Fabrizio Bruschi *Editor*

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A Linda

Preface

The helminths are comprised of two distantly related taxa, the roundworm Nemathelminthes and the flatworm Platyhelminthes, the latter being divided into cestodes (tapeworms) and trematodes (flukes). Although these groups diverged evolutionarily many hundreds of millions of years ago, their patterns of transmission, infection, and pathogenesis are in many ways similar. Furthermore, the host immune responsiveness to these pathogens follows a typical pattern which is dominated by a Type 2 (Th2) profile with a significant regulatory (Treg) component.

It was estimated that approximately one-third of the almost three billion people who live in extremely poor conditions (less than two US dollars per day) in developing regions of sub-Saharan Africa, Asia, and the Americas are infected with one or more helminths. Despite this, helminth diseases are among the *neglected tropical diseases*.

According to the Global Burden of Disease 2010 Project, more than 14 million of disability-adjusted life years due to disability plus mortality are ascribable to helminths, but since these are inclined to give chronic infections, their impact on the quality of life, estimated with the more appropriate parameter quality-adjusted life years, is certainly enormous. We should take into account that helminths can affect also livestock, with a following worsening of malnutrition in poor countries.

We may then conclude that this world is still a *wormy* one, according to the definition of Norman Stoll in 1947.

The aim of this book is that to give an overview of the impact of helminth infections on the global public health, providing informations not only on the epidemiology, immunology and immunopathology, clinical and laboratory diagnosis, treatment, and prognosis but also on the present and future perspectives of control.

Helminths not only cause diseases 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.

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

the mankind has encountered these pathogens for thousands of years, obliging to change the habits to reduce their impact on the health status.

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

Finally, a chapter on the possible exploitation of helminth-derived molecules for the treatment of human immune-mediated diseases shows how the improvement of the knowledge of the host–parasite interplay might open new ways to medicine in the future.

The reviewing work by Claudio Bandi, Jong-Yil Chai, Jorge Correale, Nilanthi de Silva, Hubert Ferté, Albis Gabrielli, Ray Gamble, Eisaku Kimura, Arne Levsen, Rick M. Maizels, Pedro Moro, K. Darwin Murrell, Alessandra Nicoletti, Karl Reinhard, Evan W. Secor, and Fernando Simon Martin is greatly appreciated.

This book is dedicated to Carlo Urbani, the Italian WHO officer well known to have given the first alarm of SARS outbreak in 2003 and dead because of that 10 years ago. He was a physician, tropicalist, and also a renowned parasitologist who gave an important contribution to the knowledge of *Schistosoma mekongi*, showing how the basic research is strictly related to field applications.

Pisa, Italy Fabrizio Bruschi

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Chapter 1
Diversity and History as Drivers of Helminth Systematics and 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 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 perturbation on parasite evolution. Collectively, these data lead to better predictive capacity for future changes in the distribution patterns and roles that parasites play in animal and human health. 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.

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1.1 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 history, 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 on 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 from extant organisms only. However, 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 (Hoberg and Brooks 2013).

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. 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 taxonomy as ongoing processes, producing a more fluid and mutable understanding of species in both space and time. Stable classifications require ample sampling, a valid comparative context, and inclusive consideration of what currently surrounds us, all of which are prone to human interpretation, frailty, and change, much as Heisenberg discovered in the movement of subatomic particles.

A wealth of reviews, chapters, and articles has been written on the taxonomy and systematics of helminths. Inasmuch as 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 (Hoberg and Brooks 2013; Brooks and Hoberg 2013). We highlight how past and current evidence provides a window to explore a future of dynamic change caused by accelerated climate warming, habitat perturbation, erosion of biodiversity, the dissemination of invasive species, changes in host adaptation, and the 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.

1.2 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 Platyhelminthes are hermaphroditic except for blood flukes (schistosomes), which are dioecious. In their adult, sexually mature stage, all helminths are considered to be 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). 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 are essential for understanding basic determinants for occurrence, emergence, and disease. The distribution of disease is often heterogeneous, local, and circumscribed within a more extensive spatial range for a parasite or host–parasite assemblage (Audy 1958; Hoberg 2010). Thus, it becomes important to use molecular phylogenetic and phylogeographic methods to understand the genetic variation within populations, 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).

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) 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 (biological species concept) (see Brooks and McLennan 2002; Wiley and Lieberman 2011). Thus, 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). Recent examples are analyses exploring diversity among species of *Taenia* and *Echinococcus* (Hoberg 2006; Nakao

et al. 2007, 2009, 2013a, b; Lavikainen et al. 2008), and studies of species richness in *Trichinella* (Zarlenga et al. 2006; Pozio et al. 2009).

1.2.1 Helminth Parasites and the World Stadium

The impact of parasites occurs at the junction of human populations, 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; Weaver et al. 2010; Brooks and Hoberg 2013). Despite thousands of years of medical and veterinary intervention, helminth parasites remain a considerable regional 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, and transformation of natural habitats have all been driving forces for the emergence of helminth and other diseases (Daszak et al. 2000; Patz et al. 2008; Rosenthal 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). Although the tipping points represented by a burgeoning human population and the development of agriculture and animal domestication have had a direct influence on the occurrence of parasites in humans, many host–parasite associations for extant parasites have considerably deeper origins extending into the Pliocene and Pleistocene (Hoberg and Brooks 2013).

Although parasites are often obscure, they represent in excess of 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 viruses and bacteria) are often associated with wildlife (Taylor et al. 2001; Cleaveland et al. 2001; Wolfe et al. 2007). This intricate web of interactions establishes the significance of human parasites as mediators of health and well-being, food sustainability, food security 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; Weaver et al. 2010).

Based on global estimates, between 75,000 and 300,000 species of helminths infect terrestrial and aquatic vertebrates (Dobson et al. 2008). 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 1.1). 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

Table 1.1 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) and Nakao et al. (2013b)]

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), *Spirometra* (4)][c]
Anoplocephalidae-
Inermicapsifer cubensis[b]
[+ species of *Bertiella* (2), *Raillietina* (2)][c]
Dilepididae-
[*Dipyllidium caninum*][c]
Hymenolepididae-
Rodentolepis nana[a]
[+ *Hymenolepis diminuta*][c]

(continued)

Table 1.1 (continued)

Taeniidae-
Taenia asiatica[a]
Taenia saginata[a]
Taenia solium[a]
[+ species of *Echinococcus* (6), *Taenia* (7)][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
[species of *Parastrongylus* (2)][c]
Oxyuridae-
Enterobius gregorii[a]
Enterobius vermicularis[a]
Ascaridae-
Ascaris lumbricoides[a]
[+ species of *Baylisascaris* (1), *Toxocara* (2), *Toxascaris* (1)][c]
Anisakidae-
[species of *Anisakis* (2), *Pseudoterranova* (1)][c]
Dracunculidae-
Dracunculus medinensis[a]
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]
Wuchereria bancrofti[a]

(continued)

Table 1.1 (continued)

[+ species of *Dirofilaria* (5)][c]
Trichuridae-
Trichuris trichiura[b]
[+ *Calodium hepaticum*, *Eucoleus aerophilus*, *Paracapillaria philippinensis*][c]
Dioctophymidae-
[*Dioctophyme renale*][c]
Trichinellidae-
[*Trichinella spiralis*, *T. britovi*, *T. murrelli*, *T. nativa*, *T. nelsoni*, *T. pseudospiralis*][c]
Acanthocephala (Ø 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
? = exact relationship as obligate human parasite requires elucidation
Ø = denotes "none"

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. Among these, only *T. solium* is considered highly pathogenic as the causative agent of human neurocysticercosis.

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 *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus*, and *Ancylostoma duodenale* are cosmopolitan and cause greater morbidity in humans than any other parasitic disease except malaria (Murray and Lopez 1996; Weaver et al. 2010). Further, the distribution of 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; Kuris 2012; Brooks and Hoberg 2013; Hoberg and Brooks 2013). An emerging challenge is seen in the disruption of socioeconomic controls on the occurrence and impact of infection. Warfare, changing climate as it affects food distribution and water resources, movement of refugees, and a breakdown in medical infrastructure all facilitate

new patterns of infection and disease (Patz et al. 2007, 2008; Weaver et al. 2010; Brooks and Hoberg 2013).

1.2.2 Host-Switching Drives Helminth Evolution

History is a defining factor in exploring and understanding contemporary distributions and disease risks 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) (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). This coevolutionary/cospeciation perspective extends to present-day ideas with regard to the difficulty for parasites to undergo host-switching with unrelated vertebrate lineages (Brooks and Ferrao 2005). It behooves us to explore this apparent paradox for cospeciation, host specificity, and colonization in the arena of pathogenic human parasites and emerging infectious diseases (Agosta et al. 2010; Brooks and Hoberg 2013).

A prevailing assumption describing complex host–parasite assemblages is that parasites coevolve with their hosts (Brooks and Ferrao 2005 and earlier papers cited therein). The interdependence of these phenomena discounts host-switching by otherwise narrowly distributed helminths. A challenge to this orthodoxy is that host-switching is common, has directly influenced parasite faunal structure for humans, and is the basis for what are recognized as emerging infectious diseases (Cleaveland et al. 2001; Wolfe et al. 2007; Brooks and Hoberg 2013). Cospeciation as the driving force behind complex host–parasite associations and faunas has had limited explanatory power. It has also hindered studies on geographic expansion, ecological perturbation, and host colonization as prominent processes in faunal assembly and diversification. Invasion is pervasive; episodes leading to the breakdown of ecological isolation and barriers to host colonization have important implications for the distribution and evolution of helminth faunas and emerging infectious diseases (Wolfe et al 2007; Hoberg 2010; Brooks and Hoberg 2013; Hoberg and Brooks 2013).

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 (Hoberg et al. 2001; Hoberg 2006). 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). 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 is 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 more to shared trophic relationships and host-switching among carnivorans than to associations with other mammals and birds that are either carnivores or piscivores (Hoberg et al. 2001; Ashford and Crewe 2003; Kuris 2012). As such, geographic proximity and ecological structure and continuity among foraging guilds are key drivers of parasite acquisition 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 at high latitudes of the Nearctic also parasitize humans (Jenkins et al. 2013). Also, 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).

1.2.3 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 the 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). In a contemporary setting, anthropogenic drivers increasingly influence invasion and the 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 our expansion out of Africa nearly 40–60 Kya. Further, the advent of agriculture and animal husbandry 10–11 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 (Riccardi 2007; Hoberg 2010; Hoberg and Brooks 2013). 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). 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).

1.3 Defining Diversity

Accurate definitions of diversity are vital to understanding the role of parasites in human and animal diseases. In addition, defining diversity is critical to studying epidemiology, developing management practices to limit transmission, and designing treatment regimes 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 have begun to resolve themselves. Although molecular-based diagnostics can now supplant preparation and microscopic examination of whole specimens, 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 applying sequences and appropriate molecular markers to diagnosing life history stages including eggs and larvae.

Continued reliance on archival museum collections as resources for biodiversity, informatics and our study of the biosphere, including history and structure, is apparent (Cook et al. 2013). Museum collections and specimens are the self-correcting records for biodiversity that document the geographic occurrence and host associations for parasites. As such, they remain highly relevant to understanding diversity and the changing patterns of distribution over time. Deposition and full documentation of specimens and their environmental niche 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). In this manner the influence of accelerated climate change, ecological perturbation, human activities and invasion, and other factors that determine the distribution 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 foundations 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).

1.3.1 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).

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*, Flockhart et al. (1982) with *Trichinella* spp.). Recently, more informative DNA approaches (gene sequences, single nucleotide polymorphisms (SNP), 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 two 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).

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* microfilaria (McNulty et al. 2008) 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 anthropophillic strains, larval parasites (e.g., microfilariae 200–275 μm 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 in order 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).

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 (e.g., see below under Sect. 1.4). 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

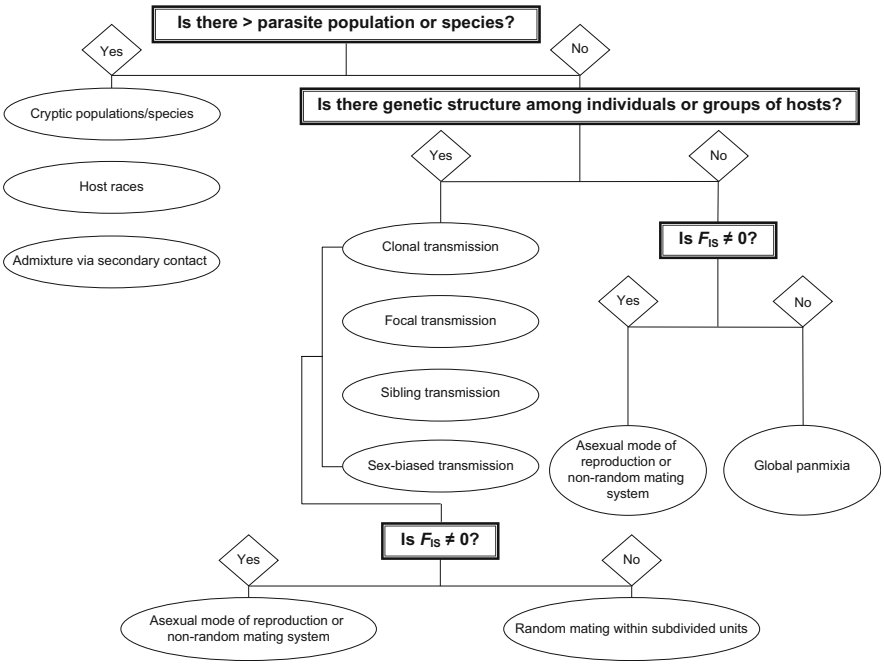

Fig. 1.1 Categorizing genetic variation among helminth parasites (From Gorton et al. 2012)

glimpse into the extent of genetic variation across broad geographic scales. In a recent review, Gorton et al. (2012) provided a flow diagram that describes the practical process of categorizing inter- and intraspecific variation for helminth parasites (Fig. 1.1).

For human helminths, the answer to the first question in Fig. 1.1, "Is there >1 parasite population or species?" has often been "yes" or "likely yes, but more 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 is 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).

1.3.2 Populations, Natural Hybrids, and Adequate Sampling

The presence of one or several populations can be detected using population genetics analyses. The same approach can be extended to address speciation. Many computer programs for population genetic analyses have been developed to estimate parameters that help infer genetically based differences between or within populations (Excoffier and Heckel 2006). For example, programs like FSTAT and GENEPOP estimate linkage disequilibrium (nonrandom 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 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* species (see Table 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 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.

1.4 Genomics, Systematics, and Parasitic Worms

Evolution is a branching process resulting from the diverging of populations over time. This process 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. Inclusion of genomic and molecular data in assessments of relationships within and among parasitic groups has dramatically expanded and has led to new insights across all helminth taxa. A molecular-based phylogenetic revision of the phylum Nematoda began in earnest back in 1998 when Blaxter et al. (1998) used a single gene (nuclear small subunit ribosomal RNA) to construct a tree that was conceptually partitioned into 5 major clades predicated upon data from 53 species. 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). 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 Mengen et al. 2009), as well as the inclusion of morphological data (De Ley and Blaxter 2002, 2004). Similar comprehensive studies have been performed on the flat worms and cestodes to estimate the phylogeny of the Digenea (Olson et al. 2003) using the complete ssrDNA and partial data from the lsrDNA focusing on expansion segments D1–D3. The analysis included 163 digenean taxa. Extensive analyses using both maximum parsimony and Bayesian inference 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 genes/protein sequences, or a small number of genes/protein sequences in 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); 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 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, 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). As such, it becomes theoretically feasible to resolve deep evolutionary relationships using phylogenomics.

1.4.1 Phylogenomics and Evolutionary Inference

In many instances it is not examining the entire genome that 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 taxons. 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. 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 the order in which genes appear in a genome (Korbel et al. 2002), among others.

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. 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. In this case, arthropods and nematodes would form a monophyletic clade independent of chordates because members of these groups undergo a homologous molting process.

In 2004, Wolf et al. (2004) used phylogenomics to address this question by examining greater than 500 protein sequences subgrouped into eight 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; 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. However, 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). Collectively these studies showed 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.

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 is an evolving and subjective science with few hardcore benchmarks. Large datasets may negate sampling errors, but systemic errors such as compositional biases and misleading data still abound (Jeffroy et al. 2006; Philippe et al. 2005a). As example, 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 analyzing 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.

The field of phylogenomics is still evolving, and 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 will 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 systems 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 macroscales when studying evolutionary trends among organisms using comparative genomics.

1.4.2 Comparative Genomics and Evolutionary Inference

In recent years, research on parasite genomes has come of age. Draft genome sequence data are now available for species of *Trichinella* (Mitreva et al. 2011), *Brugia* (Ghedin et al. 2007), *Ascaris* (Jex et al. 2011), *Schistosoma* (Berriman et al. 2009; *S. japonicum* Consortium 2009), and nearly so for the cestodes *Taenia* and *Echinococcus* (Tsai et al. 2013). Other genera encompassing key human parasitic worms are soon to follow. In addition to being used to study evolutionary relationships and processes on a grander scale (phylogenomics), genome sequences have come to better enlighten us on issues like host–parasite interactions, adaptation, and selection and have placed a genetic face to the biological diversity that abounds in this group. Comparative analyses, i.e., comparative genomics, using genome information in conjunction with the transcriptome and proteome data that usually accompanies these studies, have helped us understand the functions of genes and gene products. In essence, comparative genomics has helped link phenotypes to genotypes.

A comparative analysis of 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), was recently performed (Tsai et al. 2013). This study showed extraordinary genetic plasticity among tapeworms, 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) evaluated the use of comparative genomics in an intragenus study of *Schistosoma* phylogeography. They targeted mitochondrial 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 mitochondrial gene order. Their analysis led to the conclusion that the genus then invaded Africa with the migration of mammals. It is believed this occurred as recent as 2–3 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 and mechanisms constituting "parasitism" among nematodes (Blaxter et al. 2012; Heizer et al. 2013; Shinya et al. 2013; Strube et al. 2012; Tsai et al. 2013). 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 and 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 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 pharmacophylogenomics in the development of prophylactic and therapeutic treatments for human parasites (Caffrey et al. 2009; 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.

1.5 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. Given the extent to which parasitism permeates our ecosystem, parasitological insights must be integrated into any discussion on the unfolding and accelerating effects of climate and ecological disruption because of the potential for new and changing patterns of parasite/pathogen distribution. 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, imparts on that plasticity.

