine production in the murine normal distal intestine. Infect Immun 75:4655–4663

Sewell D, Qing Z, Reinke E, Elliot D, Weinstock J, Sandor M et al (2003) Immunomodulation of experimental autoimmune encephalomyelitis by helminth ova immunization. Int Immunol 15: 59–69

Shi M, Wang A, Prescott D, Waterhouse CC, Zhang S, McDougall JJ et al (2011) Infection with an intestinal helminth parasite reduces Freund's complete adjuvant-induced monoarthritis in mice. Arthritis Rheum 63:434–444

Shimokawa C, Kato T, Takeuchi T, Ohshima N, Furuki T, Ohtsu Y et al (2020) $CD8^+$ regulatory T cells are critical in prevention of autoimmune-mediated diabetes. Nat Commun 11:1922

Smith P, Mangan NE, Walsh CM, Fallon RE, McKenzie AN, van Rooijen N et al (2007) Infection with a helminth parasite prevents experimental colitis via a macrophage-mediated mechanism. J Immunol 178:4557–4566

Sofronic-Milosavljevic L, Radovic I, Ilic N, Majstorovic I, Cvetkovic J, Gruden-Movsesijan A (2013) Application of dendritic cells stimulated with Trichinella spiralis excretory-secretory antigens alleviates experimental autoimmune encephalomyelitis. Med Microbiol Immunol 202: 239–249

Song X, Shen J, Wen H, Zhong Z, Luo Q, Chu D et al (2011) Impact of Schistosoma japonicum infection on collagen-induced arthritis in DBA/1 mice: a murine model of human rheumatoid arthritis. PLoS One 6:e23453

Stanley RG, Jackson CL, Griffiths K, Doenhoff MJ (2009) Effects of *Schistosoma mansoni* worms and eggs on circulating cholesterol and liver lipids in mice. Atherosclerosis 207:131–138

Su CW, Chen C-Y, Jiao L, Long SR, Mao T, Ji Q et al (2020) Helminth-induced and TH2-dependent alterations of the gut microbiota attenuate obesity caused by high fat diet. Cell Mol Gastroenterol Hepatol 10:763–778

Summers RW, Elliott DE, Urban JF Jr, Thompson RA, Weinstock JV (2005a) Trichuris suis therapy for active ulcerative colitis: a randomized controlled trial. Gastroenterology 128:825–832

Summers RW, Elliott DE, Urban JF Jr, Thompson R, Weinstock JV (2005b) Trichuris suis therapy in Crohn's disease. Gut 54:87–90

Sun X, Liu YH, Lv ZY, Yang LL, Hu SM, Zheng HQ et al (2010) rSj16, a recombinant protein of Schistosoma japonicum-derived molecule, reduces severity of the complete Freund's adjuvant-induced adjuvant arthritis in rats' model. Parasite Immunol 32:739–748

Sun S, Li H, Yuan Y, Wang L, He W, Xie H, Gao S, Cheng R, Qian H, Jiang H, Wang X, Zhan B, Fang Q, Yang X (2019) Preventive and therapeutic effects of Trichinella spiralis adult extracts on allergic inflammation in an experimental asthma mouse model. Parasit Vectors 12(1):326

Sutton TL, Zhao A, Madden KB, Elfrey JE, Tuft BA, Sullivan CA et al (2008) Anti-Inflammatory mechanisms of enteric Heligmosomoides polygyrus infection against trinitrobenzene sulfonic acid-induced colitis in a murine model. Infect Immun 76:4772–4782

Trujillo-Vargas CM, Werner-Klein M, Wohlleben G, Polte T, Hansen G, Ehlers S et al (2007) Helminth-derived products inhibit the development of allergic responses in mice. Am J Respir Crit Care Med 175:336–344

van der Vlugt LE, Labuda LA, Ozir-Fazalalikhan A, Lievers E, Gloudemans AK, Liu KY, Barr TA, Sparwasser T, Boon L, Ngoa UA, Feugap EN, Adegnika AA, Kremsner PG, Gray D, Yazdanbakhsh M, Smits HH (2012) Schistosomes induce regulatory features in human and mouse CD1d(hi) B cells: inhibition of allergic inflammation by IL-10 and regulatory T cells. PLoS One 7(2):e30883

van der Werff SD, Twisk JW, Wördemann M, Ponce MC, Díaz RJ, Núñez FA et al (2013) Deworming is not a risk factor for the development of atopic diseases: a longitudinal study in Cuban school children. Clin Exp Allergy 43:665–671

van der Zande HJP, Gonzalez MA, de Ruiter K, Wilbers RHP, Garcia-Tardon N, van Huizen M et al (2021) The helminth protein ω1 improves metabolic homeostasis in obese mice through type 2 immunity-independent inhibition of food intake. FASEB J 35:e21331