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Chapter 2
Paleoparasitology of Helminths

Gino Fornaciari and Raffaele Gaeta

Abstract Paleoparasitology is an important branch of paleopathology, which is the discipline that studies ancient diseases through the use of human skeletal or mummified remains.

This science is useful from a medical perspective to understand past events of human evolution, including conditions of hygiene, sanitation, and nutritional adequacy.

A central role is 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 and the latrine soils.

In this chapter we will show the most important paleoparasitology findings in the four continents which will present the evident massive spread of the whole class of the helminths (trematodes, cestodes, nematodes) through the ages.

2.1 Introduction

Paleopathology is the discipline that studies ancient diseases through the use of human skeletal or mummified remains. For these reasons it differs from the history of medicine, which debates on past medical theories, therapies, and diseases by historical and literary sources. The paleopathology therefore is strictly linked to the application of traditional medicine methods but enriched and supported by other subjects like history, anthropology, and archaeology.

An important branch of this discipline is paleoparasitology, whose studies can be useful from a medical perspective to understand other past events of human evolution, including conditions of hygiene, sanitation, and nutritional adequacy (Bouchet et al. 2003a).

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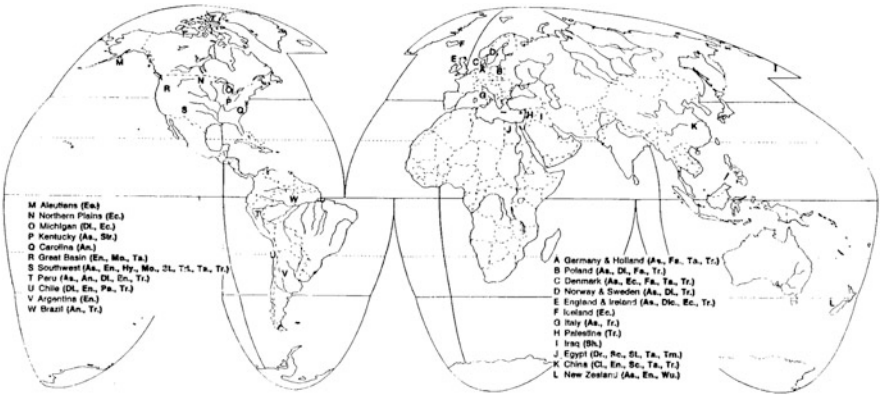

Fig. 2.1 Analyses of fecal debris from archaeological sites resulted in the find of many cases of parasitism. The finds to date are mapped below. For the Americas, the finds of many as TAENIA are considered only to be in the family Taeniidae. The identification to genus level is not yet established for these finds. *An* Ancylostoma, *AS* Ascaris, *Di* Diphylloborthrium, *Cl* Clonorchis, *Dic* Dicrocoelium, *Dr* Dracunculus, *Ec* Echinococcus, *En* Enterobius, *Fa* Fasciola, *Hy* Hymenolepis, *Mo* Moniliformes, *Pa* paragonismus, *Sc & Sh* Schistosoma, *St* strongyloides, *Ta* Taenia, *Tr* trichuris, *Tri* trichostrongylus, *Trn* Trichinella, *Wu* Wucheria. For Germany, also the presence of Dic, Di, and En could be proven. (from Reinhard et al. 1986)

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 (Fig. 2.1).

The most important sources for the study of paleoparasitology are the coprolites, i.e., desiccated or mineralized feces, and the latrine soils that can be recovered from archaeological layers or directly from mummified bodies; this encourages an increasing cooperation among archaeologists, paleopathologists, and paleoparasitologists.

To rehydrate desiccated coprolites, a trisodium phosphate solution is used (Callen and Cameron 1960), while different techniques are applied for the detection of parasites, like the modified pollen analysis technique (Warnock and Reinhard 1992), and the detection of antigen in mummies, like the ancient DNA techniques (Martinez Machado et al. 2003; Loreille et al. 2001; Leles et al. 2008).

Finally, paleoparasitology is not only a science of the past but a useful discipline for the future; because with the understanding of parasite evolution, it is possible to better comprehend modern diseases (Ewald 1996).

2.2 Africa

2.2.1 Trematodes

A major range of parasites species have been recovered in Egypt, and East Africa seems to be the center of dispersion of schistosomiasis, and from there the infection dispersed to other parts of the world (Chamot and Amat-Roze 1993) probably because of nomad caravans and the slave trade along the Nile (Nozais 1987).

In the beginning of the century, the development of a technique to rehydrate desiccated tissues allowed the finding of calcified ova of *Schistosoma haematobium* in the infected kidneys of two Egyptian mummies from the twentieth dynasty (c. 1184–c. 1087 B.C.) (Ruffer 1910).

This is the earliest demonstration of a parasitic infestation in human tissues.

Instead, the oldest mummies were infected by *Schistosoma*, identified using immunodiagnosis (ELISA), which occurred over 5,000 years ago in an Egyptian adolescent (Deelder et al. 1990). ELISA also revealed *S. haematobium* in two mummies 3,000 and 4,000 years old (Contis and David 1996).

Calcified schistosome ova were identified radiologically in several mummies from later periods by the Manchester Mummy Project that has developed a program to study the paleoepidemiology of schistosomiasis in ancient Egypt using computed tomography (CT), scanned electron microscopy (SEM), the enzyme-linked immunosorbent assay (ELISA), and immunocytochemistry.

It is no coincidence that there are so many reported cases of infestation; in fact the development of irrigation in Egypt, characterized by the Nile's annual flooding of basins, provided the convergence of the aquatic snail intermediate host with the human definitive host in surface waters, a condition favorable for schistosomiasis, especially *Schistosoma haematobium* (Kloos and David 2002).

2.2.2 Cestodes

It was possible to identify the ova of *Taenia saginata* in a mummified intestine placed in an embalming rejects jar dated from the twenty-fifth dynasty (715–656 B.C.) (Harter et al. 2003).

The presence of the *Taenia* spp. eggs was already recorded by electronic microscopy of histological sections of the intestine of the Egyptian mummy ROM, dated 1198 years B.C. (Horne and Lewin 1977).

A recent diagnosis of cysticercosis, dating back to the late Ptolemaic period (II–I century B.C.), confirms the wide diffusion in Egypt of the farming of pigs, representing the most common intermediate hosts of *Taenia solium* (Bruschi et al. 2006) (Fig. 2.2).

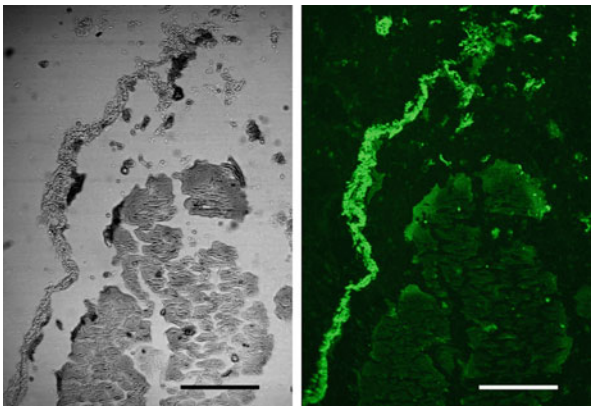

Fig. 2.2 Cysticercus cellulosae and cyst wall observed by laser confocal microscopy after incubation of sections with *Taenia solium*-positive control serum. *Left*: transmission light microscope view. *Right*: corresponding fluorescent image; the bladder wall is strongly stained. From Bruschi et al. 2006

2.3 America

As regards America, the most debated question is when the different species of helminths were introduced into the New World (Reinhard 1998).

There are three theories about this dilemma:

1. During the prehistoric migrations through the Bering land bridge (10,000 before present), many Old World human infections reached America despite the polar temperature. Arctic conditions played the role of a "filter" for many parasite infections; in fact lots of parasites need especial humidity, pH, and temperature conditions to be able to maintain eggs or larvae alive near 22 °C positive (Araùjo et al. 1988).
2. The climatic conditions of Beringia encountered by migrating people have been considered too extreme for tropical hookworms to survive, suggesting that hookworms must have been introduced into South America by "storm-tossed" fishermen or explorers from Asia.
3. Based on an analysis of the life cycle and morphology of hookworm, some paleopathologists affirmed that the presence of hookworm infestation in the Americas prior to 1492 is uncertain, so helminths are considered one of the many pathogens brought to the Americas after contact in 1492 (Fuller 1997).

Analyses of coprolites and colon contents of mummies are most frequently reported from the deserts of the southwestern USA (Moore et al. 1974; Fry 1980), central and northeastern Brazil (Araùjo et al. 1981; Confalonieri et al. 1981; Ferreira et al. 1983), and Peru (Patrucco et al. 1983). Cold, dry environments in fact result in the preservation of helminth remains in mummies.

2.3.1 Trematodes

Fluke eggs which could not be specifically identified were reported from Nevada and Utah with a single case each of *Paragonimus westermani* (Chile) and *Cryptocotyle lingua* (Alaska) (Zimmermann 1980).

2.3.2 Cestodes

Clinical data show the presence of *Diphyllobothrium pacificum* in relation with the El Niño-Southern Oscillations (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 drastic environmental changes. The abundance of human mummies and archaeological coastal sites in the Atacama Desert provides an excellent model to test the ENSO impact on antiquity (Arriaza et al. 2010).

This arid region was the place where Chinchorro culture arose about 9,000 years ago. These people used to mummify their dead starting from about 7,000 BC, so they are considered the oldest examples of artificially mummified human remains. The Chinchorro diet has been reconstructed using bone chemistry (Aufderheide and Allison 1992) and coprolite samples, revealing the consumption of a variety of maritime foods (Reinhard and Aufderheide 1990). The data show that this population was affected by *D. pacificum*, implying warmer water associated to the cyclical ENSO phenomenon. The tapeworm lives in the lower intestines and competes with the host for nutrients, particularly vitamin B12; therefore, the cases of anemia reported in ancient Chilean and Peruvian skulls could, in certain cases, be related to parasitosis (Arriaza and Standen 2009).

Two parasite species were found in the Atacama Desert 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 find in Michigan. Various reports of taeniid eggs came from Utah, Michigan, and Arizona.

2.3.3 Nematodes

Hookworm infection seems to have been common in past South Americans, as it has been found in many archaeological sites.

The oldest dating for hookworm eggs in human coprolites is from the archaeological site of Boqueirão do Sítio da Pedra Furada, Piauí, Brazil, which is 7,230 ± 80 years old (Ferreira et al. 1987), while the first record was by Allison

et al. (1974) who found *Ancylostoma duodenale* adults in the intestinal mucosa of a Peruvian mummy from 900 B.C. *Trichuris trichiura* and hookworm eggs and larvae were found in human coprolites collected in sedimental layers of an archaeological site in Minas Gerais, Brazil, dated from 3,490 ± 120 to 430 ± 70 years by radiocarbon (Ferreira et al. 1983). Later, the same parasites were also found in coprolites collected from a naturally mummified body of a child from the same site and dating (Ferreira et al. 1987). Araùjo (1987) found hookworm eggs in human coprolites also from Minas Gerais, Brazil, dated from 4,905 ± 90 to 1,325 ± 60 years.

Another case of hookworm infestation was present in a Tiahuanaco mummy dating from around 900 A.D. where *A. duodenale* was identified in the small intestine.

DNA results indirectly contributed to design a new panorama of *Ascaris* paleodistribution, showing that, in fact, this parasite has been present in South America since 8,800 years B.P. in prehistoric populations from Brazil and Chile (Martinez Machado et al. 2003; Leles et al. 2008).

The pinworm (*Enterobius vermicularis*) has been reported from Arizona, Colorado, Utah, Chile, Peru, and Argentina. The whipworm (*Trichuris trichiura*) has been reported from Brazil, Chile, and Peru with a single report from Arizona. Of the two hookworms which infect man, *A. duodenale* has been reported once from Peru and *Necator americanus* once from Brazil. *Ascaris lumbricoides* has been tentatively identified at one site in Kentucky and once each in Arizona and Peru. Eggs of the thorny-headed worm have been reported from four sites in Utah and one in Oregon (Horne 1985).

In regard to *E. vermicularis*, ancient DNA was extracted from 27 coprolites from archaeological sites in Chile and the USA. Enzymatic amplification of human mtDNA sequences confirmed the presence of the parasite in ancient American population (Iñiguez et al. 2003).

Finally, several cases of *Anisakis* infection were detected in the already-mentioned Chinchorro mummies.

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

2.4 Europe

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

Parasite infection was a common aspect of medieval life, as was evident in the numerous archaeological sites (Table 2.1). In particular the *Ascaris*/*Trichuris* pair is known for its almost systematic presence from Roman times until the Renaissance.

Table 2.1 Parasites find for the middle-age period in Europe

Authors and date	Samples	Site	Datations	Nematode	Trematode	Cestode
Taylor (1955)	Sediments Latrines	England Winchester	**XI-XII**	*Trichuris* *Ascaris*	*Dicrocoeclium*	
Grzywinski (1959, 1960)	Coprolites	Slaves	XI-XIII		*Fasciola*	
Pike (1967)	Coprolites		XI-XIII	*Trichuris* *Ascaris*	*Fasciola* *Dicrocoeclium*	
Greig (1981)	Sediments Latrines	Great Britain Worcester	XV	*Trichuris* *Ascaris*		
Moore (1981)	Sediments Latrines	England	XXV	*Trichuris* *Ascaris*		
Greig (1982)	Sediments Latrines	England Londres	XV-XVI	*Trichuris* *Ascaris*		
Legendre et al. (1986)	Latrines	France Strasbourg	XV-XVI	*Trichuris* *Ascaris*		
Rouffignac (1987)	Coprolites Sediments	Europe	**XIII-XV**	*Ascaris* *Trichuris*	*Fasciola* *Dicrocoeclium*	*Diphyllobothrium*
Herrmann (1988)	Sediments Latrines	Germany		*Ascaris* *Enterobius*		*Diphyllobothrium* *Tania*
Bouchet et al. (1989)	Coprolites Sediments Latrines	France Paris	**XII-XV**	*Trichuris* *Ascaris* *Toxocara* *Acanthocephala* *Ancylostoma*		*Tania*
Bouchet et al. (1991)	Sediments	France Paris	XVII-XVIII	*Trichuris* *Ascaris* *Toxocara* *Heterakis* *Toxascaris* *Filicolis* *Syngamus* *Ancylostoma*		

(continued)

Table 2.1 (continued)

Authors and date	Samples	Site	Datations	Nematode	Trematode	Cestode
Bouchet (1991)	Sediments Pits Garbage dumps	France Beauvais	**XIII-XVII**	*Trichuris* *Ascaris* *Heterakis* *Capillaria*		
Bouchet (1993)	Sediment	France Paris	XIV-XV	*Trichuris*	*Fasciola* *Dicrocoeclium*	
Bouchet (1994)	Sediments Animals Chara vines	France Paris	XVIII	*Toxocara canis* *Parascaris*	*Fasciola* *Dicrocoeclium*	
Bouchet and Paicheler (1995)	Sédiment Latrines	France Montbéliard	XV		*Schistosoma* sp.	*Tania*
Bouchet et al. (1995)	Latrine	Belgium Raversijde	XV	*Trichuris* *Ascaris*		*Tania*
Bouchet (1995)	Sediment Coprolites	France Paris		*Trichuris* *Ascaris*	*Fasciola* *Dicrocoeclium*	
Bouchet et al. (1997)	Sediments Channeling	France Vincennes	800 ap. J.C.	*Trichuris* *Ascaris*	*Fasciola*	
Bouchet et al. (1998)	Coprolites	France Marly le Roy	XVII-XVIII	*Trichuris* *Ascaris*	*Fasciola*	*Tania*
Bouchet et al. (2002)	Sediments Latrines	France Montbéliard	XV		*Schistosoma mansoni*	

From Bouchet et al. (2003)

2.4.1 Trematodes

Hookworm disease was a well-known problem in ancient times in the Old World, especially the lancet fluke, *Dicrocoelium* spp., that was attested in Western Europe from 550,000 years B.P. to the sixteenth century A.D.

Hoeppli (1959) published a discussion about diseases in ancient populations based on old documents, such as Hippocratic texts and the Ebers Papyrus, and mentions hookworm disease, characterized by hydropsy, anemia, and aerophagy, in ancient Rome and Greece.

In fact during the Roman period and the Middle Ages, the *Dicrocoelium* (Fig. 2.3) and *Fasciola* infections were frequent and are well identified in the material collected from excavations in archaeological sites in the French cities of Paris, Montbeliard, Reims, and Bordeaux (Table 2.1) (Bouchet et al. 2003a, b). Another demonstration of the high incidence of *Dicrocoelium dendriticum* in the Roman age derives from the study of the Zweeloo Woman, a bog mummy from the Netherlands. This find is especially significant because it is the oldest known patent infection of *D. dendriticum* in humans (Searcey et al. 2013).

As regards the *Schistosoma*, some samples of this parasite were found in a pit adjacent to a fifteenth- to sixteenth-century house in France (Bouchet and Paicheler 1995). This is an interesting case since both urogenital and intestinal schistosomiases are considered of African origin, so it is possible that Europeans became infected during a trip to Africa; otherwise, an infected African brought to France might have eliminated these eggs (Bouchet et al. 2002a, b).

2.4.2 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 homes occupied by the nobility because only wealthy people could afford to eat meat, but it was undercooked or nearly raw: all processes were insufficient to kill the encysted larvae in the muscles.

A case of *cysticercosis* was found in an anatomic piece, an encephalon, belonging to the collection of the University of Turin (Italy) dating back to 1911 (Ferrari and Micalizio 2001). In the section indeed it was possible to note some round formations 0.4 cm in diameter, i.e., a well-preserved fragment of *T. solium* comparable to modern specimens of surgical pathology.

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

Two cases of cyst of tapeworm (*Echinococcus granulosus*) were found in the fourteenth-century cemetery of the hospital of S. Maria della Scala in Siena

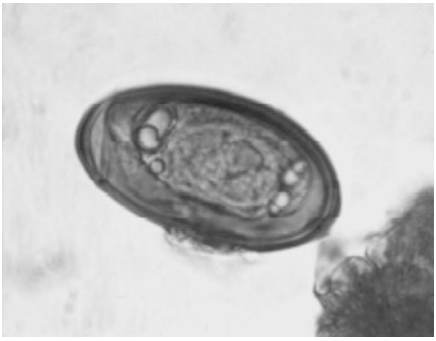

Fig. 2.3 *Dicrocoelium* spp. egg, 40 × 25 μm (×1,000)

(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).

2.4.3 Nematodes

In the medieval site of Chevenez, France (Le Bailly and Bouchet 2012), the archaeologists have done the paleoparasitological study of the inhumations through the soil located under the pelvis and abdomen of the buried. The analyses highlighted two intestinal parasites: *Ascaris lumbricoides* and *T. trichiura*.

T. trichiura eggs were identified, by immunohistochemistry and laser confocal microscopy, in the colon of a sixteenth-century Italian mummy from Naples (Masetti et al., 2008), Fig. 2.4.

In the Middle Age site of "Places d'Armes" in Namur (Belgium; Plumier et al. 1997), several coprolites mixed with soil organic matter were recovered from a particular fourteenth-century latrine. The coprolites revealed a very high concentration of parasite eggs, and some of those eggs still had embryo remains inside. After rehydration, DNA from 104 eggs was collected and extracted with an ultrasonication and phenol-chloroform-based method. The analysis of the sequences confirms the identification of the eggs as coming from *Ascaris* (Loreille et al. 2001), one of the most abundant parasites found in archaeological sites.

The oldest recorded dates for *Ascaris* are 800 to 350 B.C., the Iron Age period, in human coprolites of Hallstatt salt mines (Aspöck et al. 1973) and 600 B.C. in Prussian mummies (Szidat 1944).

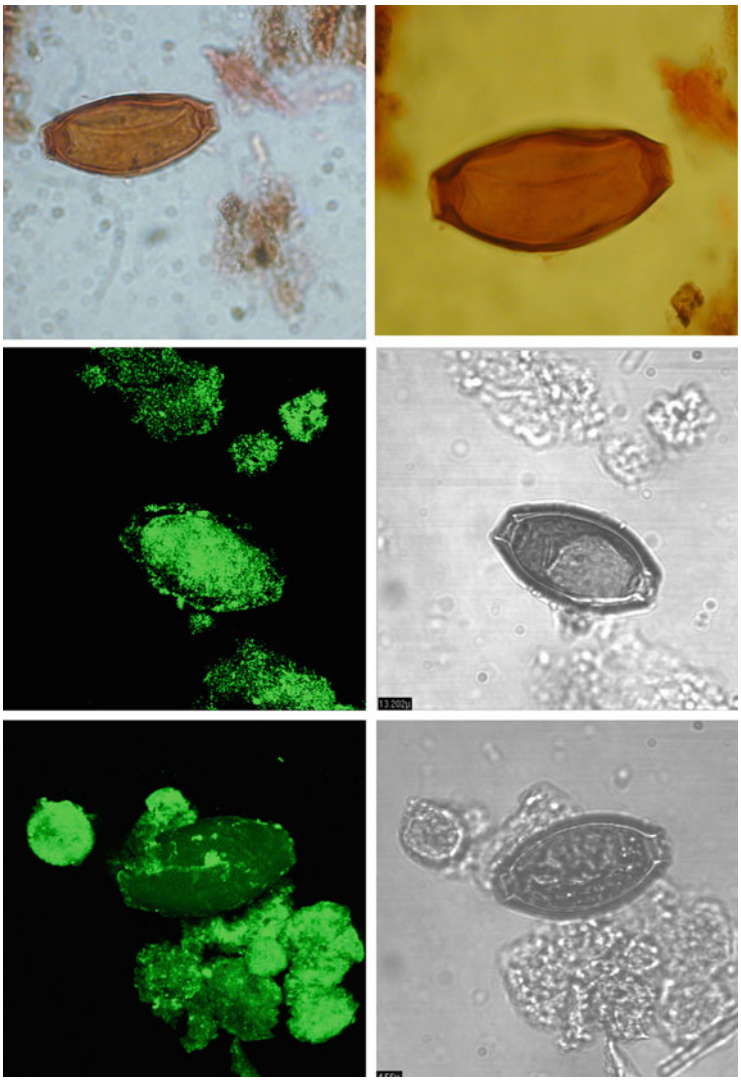

Fig. 2.4 *Upper left*, appearance of the organisms retrieved in the cecum of mummy n. 29 (×1,000); *upper right* (×1,200), H&E stain. *Middle left*, immunohistochemistry and CLSM with positive reaction, both on the surface and on the internal structure of the egg, against a specific hyperimmune serum for *Trichuris muris*; *middle right*, corresponding light microscope view (×1,000). *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 (×1,000)

2.5 Asia

The excavation of eggs from parasites from archaeological materials has been reported in several sources, mainly from Korea and 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).

2.5.1 Trematodes

The best subjects of such studies have been Korean mummies discovered in medieval Joseon tombs (1392–1910 A.D.). *Clonorchis sinensis* (Fig. 2.5) infection remained one of the most common parasite infections among Koreans even up to 1970, at which time fully 12.1 % of the population was afflicted (Kim et al. 1971). Indeed, given the historical literature's wealth of descriptions of the raw-fish-based cuisine enjoyed by the Joseon people, it is almost certain that much of that population was infected by the parasite (Seo et al. 2008).

Even in China archaeologists found the eggs of *C. sinensis,* in 1956, from coprolites of a Ming Dynasty corpse (1513) in the suburbs of Guangzhou (Chen 1956). Since then, in 1975, *C. sinensis* eggs have been found from a corpse of the Western Han Dynasty in Jiangling (Chen and Hung 1981; Wei et al. 1981) and from a tomb of the Chu Dynasty (Hu 1984; Yang et al. 1984; Su 1987).

In Japan *C. sinensis* was found also in an early medieval cesspit of a palace in Kashihara City (Matsui et al. 2003).

These means that *clonorchiasis* has been prevalent over the last 2,300 years in Korea and China.

2.5.2 Cestodes

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

2.5.3 Nematodes

It was possible to find eggs of *C. sinensis*, *A. lumbricoides*, and *T. trichiura* in the feces of a fifteenth-century child mummy from Yangju, Korea. *T. trichiura* eggs were found in far greater numbers than other parasite eggs; in fact intact bipolar plugs were clearly observed, and even the larvae were still visible in some eggs. The eggs of *C. sinensis* and *A. lumbricoides* were also well preserved. A scanning

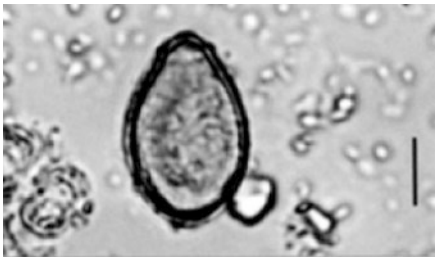

Fig. 2.5 *Clonorchis sinensis* (*bar* = 10 μm), from Eun-Taek Han (2003)

electron microscopy (SEM) study revealed *Metagonimus yokogawai*, *P. westermani*, and *Gymnophalloides seoi* eggs recovered from Korean mummies of the Joseon Dynasty (Shin et al. 2009).

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

In Mesopotamia, agricultural workers were repeatedly exposed to infection of *Schistosoma* from snail-infested irrigation canals, but in addition, the priestly caste used freshwater for religious ceremonies in temples and palaces and consequently was also at risk. The situation was further complicated by the capture of slaves, who may therefore have assisted in spreading the disease and introducing fresh strains of parasite.

2.6 Middle East

Some of the most important works in paleoparasitology of the Middle East come from the studies of medieval latrine soil useful to determine the range of helminth infections present in the crusader population.

In an attempt to help pilgrims in the Holy Land, many medical organizations were founded in the twelfth and thirteenth century, like the Knights Hospitallers, that built a network of hospitals with thousands of beds. The several parasitological findings confirm a significant rate infection because of war, plagues, and poor hygienic conditions 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."

In Israel, on the other hand, the paleoparasitological research has been carried out so far on samples previous to the Crusader Period, i.e., in the seventh–sixth century B.C. and first century A.D. Jerusalem (Zias and Mumcuoglu 1991), in the first century B.C.–first century A.D. Qumran (Harter et al. 2004), and in the second century A.D. Nahal Mishmar valley (Witenburg 1961).

2.6.1 Cestodes

The ova of *D. latum* were identified in the thirteenth-century latrine soil of the Crusader Hospital of St. John in Acre, Israel, probably the most ancient sample of this parasite in archaeological findings in the Middle East, insomuch as can be interpreted as a 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* spp.) were found in premedieval Israel.

2.6.2 Nematodes

The most common ova present in the latrine soil of Acre, as well as in pre-crusader Israel sites, were certainly *T. trichiura* followed by *A. lumbricoides* (Mitchell and Stern 2001). Referring to the Crusader Period, there are two records found in the latrine soil inside the castle of Saranta Kolones in Paphos, Cyprus, built after King Richard I of England captured the island in 1191 A.D. during the Third Crusade. The microscopic examination demonstrated the eggs of *Ascaris lumbricoides* and *Trichuris trichiura* (Anastasiou and Mitchell, 2013).

2.7 Conclusions

A recent review about the origin of human parasites (Araújo et al. 2013) demonstrates that paleoparasitology changed totally the common knowledge based on reports and interpretations of some nineteenth-century theories. For example, all the intestinal helminth infections, once considered to have been introduced to America by African slaves, already existed in American prehistoric populations.

The records of ancient human tapeworms appear very scarce. There is a unique case of cysticercosis in an Egyptian mummy of the Ptolemaic age (200–100 years B.C.) and other findings 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 *Taenia saginata* predates the domestication of pigs and cattle.

A. lumbricoides is another intestinal parasite of humans that has a long history of association with man. There was a debate about roundworm origin, if the human species, *A. lumbricoides*, originated from the parasite of pigs, *Ascaris suum*, or vice versa. This should have occurred at 10,000 years ago, after pig domestication. The techniques of molecular biology evidenced that *A. lumbricoides* infection was common in prehistoric groups, in North America as in South America, despite the rare findings in coprolites from New World archaeological sites. Due to the fact that New World wild pigs are not parasitized by roundworms, it is very probable that prehistoric migrants should have brought the parasite from the Old World to the Americas.

When comparing morphologic and genetic characteristics of both species of roundworm, there are few differences between them, found indistinctly in humans and pigs. Therefore, it was supposed that *A. lumbricoides* is the oldest species that was transferred to pigs after domestication, and so there is a single parasite species of humans and pigs.

Many nematodes that infect the human intestinal tract, and were inherited from human ancestors, were found in prehistoric populations of the Americas. The first migrants that came to the continent most probably introduced them. However, not all intestinal helminths were introduced by the same prehistoric route, and the condition of transmission of these helminths in the Americas was completely different to those in Europe.

Although *E. vermicularis* has no environmental restrictions to the transmission from one host to another individual, other intestinal helminths could not survive in the cold conditions of the Siberian-Arctic regions of the Bering land bridge. Hookworms, *T. trichiura* and *A. lumbricoides*, found in archaeological sites in both North America and South America dated as old as 9,000 years ago. For this reason, these parasites were introduced by alternative routes, such as transpacific or coastal navigation. Paleoparasitological data showed that they were common among prehistoric populations in the Americas. However, the eggs found in the coprolites were always in small number, indicating low prevalence. This is not surprising, as most of the prehistoric populations in the Americas were nomad and had hunter-gathering subsistence for living. However, when Europeans arrived, they established new conditions as colonizers, building villages, slaving Native Americans, and later bringing Africans, and changed totally the lifestyle in the Americas.

It is interesting to compare paleoparasitological data in Middle Age Europe and prehistoric times in the Americas, before and after the arrival of Europeans in the New World. While latrines and mummies in Europe are found full of intestinal worms, in the Americas the intestinal helminth eggs are very rare, although positive for the same helminth species. This should be a consequence of differences in the demographic density of both populations but also a result of different occupational patterns of the space. When the first villages were established, environment changed as well as the way of life for many people. There was a true explosion of soil-transmitted helminths caused by the growing of population and very poor sanitation conditions.

In conclusion, paleoparasitology of helminths is a multidisciplinary research, stimulating international cooperation for testing hypothesis until trustful results were obtained. Not only paleoparasitologists and archaeologists are involved, but also many other specialists contribute to the understanding of the origin and evolution of these parasite diseases.

Appendix

Presented below (Table 2.2) is a synthetic list of the paleoparasitological finds from human remains, in the New and Old World, in the pre- and post-Columbian era.

Table 2.2 Paleoparasitological finds from human remains, in the New and Old World, pre and post-Columbian

	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 cayetanenis	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
From Gonçalves et al. (2003)

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Chapter 3
Schistosomiasis

Ahmad Othman and Rashika El Ridi

Abstract Schistosomiasis is one of the most imposing and widespread 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. Techniques and tools for diagnosing schistosomiasis are either cumbersome or lack sensitivity and specificity. Accordingly, many patients remain undiagnosed and receive no treatment. Currently, the only available drug is praziquantel; the use of which in mass treatment raises concerns about development of drug resistance. Indeed, *Schistosoma* infection is a fascinating model for gaining insight about the mutual interplay between host and parasite factors, which ultimately determines the overall morbidity. 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 venues for better diagnosis, treatment, and prevention of schistosomiasis.

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3.1 Introduction

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.

3.2 The Agent

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 the order Strigeidida, characterized by a forktailed cercaria, which infects final hosts using enzymes of penetration glands. The suborder is Strigeata, superfamily Schistosomatoidea, which contains three families: Sanguinocolidae (parasites of 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. The family Schistosomatidae comprises approximately 100 species, among which *Schistosoma mansoni*, *S. haematobium*, and *S. japonicum* and to a less prevalent extent *S. guineensis*, *S. intercalatum*, and *S. mekongi* cause human schistosomiasis, also named bilharzia, a severe disease of humans in 74 developing countries of the tropics and subtropics (Platt and Brooks 1997).

3.3 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 Philippines (Leonardo et al. 2013) and the marsh and lake regions of southern China (Li et al. 2000; Ross et al. 2001; Zhao et al. 2012a, b) allows the spread of *S. japonicum*. In the Middle East and sub-Saharan Africa, *Oncomelania* spp. and schistosomiasis japonicum are not found, while the broad geographical range of susceptible snail

species of the genus *Biomphalaria* and *Bulinus* coincides with the widespread incidence of schistosomiasis mansoni and schistosomiasis haematobium, respectively (Morgan et al. 2001). 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*, *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, the occurrence of *B. truncatus* and *B. beccari* in Saudi Arabia might allow spread of *S. haematobium* (Mostafa et al. 2009, 2012).

The prevalence of schistosomiasis is also closely related to adequate financial, social, and political conditions that would permit rise in living standards and access to clear water, improved sanitary conditions, and health education to every resident of rural areas (Utzinger et al. 2009, 2011; King 2010). Thus, schistosomiasis transmission was reported to be interrupted in Japan, Iran, Jordan, Morocco, and Tunisia (Utzinger et al. 2009, 2011; World Health Organization 2011). In contrast, more than 90 % of all estimated cases of hepato-intestinal and urinary schistosomiasis reside in sub-Saharan Africa with Nigeria and Tanzania having the highest burden. Of note, the prevalence of infection increased with increase in the population in Tanzania from 19 % in 1977 to 51.5 % in 2012 (Mazigo et al. 2012). On the other hand, the zoonotic behaviour of *S. japonicum*, which may infect 40 mammalian species, renders eradication of schistosomiasis in China a most difficult task (Ross et al. 2001; Zhou et al. 2012; Hong et al. 2013).

Additionally, epidemiology of schistosomiasis is directly related to completeness and accuracy of information regarding infection prevalence. There is a prominent lack of reliable and updated information on schistosomiasis prevalence in numerous countries (Brooker et al. 2010; Utzinger et al. 2011) as the most complete global resource (Atlas of the Global Distribution of Schistosomiasis) is 26 years old (Brooker et al. 2010; World Health Organization 2010). Thus, several reports indicate that the prevalence rate of schistosomiasis in Egypt has dropped to nearly 1.5 % (EMRO 2007; Curtale et al. 2010), while no national programme for investigation of schistosomiasis prevalence was heard of since the year 2000. Thus, in the Abis area (near Alexandria), which is reportedly a region of "low" *S. mansoni* prevalence and intensity, 15.2 % of 11-year-old children were found to be infected with *S. mansoni* with infection intensity of up to 480 egg/g (Allam et al. 2009). Understandably, international travellers (not local residents) were warned that "Egypt is one of the most highly infected countries" (IAMAT 2012).

As importantly, epidemiology is dependent on the level of accuracy of diagnostic methods. Thus, the World Health Organization (WHO) estimate is of 235 million cases of schistosomiasis worldwide, with 732 million people at risk for infection in 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

accordingly, the WHO often-quoted statistics can only represent floor estimates for active and potential cases (de Vlas and Gryseels 1992; Enk et al. 2008; Coulibaly et al. 2012). King (2010) argued that probably 40–60 % of patients are likely misdiagnosed and hence suggested that the WHO values should be adjusted to between 391 and 587 million people worldwide. As a consequence, the WHO Preventive Chemotherapy and Transmission Control (PCT) databank no longer provides estimates on population infected or at risk. These have now been replaced by estimates of "population requiring preventive chemotherapy", which is the population in need of regular treatment with praziquantel, based on WHO recommendations and prevalence thresholds, calculated by "implementation unit". This indicator is now used in order to avoid the biases inherent in the calculation of number of people infected (by reason of the low sensitivity of the diagnostic tools used) or population at risk (by reason of the geographical focality of the disease) (WHO PCT databank—schistosomiasis. http://www.who.int/neglected_diseases/preventive_chemotherapy/sch/en/index.htm).

Schistosoma mansoni is endemic in a few countries in South America, principally Brazil, where prevalence rate is less than 1 %, and in around 50 countries in the Middle East (prevalence rate in Egypt less than 10 %) and sub-Saharan Africa where prevalence rates exceed 50 % in Nigeria, Ghana, Mozambique, Burkina Faso, Mali, Sierra Leone, Madagascar, and Tanzania (Utzinger et al. 2009, 2011; WHO 2010). In Tanzania, various studies have reported prevalence of up to 100 % in some areas at the Lake Victoria shores (Mazigo et al. 2012). Schistosomiasis haematobium is prevalent only in the Middle East and sub-Saharan African countries (Hürlimann et al. 2011).