Walsh KP, Brady MT, Finlay CM, Boon L, Mills KH (2009) Infection with a helminth parasite attenuates autoimmunity through TGF-beta-mediated suppression of Th17 and Th1 responses. J Immunol 183:1577–1586

Wang CC, Nolan TJ, Schad GA, Abraham D (2001) Infection of mice with the helminth Strongyloides stercoralis suppresses pulmonary allergic responses to ovalbumin. Clin Exp Allergy 31:495–503

Wang Z, Hao C, Zhuang Q, Zhan B, Sun X, Huang J et al (2020) Excretory/secretory products from Trichinella spiralis adult worms attenuated DSS-induced colitis in mice by driving PD-1-mediated M2 macrophage polarization. Front Immunol 11:563784

Wiedemann M, Voehringer D (2020) Immunomodulation and immune escape strategies of gastrointestinal helminths and schistosomes. Front Immunol 17:11

Wilson MS, Taylor MD, O'Gorman MT, Balic A, Barr TA, Filbey K et al (2010) Helminth-induced CD19+CD23hi B cells modulate experimental allergic and autoimmune inflammation. Eur J Immunol 40:1682–1696

Wohlleben G, Trujillo C, Muller J, Ritze Y, Grunewald S, Tatsch U et al (2004) Helminth infection modulates the development of allergen-induced airway inflammation. Int Immunol 16:585–596

Wu Z, Nagano I, Asano K, Takahashi Y (2010) Infection of non-encapsulated species of Trichinella ameliorates experimental autoimmune encephalomyelitis involving suppression of Th17 and Th1 response. Parasitol Res 107:1173–1188

Xia CM, Zhao Y, Jiang L, Jiang J, Zhang SC (2011) Schistosoma japonicum ova maintains epithelial barrier function during experimental colitis. World J Gastroenterol 17:4810–4816

Yan K, Wang B, Zhou H, Luo Q, Shen J, Xu Y et al (2020) Amelioration of type 1 diabetes by recombinant fructose-1,6-bisphosphate aldolase and cystatin derived from *Schistosoma japonicum* in a murine model. Parasitol Res 119:203–214

Yang J, Zhao J, Yang Y, Zhang L, Yang X, Zhu X et al (2007) Schistosoma japonicum egg antigens stimulate CD4 CD25 T cells and modulate airway inflammation in a murine model of asthma. Immunology 120:8–18

Yang Y, Liu L, Liu X, Zhang YY, Shi H, Jia W et al (2020) Extracellular vesicles derived from *Trichinella spiralis* muscle larvae ameliorate TNBS-induced colitis in mice. Front Immunol 11: 1174

Yordanova IA, Ebner F, Schulz AR, Steinfelder S, Rosche B, Bolze A, Paul F, Mei HE, Hartmann S (2021) The worm-specific immune response in multiple sclerosis patients receiving controlled trichuris suis ova immunotherapy. Life (Basel) 29(11):101

Zaccone P, Fehérvári Z, Jones FM, Sidobre S, Kronenberg M, Dunne DW et al (2003) Schistosoma mansoni antigens modulate the activity of the innate immune response and prevent onset of type 1 diabetes. Eur J Immunol 33:1439–1449

Zaccone P, Burton O, Miller N, Jones FM, Dunne DW, Cooke A (2009) Schistosoma mansoni egg antigens induce Treg that participate in diabetes prevention in NOD mice. Eur J Immunol 39: 1098–1107

Zaccone P, Burton OT, Gibbs S, Miller N, Jones FM, Dunne DW et al (2010) Immune modulation by Schistosoma mansoni antigens in NOD mice: effects on both innate and adaptive immune systems. J Biomed Biotechnol 2010:795210

Zaccone P, Burton OT, Gibbs SE, Miller N, Jones FM, Schramm G et al (2011) The S. mansoni glycoprotein ω-1 induces Foxp3 expression in NOD mouse $CD4^+$ T cells. Eur J Immunol 41: 2709–2718

Zaiss MM, Rapin A, Lebon L, Dubey LK, Mosconi I, Sarter K, Piersigilli A, Menin L, Walker AW, Rougemont J, Paerewijck O, Geldhof P, McCoy KD, Macpherson AJ, Croese J, Giacomin PR, Loukas A, Junt T, Marsland BJ, Harris NL (2015) The intestinal microbiota contributes to the ability of helminths to modulate allergic inflammation. Immunity 43(5):998–1010

Zhao Y, Zhang S, Jiang L, Jiang J, Liu H (2009) Preventive effects of Schistosoma japonicum ova on trinitrobenzenesulfonic acid-induced colitis and bacterial translocation in mice. J Gastroenterol Hepatol 24:1775–1780

Zheng X, Hu X, Zhou G, Lu Z, Qiu W, Bao J et al (2008) Soluble egg antigen from Schistosoma japonicum modulates the progression of chronic progressive experimental autoimmune encephalomyelitis via Th2-shift response. J Neuroimmunol 194:107–114