Approximately 12.7 million people are infected with schistosomes in the Middle East and North Africa with 7.2 million accounted for in Egypt, 2.9 million in Yemen, 2.3 million in Algeria, and 0.3 million in Libya (Steinmann et al. 2006; Hotez et al. 2012). More than 12,000 schistosomiasis recent prevalence survey locations from 35 Western, Middle, Eastern, and Southern African sub-Saharan countries are now compiled in one database that can be accessed at http://www.gntd.org (Hürlimann et al. 2011). *S. haematobium* (54.4 %) and *S. mansoni* (40.8 %) were the most prevalent species. 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. The 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 (Hürlimann et al. 2011).

Schistosoma 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 still report schistosome infections with prevalence rates of 6–7 % (Peng et al. 2010; Zhou et al. 2011a, b, 2013). In the Philippines, there are currently only 560,000

cases of schistosomiasis (Bergquist and Tanner 2010; Gordon et al. 2012). 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).

3.4 Immunopathogenesis of *Schistosoma* Infection

Barsoum et al. (2013) stated that "almost all clinical features of schistosomiasis are caused, directly or indirectly, by the host's immune response to different stages of the parasite's life cycle 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 difficult to be controlled and interpreted. Most authorities, however, believe that the immunopathology is rather similar in human and animal hosts.

3.4.1 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 the host skin, *S. mansoni* cercariae exposed to linoleic acid produce prostaglandin E2 (PGE2) and induce mouse and human keratinocytes to produce PGE2 and immunosuppressive interleukin (IL)-10 (Ramaswamy et al. 2000). After invasion of the 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 keratinocyte 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). Fluorescent imaging revealed uptake of cercarial and schistosomular ESP by murine skin neutrophils, dendritic cells, and macrophages, with subsequent production of IL-6, tumour

necrosis factor (TNF), IL-12, and IL-10 (Paveley et al. 2009). Indeed, skin schistosomula ESP-mediated immune responses lead to both immune priming and regulation (Mountford 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).

3.4.2 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, immune responses to schistosome antigens manifest a striking shift from a moderate T-helper type 1 (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 Th1 response is induced against migrating schistosomula and immature adult worms. This response exhibited increased levels of circulating proinflammatory 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 Th2 response with the start of egg laying, characterized by augmented 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 antigens (SAWA), soluble egg antigens (SEA), and recombinant schistosome antigens,

namely, glyceraldehyde 3-phosphate dehydrogenase (rSG3PDH), 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 (but not to rSG3PDH) only following praziquantel treatment (Barakat et al. [manuscript under preparation]). 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 ($P < 0.05$–<0.001) 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 praziquantel-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 the downregulation of granuloma formation and liver fibrosis. On the other hand, persistent production of low levels of IL-10 and IFN-γ and 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 2013).

3.4.2.1 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* periovular 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 types of granuloma could be identified during the evolution of *Schistosoma*-induced granulomatous reaction irrespective of the anatomical site (Fig. 3.1), as indicated by early studies in mice, in rhesus monkeys (Hsu et al. 1972), and, later, in pigs (Hurst et al. 2000): the weakly reactive, exudative, exudative–productive, productive, and involutional stages (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. 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 eggshells are disintegrated and the cellular elements are replaced by fibroblasts with deposition of collagen. Macrophages, lymphocytes, plasma cells, and few eosinophils are still 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).

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 the T-cell-deprived mice infected with *S. mansoni*. Interestingly, B-cell function is required for the 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 (Oliveira et al. 2006). Typically, IL-4 determines the granuloma size, induces the proliferation of Th2 cytokine-producing lymphocytes, and is important

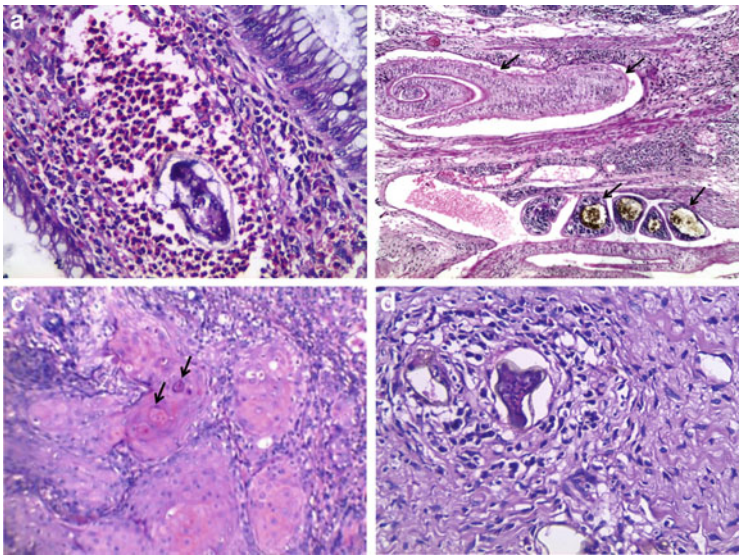

Fig. 3.1 (**a**) Early "exudative" periovular schistosomal granuloma in colonic mucosa. Note the prominent eosinophilic infiltrate (×400). (**b**) Sections of intravascular adult *Schistosoma* worms (*arrows*) in a resected colonic polyp from an Egyptian patient (×200). (**c**) Squameous cell carcinoma of urinary bladder on top of schistosomiasis in an Egyptian patient. Note *Schistosoma* eggs (*arrows*) (×200). (**d**) *Schistosoma* eggs in a resected ovarian adenofibroma from an Egyptian female, a common incidental finding in Egyptian patients (×400)

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).

3.4.2.2 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 the development of concomitant immunity and the fecundity of female worms

diminishes. 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, CD4+ CD25+ Foxp3+ regulatory T-cells (Tregs) 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 the 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 signalling 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 signalling 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). Interestingly, some sort of immunomodulation, regarding the future immune and fibrogenic responses, occurs in offsprings born to experimentally *Schistosoma*-infected hosts in case of postnatal exposure to the parasite (Othman et al. 2010). Perinatal immunological sensitization may occur by transplacental and/or transmammary passage of schistosome antigens, anti-idiotypic antibodies, or immune complexes to the offsprings.

3.4.2.3 Other Immune-Mediated Phenomena in Man

Immune complex glomerulonephritis may occur 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 2013). Moreover, *Schistosoma*-induced arthropathy has probably an underlying allergic immune mechanism (Saidenberg-Kermanac'h et al. 2005).

3.5 Pathogenesis and Clinical Features of Schistosomiasis

3.5.1 Stage of Invasion (Cercarial Dermatitis)

Penetration of 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.

3.5.2 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 pain, hepatosplenomegaly, abdominal pain, lymphadenopathy, skin rash, eosinophilia, and patchy pulmonary infiltrates on chest radiograph. 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; Jauréguiberry et al. 2010).

3.5.3 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 the 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).

3.5.4 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.

3.5.4.1 Genitourinary Schistosomiasis

Obstructive Uropathy

Healing of granulomatous lesions in the wall of the urinary bladder and lower ends of the 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 could ultimately lead to end-stage renal failure (Butterworth and Thomas 1999; Bourée 2005; Barsoum 2013).

Bladder Cancer

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 a distant site, owing to the occlusion of lymphatics by the fibrotic process (Barsoum 2013).

Associated bacterial and viral infections, rather than parasitic products, are suggested to be the main pathogenic factors. Associated infection with human papillomavirus has received considerable recent attention in this respect (el-Mawla et al. 2001), being encountered in about one-fourth of cases. Moreover, specific p53 gene mutations have been observed in one-third of cases (Warren et al. 1995), being attributed to the effect of neutrophil-generated reactive oxygen molecules, the cleavage of conjugated urinary carcinogens, or the production of nitrosamines by bacterial enzymes (Mostafa et al. 1999).

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 2013).

3.5.4.2 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 hypoproteinaemia. 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 polyps. 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 (Davis 2009).

There are contradictory reports on the contribution of *S. japonicum* to the aetiopathogenesis of colorectal or primary liver cancer (Davis 2009). The situation is more ambiguous for *S. mansoni*. The anecdotal reports of the 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.

3.5.4.3 Hepatic 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. 3.2). Portal hypertension leads to the 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. 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 the case of perisplenitis or infarction. Hypersplenism may also develop (Butterworth and Thomas 1999; Davis 2009; Elbaz and Esmat 2013).

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, severe collagen deposition in the space of Disse, ischaemic damage from repeated variceal bleeding, and severe distortion of arteriovenous relationships in the portal tracts. Alcohol

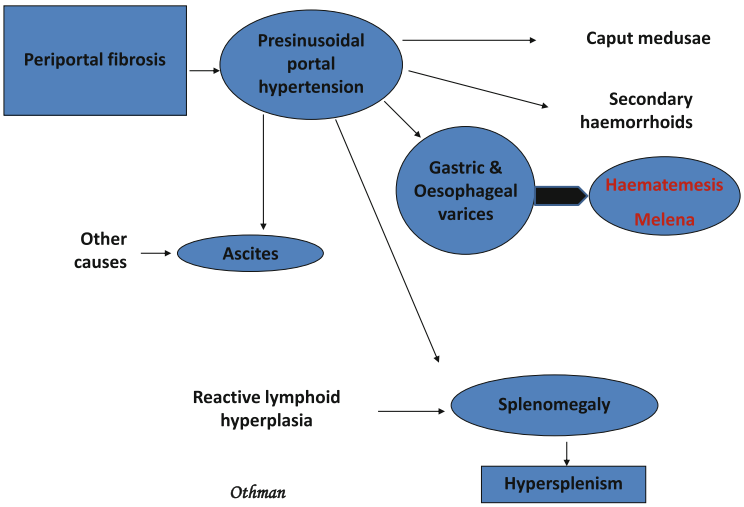

Fig. 3.2 Pathogenesis of hepatic schistosomiasis

abuse and viral hepatitis types B, C, D, or E are additional complicating factors (Watt et al. 1991; Davis 2009).

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 the 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 Disse's spaces of the liver sinusoids (Fig. 3.3), and they constitute a minor cell type, roughly 5–8 % of the total liver cells (Maubach et al. 2006). 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).

Several immunological and non-immunological determinants govern the progression of schistosomal fibrosis. The role of cytokines, such as IL-4 and IL-13, has been discussed before. Regulatory cytokines such as IL-10, IL-12, and transforming growth factor β also influence the fibrogenic response of the host (Othman et al. 2010). Alternatively activated macrophages (aaMΦ) are hypothesized to

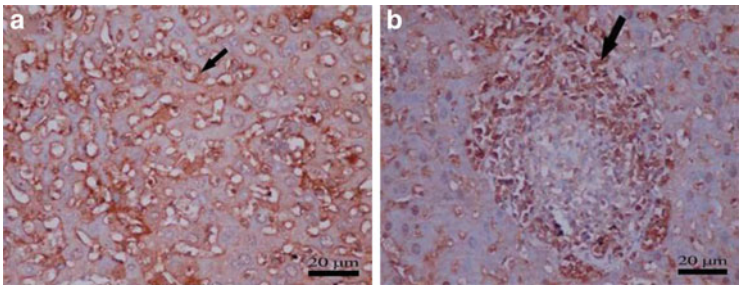

Fig. 3.3 Hepatic immunohistochemical stain for HSCs in *Schistosoma*-infected mouse showing (**a**) sinusoidal "arrow" and (**b**) mesenchymal "arrow" HSCs (immunoperoxidase GFAP ×400)

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 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, while 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).

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 translated 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 the portal tract thickness: Grade I if thickness is 3–5 mm, Grade II if it is 5–7 mm, and Grade III if it is more than 7 mm. This method reflects the haemodynamic 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, the short gastric, the paraumbilical, the splenointercostal, and the 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).

Interactions with Viral Hepatitis

Co-infection 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 more severe 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 the suppression of the intrahepatic bystander immune response to HCV. Moreover, this co-infection can produce a unique clinical, virological, and histological pattern manifested by viral persistence with high HCV RNA titres, higher necro-inflammatory 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).

3.5.4.4 Anaemia

Schistosomiasis-associated anaemia is well documented and the cause is multifactorial. Elevated levels of proinflammatory cytokines, such as TNF-α and IL-6, resulting in anaemia of inflammation, are thought to play a central role. Moreover, for intestinal schistosome species, microcytic hypochromic anaemia may occur due to 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. In schistosomiasis haematobium, anaemia may develop due to blood loss in urine (Friedman et al. 2005; Davis 2009). Interestingly, *S. mansoni* SEA has recently 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 wasting in adults and growth retardation and cognitive impairment in children.

3.5.4.5 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).

Central nervous system (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 the 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 one 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). Cerebrospinal fluid (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 nonspecific 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 nonspecific. 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).

3.5.4.6 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.

3.5.4.7 *Schistosoma–Salmonella* Interactions

Concurrent *Schistosoma–Salmonella* infections occur when enteroinvasive *Salmonella* enter the systemic circulation and attach to the tegument of adult *Schistosoma* present in the mesenteric vasculature (LoVerde et al. 1980; Melhem and LoVerde 1984). This interaction apparently provides a refuge in which the bacterium can evade systemic antibiotic therapy. 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 co-infected with *Salmonella*. Furthermore, therapy with the anthelmintic praziquantel can lead to a massive release of schistosome-associated *Salmonella* causing bacteraemia if the appropriate antibiotic is not co-administered to co-infected individuals. Finally, the use of ineffective antibiotics contributes to antibiotic resistance development and the phenomenon of bacterial persistence (Barnhill et al. 2011).

3.5.4.8 Other Manifestations

Deposition of eggs in the skin causes papular dermatitis. Growth retardation and infantilism may occur in schistosomiasis partly due to decreased levels of somatomedins. Moreover, polyarthritis may occur in *S. mansoni* infection, while *S. haematobium* may cause osteomalacia resulting from tubular lesions in association with obstructive uropathy (Butterworth and Thomas 1999).

3.6 Diagnosis

3.6.1 Parasitological Techniques

The 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. 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 × 100 μm) than those of *S. mansoni* (61 × 140 μm; prominent lateral spine) and *S. haematobium* (62 × 150 μm, prominent terminal spine), which are both ovoid. The 10 ml urine sedimentation and 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 the diagnosis of intestinal schistosomiasis, despite its limited 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 the 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 eggshell (Davis 2009).

3.6.2 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 schistosome adult worm (SAWA)- or egg (SEA)-derived antigens or selected antigens, e.g. species-specific microsomal antigens, cathepsin B, etc., have been used for immunodiagnosis by indirect haemagglutination assay (IHA), enzyme-linked immunosorbent assay (ELISA), and/or dipstick dye immunoassay, all of which showed high sensitivity (Al-Sherbiny et al. 1999; Noya et al. 2002; Xiang et al. 2003; Zhou et al. 2007, 2008, 2011a). 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. 2012a, b). Antibody detection methods are limited in their specificity as they 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 (Lei et al. 2011; Demerdash et al. 2013). 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 access to 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* (Stothard et al. 2006; Ashton et al. 2011; Shane et al. 2011; Utzinger et al. 2011; Navaratnam et al. 2012; Tchuem Tchuenté et al. 2012; Bergquist 2013; Coulibaly et al. 2013; Erko et al. 2013; Grenfell et al. 2013).

3.6.3 Molecular Techniques

Conventional or real-time polymerase chain reaction (PCR) amplification of repetitive elements specific to each schistosome species has been used for the diagnosis of *S. mansoni* (Pontes et al. 2002, 2003; Rabello et al. 2002; Abath et al. 2006; Gomes et al. 2006; Allam et al. 2009), *S. haematobium* (Abbasi et al. 2007; Obeng et al. 2008;

Aryeetey et al. 2013), and *S. japonicum* (Xu et al. 2010; Zhao et al. 2012a) using stool, urine, or serum samples.

3.7 Treatment of Schistosomiasis

3.7.1 Schistosomicidal Agents

3.7.1.1 Praziquantel (PZQ)

Praziquantel 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). 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 the interior of the parasite early after treatment (Mehlhorn et al. 1981; Andrews 1985; 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 include direct and dose-related effects such as nausea and abdominal pain, headache, and dizziness, as well as indirect effects attributable to the death of worms such as fever, urticaria, pruritus, 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-age children (WHO 2006).

Two problems are noteworthy with PZQ: first, the emergence of drug resistance—isolates with decreased sensitivity to praziquantel have been reported from different epidemiological settings, e.g. Egypt, Kenya, and Senegal (Ismail et al. 1996; Cioli et al. 2004; Melman et al. 2009), and may be harbingers of more to come. Second, the drug is not effective against juvenile forms of the parasite. It has little activity against eggs or immature worms (schistosomula) 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).

3.7.1.2 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). Oxamniquine is no longer used on a large scale (El Ridi and Tallima 2012).

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 adult *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).

The most successful plant product in the treatment of human schistosomiasis was myrrh, an oleo–gum–resin extracted from the stem of *Commiphora molmol* (Tonkal and Morsy 2008). The product is believed to affect schistosome musculature, leading to uncoupling of male and female couples and their extravasation to the liver (Badria et al. 2001). The product has been licensed for human use by the Egyptian Ministry of Health and introduced in the Egyptian market. However, conflicting reports on its efficacy shed doubts upon the usefulness of its use as a novel therapy for schistosomiasis (Osman et al. 2010).

3.7.1.3 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). Curcumin, the major constituent in the rhizome of *Curcuma longa*, has been shown to display potent schistosomicidal activities in vivo and in vitro against adult *S. mansoni* worms (El-Ansary et al. 2007; El-Banhawey et al. 2007).

An essential fatty acid, a component of our diet and cells, the polyunsaturated fatty acid arachidonic acid 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 attrition. This concept was convincingly supported using larval and adult *S. mansoni* and *S. haematobium* worms in in vitro experiments and in vivo studies in inbred mice and outbred hamsters (El Ridi and Tallima 2013a, b).

Of great interest is the class of compounds targeting schistosome histone-modifying enzymes, namely, histone acetyltransferases and histone deacetylases, leading to parasite apoptosis and death in in vitro 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 (Keiser and Utzinger 2007; Xiao et al. 2007).

Recently, there has been an 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 praziquantel (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 antischistosomals on their own.

Follow-Up 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 (Davis 2009). 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 an excellent test for establishing cure.

3.7.2 Symptomatic Therapy and Management of Complications

For the 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 the 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. Other procedures for portal hypertension include the minimally invasive transjugular intrahepatic portosystemic shunt (TIPS). 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).

3.8 Prognosis

Schistosomiasis remains a major cause of morbidity in tropical and subtropical countries (Engels et al. 2002). Clinical observations in schistosomiasis endemic areas show that major complications of *Schistosom*a infection develop in

approximately 4–12 % of the population, while the majority of infected people remain asymptomatic or exhibit mild nonspecific 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 reexposure 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 (Ohmae et al. 1992; Butterworth and Thomas 1999; Lucas 2002; Andrade 2008).

3.9 Prevention and Control

3.9.1 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 morbidity and prevent infected individuals from developing severe, late-stage morbidity due to schistosomiasis. For the assessment of prevalence of *Schistosoma* infection in a suspected endemic area, schoolchildren 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-age children and adults at risk in high-risk communities (≥50 % prevalence of infection by parasitological methods), once every 2 years to all school-age children and adults at risk in moderate-risk communities (≥10 % but <50 % prevalence by parasitological methods), and twice during primary schooling age to all school-age children and as a 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 as follows: 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).

3.9.2 Vaccine Development

3.9.2.1 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). Health and hygiene education and improving sanitary and living conditions are required, yet are not available and are not expected to be available in a near future in most countries where schistosomiasis is endemic (Utzinger et al. 2009, 2011; King 2010). 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.

3.9.2.2 The Prospects of the Candidate Vaccine Antigens

During the 1990s of the last century, 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) and residents of endemic countries, such as Egypt and Brazil, that are susceptible or resistant to reinfection after praziquantel treatment (de Jesus et al. 2000; 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 or alum adjuvant, failed to induce the benchmark of 50 % protection in mice in independent trials sponsored by the World Health Organization (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). Glyceraldehyde 3-phosphate dehydrogenase (SG3PDH) in a recombinant, linear peptide, di- and tetrabranched MAP construct, administered to inbred and outbred mice intramuscularly or subcutaneously without adjuvant or emulsified in Freund's, alum, Allison's, or Ribi adjuvant, led to only 10–35 % protection against challenge *S. mansoni* infection [reviewed in El Ridi (1998) and 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 are currently 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). More recently, other members of the tetraspanin family of integral membrane proteins, namely, 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 not reproduced on using *S. japonicum* tetraspanins (Zhang et al. 2011). However, large amounts of the extracellular domain of Sm-TSP-2 are being now generated at Baylor College of Medicine, ready for phase I clinical studies (Curti 2012), notwithstanding its poor performance (Cai et al. 2008; Zhang et al. 2011; Pearson et al. 2012) and the lack of highly significant protective potential of other integral membranes such as Sm23 (Ganley-Leal et al. 2005), *S. mansoni* glucose transporter SGTP4 (Mahana 2006), and numerous other molecules residing in the tegument of the parasite (Cardoso et al. 2008; Rofatto et al. 2013; Teixeira de Melo et al. 2013).

Of note, glutathione S-transferase of *S. haematobium* progressed to clinical trial phase I using alum as adjuvant; safety and tolerability were documented, but no reports on the efficacy against urinary schistosomiasis are available as yet (Riveau et al. 2012). The fatty acid-binding protein Sm14 also progressed to preclinical and clinical studies (Tendler and Simpson 2008); yet, no information concerning efficacy against infection with *S. mansoni* were reported.

Surprisingly, most if not all schistosomiasis vaccine experiments aimed to achieve preponderance of Th1-related cytokines and antibodies (Da'Dara et al. 2003; Cardoso et al. 2008; Siddiqui et al. 2011; Teixeira de Melo et al. 2013; Wang et al. 2013), while resistance to schistosome reinfection in humans, to whom the vaccine is supposedly dedicated, was previously and recently shown to be dependent on type 2 immune responses (Hagan et al. 1991; Roberts et al. 1993; Fallon et al. 2003; Ganley-Leal et al. 2006; Jiz et al. 2009; Figueiredo et al. 2012; Fitzsimmons et al. 2012).

3.9.2.3 The Breakthrough

A Plausible Mechanism of Immunity

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). The immune system effectors may, however, recognize and interact with the "scent" molecules, the ESP 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 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 ESP might stagnate, attracting the hunting cells in close proximity to the migrating schistosomula. Accordingly, ESP released by developing schistosomula should be excellent vaccine candidates. Indeed, many of the candidate vaccine molecules are larval ESP (El Ridi and Tallima 2009).

To develop an ESP-based vaccine, it is important to examine the "hunting" team summoned upon parasite invasion (von Lichtenberg et al. 1977). Larval ESP induce 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. Accordingly, circulating monocytes and neutrophils would be readily recruited and activated and certainly contribute to the 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 skews 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. Four consecutive experiments were set up whereby groups of 5–10 outbred CD1 mice were injected subcutaneously with 200 µl Dulbecco's phosphate-buffered saline (D-PBS), pH 7.1, or 50 µg papain in 200 µl D-PBS, 1 h before whole-body exposure to 125 cercariae of *S. mansoni*. Worm burden was evaluated 6 weeks after infection. For every experiment, papain injection consistently and reproducibly elicited highly significant ($P < 0.0001$) reduction in worm burden of 70 % ± 3 (mean ± SD for four independent experiments). The reduction was also highly significant for worm liver ($P < 0.0001$) and small intestine ($P < 0.001$) egg counts but only of approximately 50 % (Table 3.1). 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 ($P < 0.0001$) protection against schistosomiasis mansoni in every test mouse as compared to controls. These findings gave a sound proof that a large proportion of invading *S. mansoni* larvae would succumb if met upon host invasion with type 2 immune responses.

A Vaccine Formula

A series of 8 successive experiments led us to define a formula for an efficacious vaccine for schistosomiasis, leading to consistent, reproducible, and highly significant ($P < 0.0001$) reduction in challenge *S. mansoni* worm burden and worm egg load in the liver and small intestine of 62–75 % in immunized outbred, akin to man, mice as compared to controls (El Ridi and Tallima 2013c). The formula is as follows: larval ESP mix + type 2 cytokine such as TSLP, IL-25, or IL-33. It is important to examine the different ESP for optimal results. In our hands, a mixture of rSG3PDH and a MAP construct based on a peptide of *S. mansoni* 2-cys peroxiredoxine (TPX MAP Mix) was more protective than a mixture of rSG3PDH and a MAP construct based on a peptide of *S. mansoni* aldolase (ALD MAP Mix) upon immunization of outbred mice in conjunction with TSLP (200 ng/mouse) or papain (10 µg/mouse) as adjuvant (El Ridi 2012; El Ridi and Tallima 2013a).

Table 3.1 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 ± 7,777	17,187 ± 3,575 (<0.0001; 52.71 %)
Small intestine egg counts	31,035 ± 9,251	15,187 ± 3,835 (0.0002; 51.06 %)

Representative of four independent experiments whereby ten mice/group were subcutaneously injected with 100 µl phosphate-buffered saline, pH 7.1 (PBS) or 50 µg papain in 100 µl PBS one hour before exposure to *S. mansoni* cercariae via whole body exposure. Parasitological parameters were evaluated six weeks after infection, and data analyzed using Student's *t*-test

A Proof of Concept Leads to an Adjuvant-Free Vaccine Formulation

Immunization of outbred mice with schistosome antigens, which are both ESP and type 2 immune response inducing, consistently and reproducibly elicited highly significant ($P < 0.0001$) reduction (60–75 %) of challenge *S. mansoni* worm burden and worm egg load in the liver and small intestine as compared to unimmunized mice. It is, thus, fortunate that we developed an adjuvant-free Th2 immune response-based vaccine effective in outbred mice, since it is destined to the outbred human population, where adjuvant use remains a considerable challenge and where immunological correlates of resistance to the 3 species of schistosomes are associated with type 2 responses (El Ridi et al. 2014).

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Chapter 4
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 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 fascioliasis. 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. Serious complications, sequelae and death causes should be highlighted. New knowledge has allowed improvement of individual infection prevention measures and community control. Challenges appear in vaccinology, indicating that a human vaccine is still far from affordable.

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4.1 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. 1999a, 2009a), including estimations of 2.4 million up to 17 million people or even higher depending on the hitherto unknown situations in mainly 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, 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 (WHO 2013).

4.2 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 characterised by the branching of their caeca, testes and ovary; the very numerous small vitellaria extending bilaterally up to the hind body; and a short uterus located between the ovary and the caecal bifurcation (Fig. 4.1). The eggs are operculated, ovoid, yellow and non-embryonated when laid.

Fasciola gigantica is more elongated and narrower, with lateral walls tending to be parallel and with non-existent or less marked shoulders of the cephalic cone (Fig. 4.1). 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).

Adult worms parasitise the large biliary passages and the gall bladder of ruminants, mainly sheep, goats, cattle and many other herbivorous domestic and wild animals, including horses, donkeys, mules and also Old and New World camelids. Buffalo, deer, wild sheep, wild pig, various marsupials, rabbit, hare and nutria are also susceptible hosts. Grazing domestic pigs may also be infected, but this host usually shows a higher natural resistance against the liver fluke (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*. Among wild definitive hosts in Europe, *F. hepatica* seems to be less adapted to the roe deer (*Capreolus capreolus*) when compared to other deer (red and fallow deer), and the introduced nutria (*Myocastor coypus*) has become an important reservoir in France and may also be so in areas of South America where it originated.

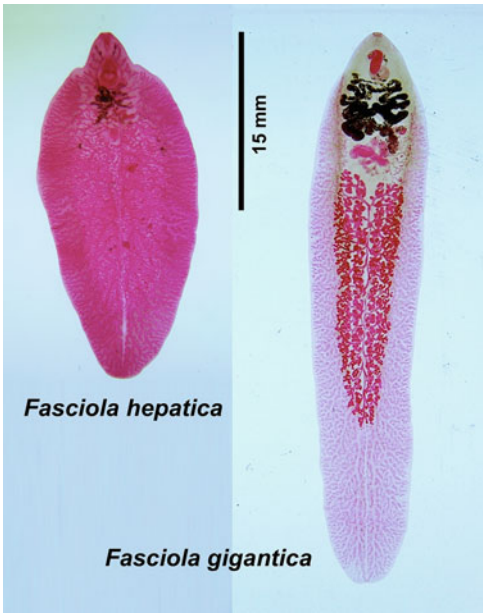

Fig. 4.1 Adult stages of pure *Fasciola hepatica* and pure *F. gigantica*. Note different size and shape; hybrid forms usually show an intermediate form. The two photographs are at the same scale (Orig. S. Mas-Coma)

Humans are susceptible hosts to the infection by both *Fasciola* species (Mas-Coma et al. 2009a).

The two-host life cycles of both fasciolids are similar and take about 14–23 weeks. These comprise four phases (Mas-Coma and Bargues 1997):

(a) The definitive host harbours fluke adults producing eggs which reach the external milieu by way of 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 physico-chemical 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 water; the prepatent period (38–86 days) is dependent on temperature, and higher temperatures reduce 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, independent 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. 4.2); metacercarial cysts become infective within 24 h. Liver fluke development is very

Fig. 4.2 Metacercarial body of *Fasciola hepatica* shortly after detaching from its swimming tail and already starting encystment process (Orig. S. Mas-Coma)

dependent on 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. *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 colonised all continents and appears to be able to transmit both *Fasciola* species (Bargues et al. 2011c).

The presence of lymnaeid vectors not only defines 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. 2011b) and Chile (Artigas et al. 2011), and within an endemic area, as well as its seasonality or permanent transmission (Mas-Coma et al. 1999c). 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 waterbodies presenting lymnaeids (Mas-Coma et al. 1999c).

4.3 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. 2005, 2009a).

In the human hyperendemic areas, though more prevalent and intense in children (with a peak in the 9- to 11-year age group), adult subjects are also infected. 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).

4.3.1 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. (1999a) 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 4,200 m; hot and cold weathers; seasonal and yearly constant temperatures; scarce to pronounced annual rainfall; low and high mean annual potential evapotranspiration; and seasons 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 waterbodies (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. 2012c), (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.

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.

4.3.2 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 waterbodies instead of temporary ones due to the high evapotranspiration rates at the very high altitude (Mas-Coma et al. 1999c). In other areas, the transmission appears mono-seasonal, due to the existence of only one intra-annual 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). 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).

4.3.3 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):

- Ingestion of freshwater wild plants: important in animal endemic areas
- Ingestion of freshwater cultivated plants, mainly watercress
- Ingestion of terrestrial wild plants: collected in dry habitats but which were submerged in water a few weeks or months before
- Ingestion of terrestrial cultivated plants needing frequent irrigation
- Drinking of contaminated water
- Ingestion of dishes and soups made with contaminated water
- Washing of kitchen utensils or other objects with contaminated water
- Ingestion of raw liver infected with migrating metacercariae which may keep the capacity to restart migration

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).

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).

4.4 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).

4.4.1 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 fascioliasis too. The results indicated that during early chronic infection there was a predominance of a Th2 response, which decreased in the advanced chronic infection characterised 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. 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 post infection and is characterised 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 by 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 metabolises L-arginine and are important in promoting Th2 responses and facilitating tissue repair and fibrosis (Dalton et al. 2013).

4.4.2 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 co-infection 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, b, 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 recognised pathogenic parasites were recorded. A significant positive association of great health importance in children was found with *Giardia intestinalis*, in both Bolivia and Peru (Esteban et al. 1997a, b, 1999, 2002).

4.5 Pathology

Pathogenesis depends on the number of flukes. 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. 4.3) (Chen and Mott 1990). The ultrastructural picture revealed bile ductular

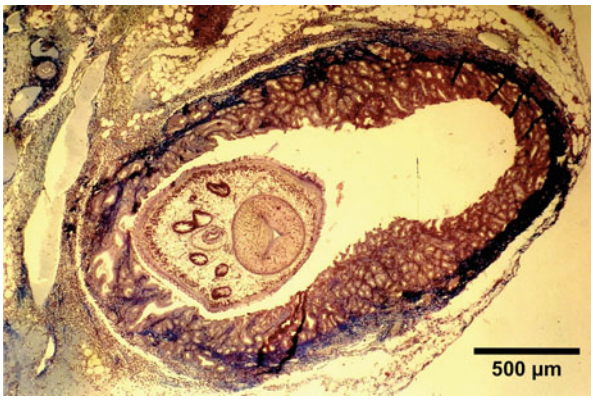

Fig. 4.3 Histopathological section showing a specimen of *Fasciola hepatica* in a rat biliary duct. See section of large sucker inside and prominent spines in the tegumental external surface of the worm (Orig. S. Mas-Coma)

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.

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 % 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 summarised as follows: (a) the fluke is a blood feeder but may also feed on tissue; (b) haemorrhages may occur from the erosion of the biliary epithelium due to the infection; (c) reticulocytes are increased in the peripheral blood; (d) generalised haemolysis is absent; (e) 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 fascioliasis.

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).

4.5.1 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. 4.3) and dilated, and the wall is thickened upon palpation. The gall bladder wall is greatly thickened and oedematous. Multiple greyish-white subserous nodules are present, and adhesions of the gall bladder to adjacent structures are common. The mucosal folds of the gall bladder appear prominent. The wall of the gall bladder 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 gall bladders 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, necrotising arterial vasculitis and portal venous thrombosis are frequent (Acosta-Ferreira et al. 1979; Chen and Mott 1990).

4.5.2 Other Locations and Ectopic Fascioliasis

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 emphasise 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).

4.6 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).

4.6.1 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 localised or generalised 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 generalised at the outset but is usually localised 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, haemoptysis 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 is 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.

4.6.2 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 gall bladder 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 the 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 gall bladder is usually enlarged and oedematous with thickening of the wall. The gall bladder may measure $12 \times 7 \times 7$ cm and the lower edge reaches the umbilicus. Fibrous adhesions of the gall bladder to adjacent organs are common. Lithiasis of the bile duct or the gall bladder is frequent, and the stones are usually small and multiple (Chen and Mott 1990; Arjona et al. 1995). The bile duct and the gall bladder may contain blood mixed with bile (haemobilia), blood clots and fibrinous plugs.

Symptomatology in children from human endemic areas of Peru includes abdominal pain localised 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 fascioliasis may be related to biliary sepsis. These results lead to the reconsideration of treatment features in human disease, i.e. therapeutic strategies should also consider the possibility of bacterial co-infection (Valero et al. 2006b).

4.6.3 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/mm^3 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. 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 in both 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 utilised, 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.

4.7 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 the haplotyping of the two pure fasciolid species and the detection of hybridisation 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).

4.7.1 Direct Techniques

Detection and identification of fasciolid eggs in stool sample, duodenal contents or bile continue to be the most appropriate diagnostic strategy for both detection of infection and estimation of intensity. This is even in spite of the recognised 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 the egg counts per g of faeces and the fluke burden (Valero et al. 2006a, 2009). Identifying fluke adults obtained during an endoscopy after surgical intervention by either 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. 1999a).

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 spaced mid-dose is recommended in patients shedding more than 400 eggs (WHO 2007; Valero et al. 2012a). The second half of the regimen is administered 24 h later, once the absence of secondary effects 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 and duodenal and biliary aspirates, surgery (laparotomy, cholecystectomy, sphincterotomy), histological examination of liver and/or other organ biopsy materials (Mas-Coma et al. 1999b).

4.7.2 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 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 post infection), intermittent egg output dynamics, very low or even absence of egg shedding in cases of only one or a few adult flukes and old, chronic infections, ectopic infections and "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. 2012a). 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 commercialised 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. 2012b).

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.

4.8 Treatment

Emetine and the better tolerated dehydroemetine were used widely and still continue to be in use 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 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). This drug is better adsorbed if administered after meals (Lecaillon et al. 1998). The recommended dosage is two separate regimens of 10 mg/kg. Cure rates of 79.2 % when first used and 100 % after a second round of therapy were 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).

Unfortunately, the risk of appearance of resistance to triclabendazole cannot be forgotten. Triclabendazole resistance was first described in Australia, 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).

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 for several years. Nitazoxanide had demonstrated its efficacy 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 Barrios 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).

4.9 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 normalisation 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 gall bladders.

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 serious complication is the multiple extrahepatic venous thromboses. In the post-mortem examination of one patient who died suddenly, multiple thromboses of the ovarian, suprahepatic, mesenteric and myocardial veins, along with massive pulmonary embolism, were disclosed. During the invasion period, another patient developed a complete thrombosis of the superior vena cava (Mas-Coma et al. 1999b, 2000).

The seriousness of fascioliasis was emphasised 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 fascioliasis 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 fascioliasis. 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 fascioliasis 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).

4.10 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.

4.10.1 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 cities, plants carrying metacercariae can be sold in non-controlled city markets giving rise to urban infection (Fig. 4.4) (Mas-Coma 2004).

Fig. 4.4 Uncontrolled sale of vegetables involved in the transmission of human fascioliasis in a city market in Quy Nhon, Vietnam (Orig. S. Mas-Coma)

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.

4.10.2 Control Measures at Community Level

The availability of triclabendazole prompted the WHO to launch a decisive step forwards 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 serious 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 utilisation 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).

4.10.3 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 characterising 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.

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Chapter 5
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 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 industrialized countries are not exempted to acquire these pathogens due to an increasing consumption of raw fish. Near one third of infected persons are asymptomatic. 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. The drug of choice is praziquantel. Health education and implementation of food safety measures can prevent infections and morbidity.

5.1 History

An early documentation of clonorchiasis in the human beings dates back some 2,000 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 XV 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

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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 viverrinus*, 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).

5.2 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. 5.1). About 1–2 months after infection, operculate eggs (22–35 × 10–22 μm) containing the larval stage, or miracidium, are shed with feces (Fig. 5.1). An adult worm can produce from 1,000–4,000 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 *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 rediae, 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. 5.1). 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 carcariae lose their eyespots and develop a saclike excretory bladder filled with coarse, refractile granules. The metacercarial stage is usually

ovoid (140 × 120 μm) with a thin wall (Fig. 5.1). 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).

5.3 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). Different populations were identified in each fluke species according to the region of origin, but the genetic differences were in general low. The study of microsatellite markers of *O. viverrini* allowed to identify different populations in Lao People' s Democratic Republic (PDR) and Thailand (Laoprom et al. 2010, 2012).

5.4 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, Ukraine, and in 13 countries of the European Union (EU) (Pozio et al. 2013) (Fig. 5.2, panel A).

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. 5.2, panel B).

O. viverrini is endemic in Cambodia, Lao PDR (mainly southern areas), Thailand (mainly northeast areas), and southern Vietnam (Andrews et al. 2008; Sohn et al. 2011, 2012; Yong et al. 2012) (Fig. 5.2, panel B).

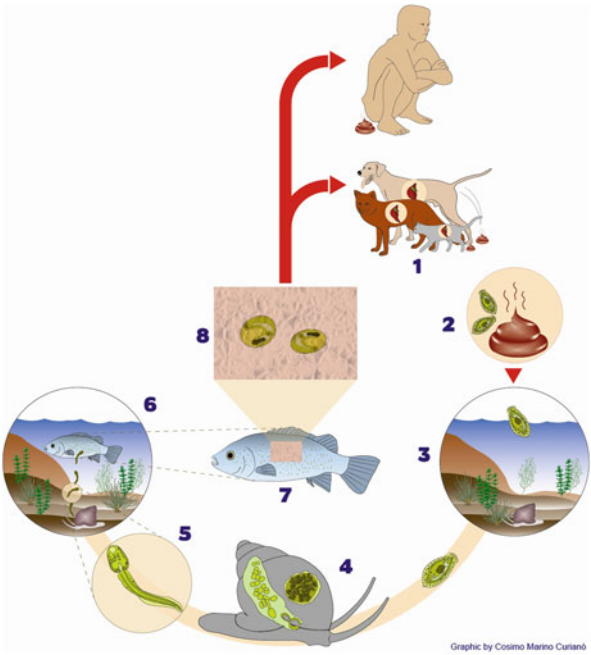

Fig. 5.1 The natural life cycle of *Clonorchis sinensis*, *Opisthorchis felineus*, and *O. viverrini*

5.5 Intermediate Hosts

The prevalence of infection of gastropoda molluscus 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). An increasing number of reports suggest that the genetic diversity of the gastropoda molluscus 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. leachi*, 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 goniomphalos*) (Petney et al. 2012; Sithithaworn et al. 2012).

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

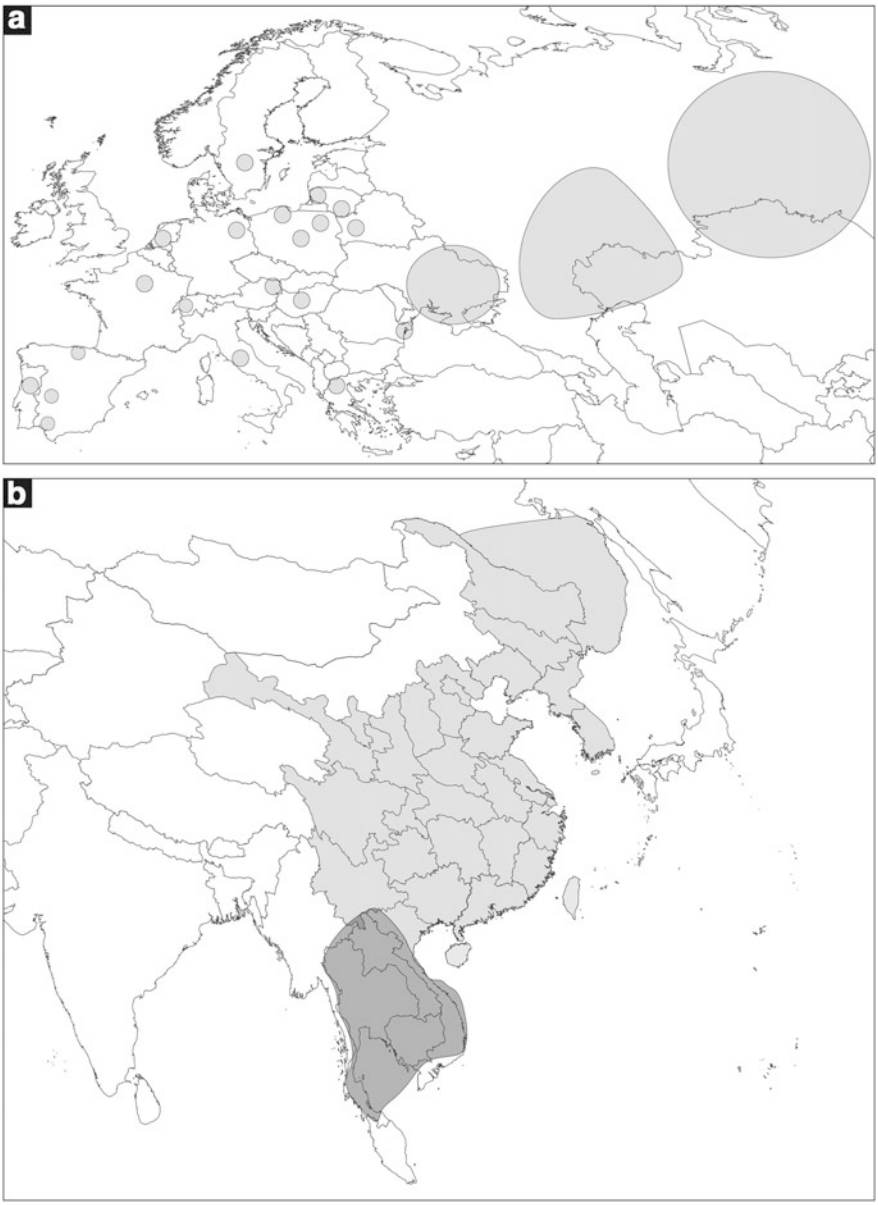

Fig. 5.2 Distribution areas of *Clonorchis sinensis*, *Opisthorchis felineus*, and *Opisthorchis viverrini*. Panel A, *O. felineus* distribution area. Panel B, *C. sinensis* distribution area (*light gray*); *O. viverrini* distribution area (*dark gray*)

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 one to hundreds, but over 30,000 parasites have been detected per fish with more than 6,000 metacercariae/g depending on species and 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.

5.6 Final Hosts

All fish-eating mammals, including humans, can act as final hosts of *C. sinensis*, *O. felineus*, and *O. viverrini* (Table 5.1), but their role as reservoir hosts is strongly influenced by biological, ecological, and epidemiological factors, including the human impact on fishing. 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.

Table 5.1 Epidemiological pattern of the etiological agents of opisthorchiasis and clonorchiasis

Etiological agent	Distribution	Main hosts 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)
		II intermediate hosts 132 species	Lun et al. (2005)
		Final hosts Human, dog, cat, pig, rat	Lun et al. (2005)
Opisthorchis felineus	Belarus, Russia, West Siberia, Ukraine, Austria, France, Germany, Greece, Hungary, Italy, Lithuania, the Netherlands, Poland, Portugal, Romania, Spain, Scandinavia	I intermediate hosts *Bithynia inflata*, *B. leachi*, *B. troscheli*	Erhardt et al. (1962) Hering-Hagenbeck and Schuster (1996)
		II intermediate hosts *Alburnus alburnus*, *Abramis brama*, *A. ballerus*, *Blicca bjoerkna*, *Idus idus*, *Rutilus rutilus*, *Scardinius erythrophthalmus*, *Tinca tinca*	Lazuthina et al. (2009) Mordvinov and Furman (2010)
		Final hosts Human, dog, cat, fox (3 species), seal (3 species), wolf, mustelids (11 species), raccoon dog, raccoon, wild boar, rodent (4 species)	Mordvinov et al. (2012) Pozio et al. (2013)
Opisthorchis viverrini	Cambodia, Lao PDR, Thailand, Vietnam	I intermediate hosts *Bithynia funiculate*, *B. siamensis siamensis*, *B. siamensis goniomphalos*	Andrews et al. (2008), Petney et al. (2012)
		II intermediate hosts *Cyclocheilichthys*, *Hampala*, *Puntius*	Sithithaworn et al. (2012)
		Final hosts Human, dog, cat	Wykoff et al. (1965)

5.7 Epidemiology

Over 10 % of world inhabitants, i.e., 750 million people, was estimated to be at risk of foodborne trematodiases (WHO 1995). According to Sithithaworn et al. (2007), 35 million people are infected with *C. sinensis*, and of them, 15 million are Chinese people (Lun et al. 2005). In Southeast Asia, 67 million people were estimated to be at risk of *O. viverrini* infection (Keiser and Utzinger 2005). In 2003, Sithithaworn and Haswell-Elkins estimated a prevalence of *O. viverrini* infection in the Mekong region of eight million in Thailand and two million in Laos. No prevalence data are

available in Vietnam even if the presence of *O. viverrini* infection in humans has been documented (Sithithaworn et al. 2006; Sayasone et al. 2007). In Cambodia, a high endemicity of *O. viverrini* infections in humans has been documented. In a southern area of the country, the prevalence was assessed as high as almost 50 % among the surveyed people (Yong et al. 2012). The number of *O. felineus* infections in humans has been estimated to be 1.2 million (WHO 1995).

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 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 1 million people are estimated to be infected (Figurnov et al. 2002). In North Vietnam, 1 million people were estimated to be infected (Dang et al. 2008). Globally, 1.5–2 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 recent investigation shows that cats are the most important animal reservoir of human opisthorchiasis in Northeast Thailand with a prevalence of infection of 35.51 % (Aunpromma et al. 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. 2012; Sripa et al. 2012).

Opisthorchis felineus shows two well-distinct transmission patterns in the EU, Eastern Europe (Byelorussia, Russia, and 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 (five cases in Germany and two 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). In Russia, Ukraine, and Kazakhstan, 12.5 million people have been considered to be at risk for *O. felineus* (Keiser and Utzinger 2005). In these foci, both humans and domestic animals (cats and dogs) play the role as final hosts (Mordvinov et al. 2012). In the Tomsk region of Siberia, the prevalence of

opisthorchiasis in humans increased from 495 cases per 100,000 inhabitants to 649 cases per 100,000 inhabitants between 1997 and 2006 (Mordvinov et al. 2012). Other endemic foci of *O. felineus* in Siberia are the Ob river and the Irtysh river basins.

5.8 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.

5.8.1 Clonorchis sinensis

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. 5.3, 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. Periductal infiltrates of mononuclear cells are frequently found; however, inflammation of the bile duct walls is slight in uncomplicated cases. On the contrary, 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. These changes are distinctive features of clonorchiasis; therefore, when they are observed in patients in an area of endemic infection, they are suggestive of infection (Sithithaworn et al. 2007). 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).

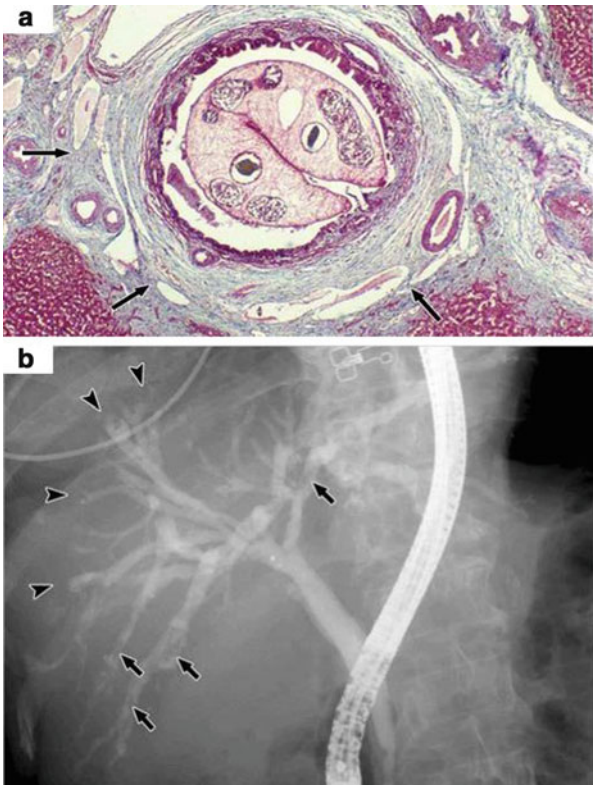

Fig. 5.3 (**a**) Photomicrograph of a pathologic specimen shows an adult fluke of *Clonorchis sinensis* in an intrahepatic bile duct. Note adenomatous hyperplasia of mucosa and severe fibrous thickening of bile duct wall (*arrows*). Masson's trichrome stain X 40. (**b**) Endoscopic retrograde cholangiogram 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, with permission)

5.8.2 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 (3,000–3,500 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-size 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 either

in autopsy (Riganti et al. 1989) or ultrasonographic studies (Mairiang et al. 1992, 2012). Following antihelmintic treatment, many of the gallbladder abnormalities can be eliminated, as indicated by the reduction of the length and regained contractility (Mairiang et al. 1993). Granulomatous inflammation around the parasite eggs is occasionally seen in the gallbladder wall during *O. viverrini* infections (Riganti et al. 1989).

5.8.3 Opisthorchis felineus

In *O. felineus* human infections, pathological changes seem to be similar to those induced by the others 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 recently 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).

5.8.4 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).

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 III (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 et al. 2012; Sripa et al 2012).

5.8.5 Carcinogenesis Induced by Liver Flukes

Infection in hamsters with *O. viverrini* closely mimics the carcinogenic processes in humans. This process starts by 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, 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 advancing fibrosis along its length. Periductal fibrosis is considered the precursor event to CCA and, similarly to human opisthorchiasis, progression of infection to CCA is accelerated by the inclusion of dietary nitrosamines (Sripa et al. 2007, 2012).

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 to the biliary epithelia (see Sect. 5.9), toxic effects of parasite excretory/secretory (ES) products, and the immunopathology due to infection-related inflammation (Sripa et al. 2012).

5.8.6 Toxic Effects of Parasite Excretory/Secretory 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* can induce cell proliferation which corresponds to hyperplasia of biliary epithelial cells in opisthorchiasis (Sripa and Kaewkes 2000). To understand the cellular response to *O. viverrini* ES products, gene expression analysis of NIH-3 T3 non-contact 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). Recently, 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*. It is the only helminth-derived growth factor reported to date that causes proliferation of mammalian cells (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).

5.8.7 Immunopathology Due to Infection-Related Inflammation

During liver fluke infections, macrophages, mast cells, eosinophils, epithelial cells, and neutrophils that infiltrate the sites of inflammation activated by the parasite-specific T cells and cytokines synthesize nitric oxide (NO) (Haswell-Elkins et al. 1994). Further, 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. Recently, a 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).

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, precancerous or cancerous lesions have been found during autopsy in *O. felineus* naturally infected animals, in particular cats and dogs (Pozio et al 2013).

5.9 Immune Response

5.9.1 Opisthorchis 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 post infection), whereas the expression of the Th2-inducing cytokine, IL-4, and the regulatory cytokines, TGF-β and IL-10, are 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 antihelmintic treatment (Wongratanacheewin et al. 1987).

5.9.2 Clonorchis 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 reinfected or superinfected rats; however, the rat resistance was not observed in immune-suppressed or nude rats (Zhang et al. 2008a, b). 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, b). 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).

5.9.3 Opisthorchis 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-immune response in man (Kotelkin et al. 2001).

5.10 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).

5.10.1 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 is 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).

5.10.2 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 1,000 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).

5.10.3 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 frequently reported symptoms include 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, and increased transaminases, e.g., aspartate aminotransferase, 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).

5.11 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. 5.3, panel B). However, in any case, the clinical diagnosis of liver flukes infections should be confirmed by the detection of eggs in stools.

5.11.1 Parasitological Diagnosis

Fecal examinations by the Kato-Katz (KK), the formalin–ether concentration technique (FECT), and 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). 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).

5.11.2 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 highly sensitive (limit of detection 20 ng/mL) polyclonal antibody capture ELISA for the detection of *C. sinensis* coproantigens in rat feces has been developed (Rahman Mazidur et al. 2012), but further evaluation of the method on a human large population from endemic areas has to be still carried out.

5.11.3 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), *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 it reaches 100 % in moderate to severe infections (eggs per gram >1,000), 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).

5.11.4 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 around 2 weeks for *O. viverrini* (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 sensitivity (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. 2010) and has a 93.1 % sensitivity when ES are used as antigen 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. 2010; 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), ELISA shows the best performance among all the serological tests. Recently, ELISA based on ES products has been validated for *O. felineus* infection in humans from low endemic areas (Gómez-Morales et al. 2013).

5.12 Human Treatment

Currently, the drug of choice to treat people with clonorchiasis or opisthorchiasis is praziquantel (2-(cyclohexylcarbonyl)-1,2,3,6,7,*11b*-hexahydro-4H-pyrazine-[2,1-a]-isoquinoline-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 antihelmintic 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 absorbed rapidly 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 (1995), the recommended dose for mass treatment is 40 mg/kg body weight. Praziquantel should be administered at a daily dose of 75 mg/kg body weight divided into three subdoses of 25 mg/kg body weight at 4–5 h intervals. 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, and drowsiness.

The second drugs of choice are the benzimidazole derivates 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.

5.13 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).

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Chapter 6
Echinococcosis

Francesca Tamarozzi, 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* 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, or 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.

6.1 Introduction

Cystic echinococcosis (CE) and alveolar echinococcosis (AE) are zoonoses of great medical and veterinary importance, caused by *Echinococcus granulosus* and *E. multilocularis*, respectively. The life cycle of these parasites develops between

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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, or 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 et al. 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 2,190 million US$ (Budke et al. 2006) (2). Despite these figures, these infections are still neglected (Budke et al. 2006).

CE is a chronic, complex, and neglected disease (Brunetti et al. 2011; Craig et al. 2007a) for the following reasons: (1) *E. granulosus* 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 impact on its spread as opposed to control in animals, but CE is not perceived as an important animal health problem, which impairs 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 record systems; and (4) it affects mostly poor pastoral communities, with a low fatality rate and with a difficult and expensive diagnosis and treatment.

AE is also a chronic, complex, and neglected disease similar to CE for the following reasons, among others: (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, requires a multidisciplinary approach, and is not available in many countries (Brunetti et al. 2010).

"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.

6.2 The Agent

6.2.1 Life Cycle

Echinococcus spp. are cestodes (tapeworms) of the family Taeniidae, with a predator–prey life cycle and a complex biology (Eckert and Deplazes 2004; Eckert et al. 2001).

The adult worm is 2–7 mm long and resides in the intestine of dogs, foxes, and other canids (Deplazes and Eckert 2001). It is composed of a scolex with four suckers and a rostellum with a double ring of 25–50 hooklets and a strobilus composed of 2–6 proglottids. These mature progressively from the scolex end, and the last one, gravid with hundreds of oncospheres (eggs), is released every 1–2 weeks. Eggs are 25–30 μm, round, with a striated embryophore which contains the

hexacanth larva, indistinguishable from eggs of other taenie of dogs. Eggs are dispersed in the environment, where they can survive up to two 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*, usually wild rodents of various species, and the lagomorph *Ochotona curzoniae*, on the Tibetan plateau of China (Vuitton et al. 2003), 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,* or 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, depends on 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 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.

6.2.2 The Hydatid Cyst and Its Natural History

The hydatid cyst is the form that develops in humans. It has a complex structure encompassing a parasitic part (hydatid) and a host-derived adventitia. From the outer to the inner surface, there are as follows: (1) the host-derived fibrous or adventitious layer (commonly called pericyst) 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 that form the endocyst; 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 multilaminated 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. 2011b) and has a pivotal role in immune evasion (Diaz et al. 2011a). From the germinal layer form brood capsules containing PSC, and sometimes daughter vesicles (commonly and improperly denominated daughter cysts) (da Silva 2011; Rogan et al. 2006; Galindo et al. 2002), which can be released in the HCF ("hydatid sand"). Each viable protoscolex (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 hydatidosis). 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). 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 the central nervous system, but rarer localizations have been described; in about 40 % of cases there are multiple localizations (Pedrosa et al. 2000; Eckert et al. 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. 2008a; Stojkovic et al. 2009).

CE cysts have been classified by the WHO Informal Working Group on Echinococcosis (WHO-IWGE 2003; Brunetti et al. 2010) in five groups: CE1 (unilocular), CE2 (with daughter vesicles), CE3 (with detached endocyst or with daughter vesicles in a solid matrix), CE4 (folded endocyst in a solid matrix), and CE5 (solid with calcifications). CL (cystic lesion) allocates cysts whose parasitic nature is unclear based on ultrasound only and deserves further evaluation (Fig. 6.1). CE1 and CE2 cysts are classified as active; CE3, transitional; and CE4 and CE5, 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 are further classified into CE3a (with detached endocyst) and CE3b (with daughter vesicles 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. 2008a; Golemanov et al. 2011).

If not modified by treatment, CE cysts are likely to pass spontaneously through several stages, from active to inactive (Romig et al. 1986; Keshmiri et al. 1999; Rogan et al. 2006; WHO-IWGE 2003), and tend to be stable over time (Frider et al. 1999) (Fig. 6.2).

About 20 % of patients show evidence of spontaneous involution of cysts (CE4-CE5) (Li et al. 2011b; Larrieu et al. 2004; Wen et al. 1994; Wang et al. 2006; Keshmiri et al. 2001).

6.2.3 *Alveolar Echinococcosis Lesions and Their Natural History*

The metacestode of *E. multilocularis* has a multicystic appearance, each cyst being 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 "microvesicles" (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

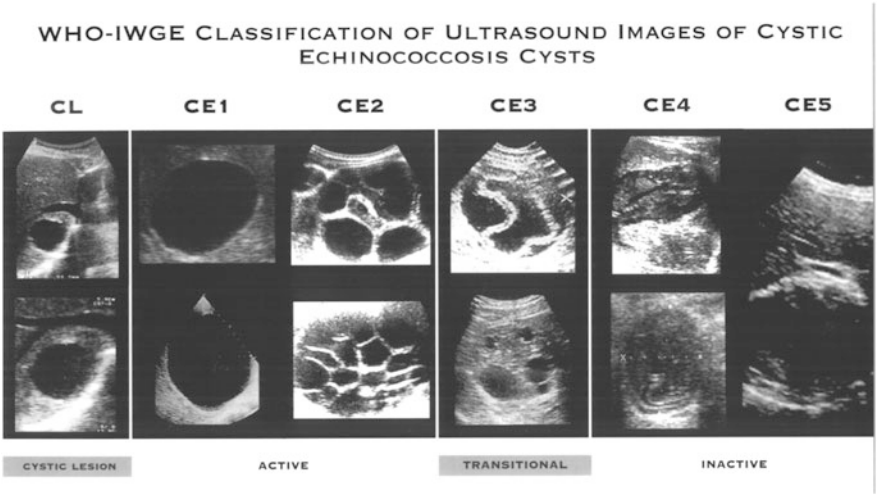

Fig. 6.1 WHO Informal Working Group Classification of Echinococcal Cysts. Cysts are grouped according to their activity. CE1 (unilocular) and CE2 (with daughter vesicles) are active, CE3 (with detached endocyst or with daughter vesicles in a solid matrix) are transitional, and CE4 (folded endocyst in a solid matrix) and CE5 (solid with calcifications) are inactive. CL (cystic lesion) is a cyst whose parasitic nature is uncertain and that requires further investigation

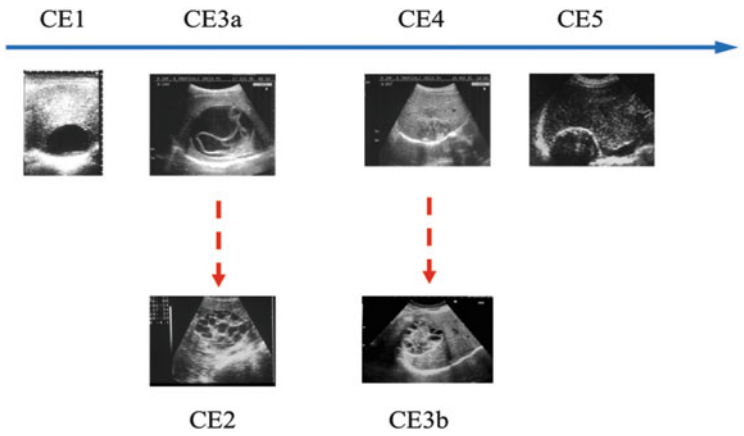

Fig. 6.2 Sequence of cyst stages as seen from diagnosis to successful nonsurgical treatment (From *L* to *R upper row*). This is also thought to be the same course followed by spontaneous involution. The *dashed lines* show the reactivation of CE3b producing a CE2 and the reactivation of CE4 producing a CE3b

(<20 %) in resistant hosts, such as humans, or most domestic 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

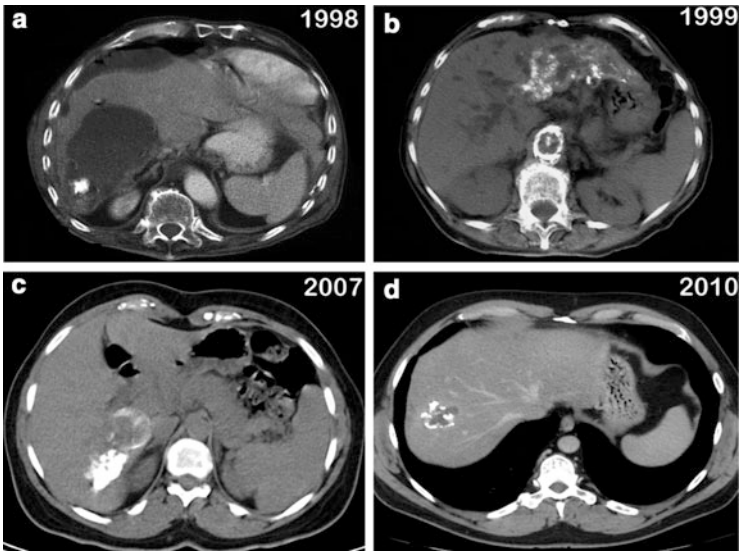

Fig. 6.3 Alveolar echinococcosis. CT-scan. (**a**, **b**) Typical images of AE lesions in symptomatic patients. (**a**) a huge AE lesion with central necrosis in the right liver of a 75-year-old man is diagnosed in 1998; (**b**) 1 year later, a very advanced AE invading both lobes of the liver, with 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, Besançon, France)

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 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. 2006) (Fig. 6.3).

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 behave 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 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).

6.3 Epidemiology of Infection

6.3.1 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 (Eckert and Deplazes 2004). 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 (Eckert et al. 2001). 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).

Recent figures (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; Eckert et al. 2001). Annual economic losses due to diagnosis and treatment costs in humans have been estimated at over 763 million US$, while global annual livestock-associated losses due to liver condemnation and decrease productions by infected animals were calculated at over 2,190 million US$ (Budke et al. 2006).

AE is only observed in the northern hemisphere, in geographical areas where *E. multilocularis* sylvatic life cycle can occur (Fig. 6.4). AE is a rare disease in most endemic regions where the cycle is maintained in the wild life only (<10/100,000 in

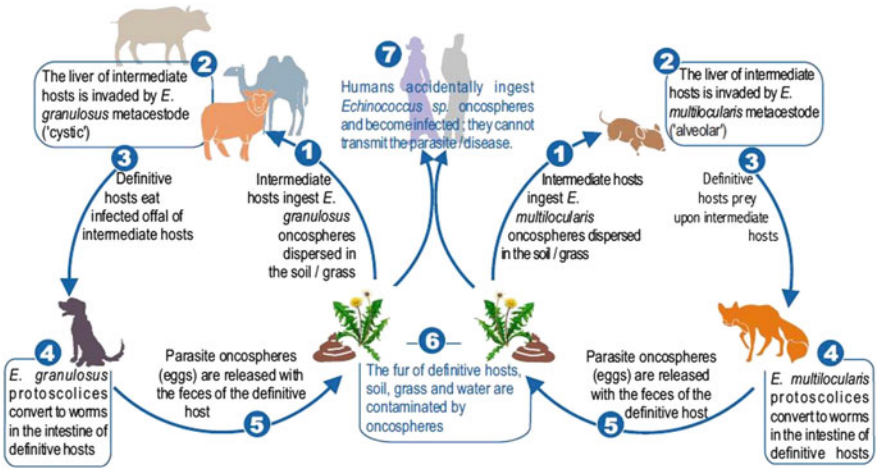

Fig. 6.4 Parasite cycle of *Echinococcus granulosus* (*L*) and of *Echinococcus multilocularis* (*R*). The adult stage, present in the intestine of various species of carnivores, is only killed by praziquantel, which, however, is unable to kill the oncospheres (eggs) released in the feces with the last 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, Besançon, France*)

regions with 70 % of foxes infected) (Piarroux et al. 2013), due to both the rare encounter of humans with infected fox feces and by their natural resistance as an intermediate host (Vuitton and Gottstein 2010; Bresson-Hadni et al. 2007; Vuitton 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).

Compared to CE, the burden of human infection is lower; 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 to 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 et al. 2003; Scharf et al. 2004; Bottcher et al. 2013; Ueno et al. 2012), and in zoo animals (Rehmann et al. 2005).

6.3.2 Transmission and Risk Factors for Human Infection

Humans get infected by ingesting *Echinococcus* eggs. These may be dispersed for >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) (Eckert et al. 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 dogs may be a source of infection especially for children (Matoff and Kolev 1964; Larrieu et al. 2002; Yang et al. 2008; Campos-Bueno et al. 2000). On the contrary, dog ownership was generally not associated with CE infection in studies investigating adults or whole communities (Carmona et al. 1998; Dowling et al. 2000; Harandi et al. 2011). Female sex, use of unsafe water source, and not washing hands before eating have been associated with CE infection, while other potential risk factors such as livestock ownership and home slaughter or eating raw vegetables were inconsistently or not associated with infection (Campos-Bueno et al. 2000; Carmona et al. 1998; Dowling et al. 2000; Harandi et al. 2011; Larrieu et al. 2002; Yang et al. 2008; Bakal et al. 2012).

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). 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 favor *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 et al. 2003; Eckert et al. 2001; Robardet et al. 2011; Deplazes et al. 2004).

6.3.3 Genetic Diversity

E. granulosus strains were first recognized due to phenotypic variations (Thompson and Lymbery 1990; Thompson and McManus 2002). Currently, ten genotypic strains have been identified (G1–G10), which are grouped in 4 species that constitute the *E. granulosus* complex: *E. granulosus* sensu stricto (G1–G3), *E. equinus*

(G4), *E. ortleppi* (G5), and *E. canadensis* (G6–G10). The "lion strain" has been now assigned to a different species, *E. felidis*, not included in the *E. granulosus* complex (McManus 2013). *E. granulosus* strains show different host range and geographical distribution (Eckert and Thompson 1997; McManus 2013; Thompson and McManus 2002; Eckert et al. 2001).

The capacity of developing fertile cysts in different intermediate hosts varies between and within strains. For example, horses and cattle can be infected both with G1 (sheep strain) and their "specific" strains (G4 and G5 respectively), but only infection with these 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 (Piccoli et al. 2013); however, all other strains, with the exception of *E. equinus*, 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) (80). 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).

6.4 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).

6.4.1 Immune Response to E. granulosus

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 (Baz et al. 2006; Breijo et al. 2008).

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. 2003a). PSC become refractory to complement-mediated killing once they start to vesiculate (Irigoin et al. 1996; Kassis and Tanner 1976).

Protection to infective stages is antibody and complement dependent (Dempster et al. 1992; Dempster and Harrison 1995; Heath and Lawrence 1996; Li et al. 2011a), is enhanced in the presence of neutrophils (Rogan et al. 1992) and requires the presence of lymphocytes (Dixon 1997a). 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. 2003b; 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 hydatid cyst fluid (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 (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 T-regs 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, 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). Recently, 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 (1,000–10,000 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 immune-modulatory 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 2012; Siracusano et al. 1988). Other potentially immunomodulatory HCF molecules include EgTeg, EgTPx, paramyosin, and tetraspanin (Monteiro et al. 2010; Ortona et al. 2005).

6.4.2 *Immune Response to* E. multilocularis

Opposite to 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).

As for *E. granulosus*, *E. multilocularis* can modulate the immune response at the very early stage of antigen presentation. *E. multilocularis* modifies macrophage and DCs function and interferes with antigen presentation and T cell proliferation (Dixon 1997b; 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/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 T-reg function of the CD8-Tcell 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). Immune-modulatory mechanisms by T-regs 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-Blum et al. 1996; Wang et al. 2013).

As for *E. granulosus* infection, the LL has an important role in immune modulation (Vuitton and Gottstein 2010). Immune-modulatory 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.

6.5 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 the central nervous system, but rarer localizations such as the thyroid, pancreas, heart, muscles, breast, orbit, surrenal glands, etc., have been described; about 40 % of cases have multiple locations (Pedrosa et al. 2000; Eckert et al. 2001; Polat et al. 2003). Prevalence increases with age and generally is higher in females (Craig et al. 2007a; Eckert et al. 2001).

CE symptomatology is extremely variable and nonspecific (Brunetti and White 2012; Eckert et al. 2001; Pedrosa et al. 2000). On average 60–75 % of patients with hepatic CE are asymptomatic and can remain so for up to 10–12 years (Caremani et al. 1993; Frider et al. 1999). When present, symptoms depend on cyst's size, number, organ infected, localization within the organ, and complications (Larrieu and Frider 2001).

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 superinfection (Eckert et al. 2001; Pedrosa et al. 2000). 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, anaphylactic reaction or may result in superinfection or dissemination (secondary hydatidosis). 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 membranes. Bacterial infection of the cyst is the most serious complication commonly seen after rupture. A comprehensive review of clinical manifestations and complications can be found in Eckert et al. (2001) and Pedrosa et al. (2000), while the reader can refer to the following selected reviews on the clinical aspects of CE in the most common extrahepatic locations: lungs (Santivanez and Garcia 2010), bone (Papanikolaou 2008; Zlitni et al. 2001), and central nervous system (Nourbakhsh et al. 2010; Neumayr et al. 2013a, b).

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 (Eckert et al. 2001).

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).

6.6 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 (Kern et al. 2011; Gruener et al. 2008; Chauchet et al. 2013).

The most frequent complications of AE are bacterial or fungal infection of the bile ducts and/or of the central necrotic area of lesions, with abscesses, cholangitis, and septic shock (Bresson-Hadni et al. 2007; Kern 2010). 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. 2006, 2007).

6.7 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 evaluation analysis of parasitic material can confirm the parasitic etiology of the CE lesion, while PCR on tissue samples is mostly used in AE doubtful cases.

Monoclonal antibodies for the detection of parasite circulating antigens and techniques for the identification of parasite nucleic acids in blood have also been

developed; however, they are at present not sensitive enough to be used in clinical diagnosis (Gottstein 1984; Liu et al. 1993; Siles-Lucas and Gottstein 2001; Devi and Parija 2003; Sunita et al. 2011; Kanwar and Vinayak 1992).

6.7.1 Imaging

Imaging techniques are the mainstay of the diagnosis of CE and AE (Pedrosa et al. 2000; Polat et al. 2003), which are often suspected after the accidental discovery of lesions during imaging exams carried out for other reasons.

Ultrasonography (US) is the cornerstone of abdominal CE screening, diagnosis, staging, and follow-up (Stojkovic et al. 2012; Del Carpio et al. 2012; Giordani et al. 2012; Macpherson and Milner 2003; Eckert et al. 2001; Torgerson and Deplazes 2009) and is the current screening method of choice for diagnosis and regular follow-up imaging in AE (Bresson-Hadni et al. 2006; Macpherson et al. 2003; Bartholomot et al. 2002; Yang et al. 2006a; Kantarci et al. 2012), while computed tomography (CT) and magnetic resonance imaging (MRI) techniques are needed for an accurate staging of the disease (Brunetti et al. 2010).

The WHO-IWGE classification of CE cyst stages is based on US CE-specific imaging features (Fig. 6.1). US can visualize cysts of <5 mm with a sensitivity of 93–100 % and specificity of 88–96 % for hepatic cysts (WHO-IWGE 2003; 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 LL and the pericyst; (2) "water lily sign" of CE3a cysts, that is, the fluctuation of the detached endocyst in the cyst fluid content; and (3) multivesiculated cysts (CE2, CE3b) with honeycomb appearance where the "septa" are formed by the adjacent walls of daughter vesicles (CE2) or where daughter vesicles form in a pseudosolid cyst content (CE3b). The "ball of wool" sign of CE4 cysts, indicative of degenerating membranes folded in a pseudosolid cyst content, although not pathognomonic is highly suggestive (WHO-IWGE 2003; Grisolia et al. 2009). On the contrary, in 2/3 cases 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. 2006). 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).

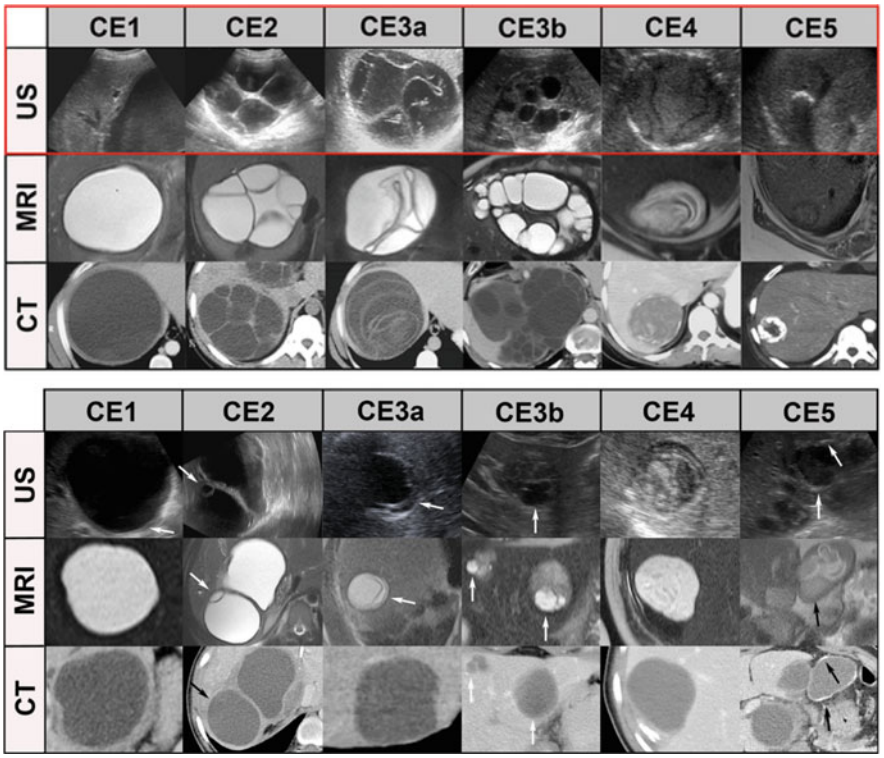

Fig. 6.5 "Best case (**a**) and "Worst case"(**b**) comparison of US, MRI, and CT. The "*double line sign*", typical for CE1 is often seen in US (CE1/US, *arrow*), but less reliably in MRI and CT. Daughter cysts and detached endocyst ("*water-lily-sign*") are often missed by CT, but are clearly visible in US and MRI (see CE2, 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 type CE1 cysts, i.e., staged "active" instead of "inactive." The identification of calcifications is the domain of CT imaging. MRI does not differentiate well between thick hyaline walls and calcifications. US picks up calcifications only when a dorsal echo shadow is produced (see CE5, *arrows*). MRI: HASTE sequence, CT: post contrast-enhanced images (From Stojkovic M et al. PLOS NTD 2012, with permission)

CT and MRI are necessary before surgery to determine the anatomic relationships 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. 6.5). On CT and MRI T1WI, CE cyst membranes are hyperdense/intense, with hypodense/intense intracystic fluid, and hypointense outer ring representing the fibrous capsule, which is better seen on MRI. On T2WI, cyst content is hyperintense and the hypointense rim sign is more evident. Cyst membranes may induce non-specific enhancement 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. 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. 2006; Reuter et al. 2001; Kodama et al. 2003). It shows the pathognomonic aspect of multiple small vesicles, as 'honeycomb' of '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. 2008b) 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. 2006).

Chest X-ray is generally the primary exam posing a presumptive diagnosis of pulmonary CE and may show the "water lily sign" in case of detached membranes or a "meniscus" between pericyst and laminated membrane 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, metastases, postsurgical cavities, AE pseudocysts, and other parasitic lesions in the brain (Abdel Razek et al. 2011), hematomas, and abscesses. Differential diagnosis may be difficult, as, for example, the vascularization pattern is not always specific and peripheral egg shell-like calcification characteristic of CE5 cysts can be observed also in AE, albeit rarely, and in neoplasms. The most difficult differential diagnostic problems for AE are hepatocellular carcinoma and metastases.

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).

6.7.2 Serology

Detection of circulating specific antibodies complements image-based diagnosis of CE and AE. Many techniques have been used for serodiagnosis, based most commonly on HCF. For CE, purified or recombinant AgB and Ag5 or their peptides and several other parasite-derived recombinant molecules are not yet routinely used in diagnostic laboratory practice (Carmena et al. 2006; Zhang et al. 2003a). For AE, a commercially available ELISA test based on Em2 antigen and rII/3-10 is in routine use (Em2PLUS; Bordier, Switzerland). A rapid test, easy to use for mass screening, is also available but is marketed only in China (Feng et al. 2010).

Reported diagnostic performances for CE are extremely variable: sensitivity 80–99 % and specificity 60–97 % for HCF and sensitivity 38–93 % and specificity 80–100 % for native purified or recombinant proteins (Carmena et al. 2006; Eckert and Deplazes 2004; Barbieri et al. 1998; Hernandez-Gonzalez et al. 2008, 2012; Lorenzo et al. 2005; Ortona et al. 2000; Poretti et al. 1999; Schweiger et al. 2012; Tawfeek et al. 2011; Virginio et al. 2003; Zhang et al. 2003a; Gonzalez-Sapienza et al. 2000; Jiang et al. 2012; Rott et al. 2000). On the contrary, serology is positive in more than 90 % of immunocompetent AE patients.

In clinical practice, two paired tests are performed (e.g., ELISA and indirect hemagglutination—IHA), with immunoblotting (IB) as a confirmatory test and to discriminate *E. granulosus* from *E. multilocularis* (Liance et al. 2000; Brunetti et al. 2010). It is important to note that positive serology in the absence of suggestive lesions on imaging does not justify treatment (Siles-Lucas and Gottstein 2001). 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).

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). 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 et al. 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). 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 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. 1992).

   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 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. Antigens used for the specific serological diagnosis of CE are not standardized. The use of highly sensitive and specific tests based on recombinant proteins, such as rAgB or derived peptides rAgB/2 and 2B2t, appears promising in this sense (Carmena et al. 2006; Hernandez-Gonzalez et al. 2012; Virginio et al. 2003).
4. At present, no reliable marker of infection activity is available. In CE, specific antibodies may be detectable for over 10 years even after radical surgery (Hernandez-Gonzalez et al. 2008); therefore, only monitoring of titers over time can 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. 2011b; Rigano et al. 2002; Zarzosa et al. 1999). Generally antibody titers increase upon relapse, but this does not always occur (Lawn et al. 2004; Zarzosa et al. 1999; Gollackner et al. 2000). 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).

6.8 Treatment

Treatment approaches of CE and AE are very different and will be treated here separately. However, in both cases, the therapeutic management of CE 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).

6.8.1 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 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

Table 6.1 Treatment indications based on cyst stage and size[a]

Stage	Size	Treatment
CE1 and CE3a	<5 cm	Albendazole
	5–10 cm	Percutaneous aspiration + albendazole
	>10 cm	Permanent catheterization + albendazole
CE2 and CE3b	<5 cm	Albendazole (rarely successful) surgery + albendazole
	>5 cm	Surgery + albendazole
CE4 and CE5	All	Watch-and-wait

[a]Albendazole = generally 6-month treatment as a single therapy, 1 month after percutaneous or surgical treatment

stage-specific approach to cysts (Brunetti et al. 2010). This should take into consideration cysts 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; Menezes da Silva 2003). 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 6.1.

6.8.2 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 poorly accessible anatomical locations, 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 ("cystectomy," previously known as "pericystectomy") or, conservatively, leaving the adventitious layer in place ("hydatidectomy" or "endocystectomy"). The procedure is said "open" when the cyst content is evacuated and the cavity sterilized using a scolecidal agent (if no

communication with the biliary system is present). Laparoscopic interventions are also performed in selected cases with good results (Dziri et al. 2004).

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). The WHO-IWGE suggests that parasitic material should be removed as much as possible. Results of meta-analysis and single-center studies indicate that radical surgery is superior to conservative, 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). Other factors associated with poor surgery outcome are large cyst size, presence of biliary fistulae (significantly higher for cysts >7.5 cm) (Kilic et al. 2008), 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 bone infiltration with microvesicles (Papanikolaou 2008). Perioperative albendazole prophylaxis is needed 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) (Arif et al. 2008; Gollackner et al. 2000). Bile leakage from biliary fistulae and cyst cavity superinfection are the most common complications of surgical interventions (4–14 %) and are managed either conservatively 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 scolicidal 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 by cholangiography); and (3) appropriate management of the residual cavity (Junghanss et al. 2008). 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).

6.8.3 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 its active form, 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).

ABZ should be administered continuously for ≥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. Moreover, it should be administered perioperatively in case of percutaneous or surgical treatment, from a minimum of 4 h before until 1 month after, to prevent recurrence and secondary CE (Brunetti et al. 2010). 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).

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). 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) and breastfeeding, and bone marrow depression, while they should be used cautiously in patients with chronic hepatic diseases (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 method heterogeneity. Reported outcome rates for hepatic cysts are as follows: 28,5–58 % cure/marked improvement, 10–51 % partial response, 13–37 % no change, and 4–33 % worsening (Todorov et al. 1992a, b; Nahmias et al. 1994; Horton1989; Wen et al. 1994; Salinas et al. 2011; Li et al. 2011b; 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 multivesiculated (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. 2011b). 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).

6.8.4 Percutaneous Treatment

Percutaneous interventions aim to evacuate the cyst content and destroy the germinal layer by means of scolecidal agents. The most widely used technique is PAIR (puncture, aspiration, injection of scolecidal agent, re-aspiration) (Anonymous 2001); however, several other techniques have been described, mostly applied to the treatment of partially solid or complex cysts (Brunetti and Filice 2001; Bastid et al. 2005; Saremi and McNamara 1995; Haddad et al. 2000; Schipper et al. 2002; Akhan et al. 2007).

Percutaneous treatment has been increasingly used for hepatic and abdominal CE and is now recommended by the WHO-IWGE guidelines in selected cases (Brunetti et al. 2010). These include CE1 and CE3a cysts of >5 cm, inoperable patients, failure to respond/relapse after other treatments, and in 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). It has also been applied in cysts in other organs but is contraindicated in lung cysts (Brunetti et al. 2010; Junghanss et al. 2008). In case of giant cysts (>10 cm), permanent catheterization with catheter removal when daily drainage is <10 mL is recommended as classic PAIR is less successful in these cases (Ustunsoz et al. 1999; Men et al. 2006; Golemanov et al. 2011). PAIR needs to be performed in the presence of resuscitation equipment, and ABZ peri-interventional therapy is mandatory, as is for surgery. The most commonly used scolecidal agents are 20 % saline and 95 % ethanol (Smego et al. 2003). These should be applied only after excluding 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 in PAIR, several reports have documented it after surgery (Taranto et al. 1995; Belghiti et al. 1986; Castellano et al. 1994).

Fear of anaphylactic shock as a consequence of cyst puncture has been the main reason for reluctance to percutaneous treatment (Yaghan et al. 2004). However, to date more than 5,500 cysts have been punctured accidentally or intentionally with only 99 cases of anaphylaxis (1.6 %), of which only two 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 has not been evaluated.

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). 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). As for surgery, biliary fistula and superinfection are the most common complications, although with lower rates (2–6 %) (Smego and Sebanego 2005).

Mean hospital stay is 1–4 days compared to 12 days in case of 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).

Overall response rates range from 72 to 97 %, with relapse rates of 1.6–15 % (Giorgio et al. 2008; Khuroo et al. 1997; Smego et al. 2003; Ustunsoz et al. 1999; Smego and Sebanego 2005). 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 multivesiculated CE2 and CE3b cysts have a success rate <40 % (Kabaalioglu et al. 2006; Golemanov et al. 2011; Giorgio et al. 2008).

6.8.5 Watch and Wait

Recent expert opinion recommends 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). 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.

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.

6.8.6 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 6.2), designed on the model of the TNM classification of cancers, helps clinicians to choose the appropriate

Table 6.2 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[c]
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

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.

6.8.7 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 for life) significantly prolongs survival (80 % at 10 years, compared to less <25 % in historical controls) (Brunetti et al. 2010). As for 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. 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). 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).

6.8.8 Surgery

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. 2006; 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. 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 (Bresson-Hadni et al. 2006).

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). Autotransplantation, which allows easier resection of large-sized lesions with vascular involvement, awaits long-term evaluation (Wen et al. 2011).

6.8.9 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 on the basis of serological and PET/CT negative results and in patients with radical resection after 2 years of ABZ or MBZ (Brunetti et al. 2010).

6.9 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, 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 et al. 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 make possible 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 (Brunetti et al. 2010). 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 (Torgerson et al. 2008; Piarroux et al. 2011). 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.

6.10 Prevention and Control

Individual prevention of CE and AE relies on hygiene: washing hands before eating, avoiding mouth–hand contact, thorough washing or cooking of vegetables, and use of safe water are all important. Chlorination and household freezing does not inactivate eggs, which are instead sensible to heat.

Control programs for CE are complex, with multiple targets, and require a large amount of time (minimum 10 years of "attack phase" followed by a "consolidation" and "maintenance" phase) and resources (Eckert et al. 2001; Huang et al. 2011). The principal points of intervention are: (1) veterinary public health actions, (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 (WHO-IWGE 2011). 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 and Zanini 2012). Elimination is difficult to obtain and experts believe that with the current control options, achieving such a goal will take around 20 years of sustained efforts (Craig et al. 2007b). However, livestock vaccination using the highly effective EG95 vaccine, together with the incorporation of improvements made in the diagnosis and treatment of human and animal CE and the genetic characterization of strains, could be a useful tool to shorten the length of control programs (Huang et al. 2011; Craig et al. 2007b).

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 wild life, 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 and northern Japan; fox baiting with praziquantel got variable results, depending mostly on the contamination pressure in the rural areas surrounding the targeted city (Hegglin and Deplazes 2008; Comte et al. 2013). More studies are needed to assess the best strategy to tackle this emerging public health problem.

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Chapter 7
Taeniosis and Cysticercosis

Elizabeth Ferrer and Teresa Gárate

Abstract Taeniosis and cysticercosis are zoonotic diseases produced by *Taenia saginata* and *Taenia solium*. The adult tapeworms are parasites of human intestine and show a wide geographical distribution. *Taenia asiatica*, another tapeworm species, was described in Southeast Asia. The larval stages of these cestodes (metacestodes or cysticerci) cause cysticercosis; *T. saginata* causes bovine cysticercosis, *T. asiatica* larvae develop in the pig viscera, and *T. solium* is able to produce cysticercosis in both pig and man. When the parasite larva invades, the central nervous system (CNS) can provoke neurocysticercosis (NCC), one of the most frequent parasitic infections of human CNS. These diseases continue to cause health problems and livestock industry losses in areas where the parasites are endemic and also in non-endemic regions as a consequence of travel and migrations. There are few symptoms associated with taeniosis; in contrast, NCC (pleomorphic pathology) could be a life-threatening disease, depending on the location, number, stage of cysticerci, and the host immune response. Diagnosis of taeniosis is generally achieved by stool microscopic examinations, and the detection of cysticercosis is generally performed by neuroimaging and immunoassays. Both conventional coprological techniques and immunological assays show limitations, and new diagnostic tools have been developed, more specific and sensitive, such as specific monoclonal antibodies, recombinant antigens, synthetic peptides, and PCR. Considering the clinical impact, veterinary problems, and economic losses derived from taeniosis/cysticercosis, control programs have been implemented. In addition, several vaccine candidates have been characterized that could complement the control measures.

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7.1 Introduction

Taeniosis is a human intestinal parasitic infection caused by *Taenia saginata* and *Taenia solium* adult stages. Human being is the only definitive host for both taeniids. Cysticercosis occurs as a result of the development of parasitic larval stages (cysticerci or metacestodes) in the intermediate host tissues. The cysticerci of *T. saginata* (*Cysticercus bovis*) cause bovine cysticercosis, and the cysticerci of *T. solium* (*Cysticercus cellulosae*) provoke porcine and human cysticercosis, since man can also accidentally act as an intermediate host of *T. solium* (Náquira 1999).

Taenia asiatica is the last species found to be a human-infecting tapeworm, although at the beginning there were controversies about its taxonomic status that were finally overcome. The larvae of *T. asiatica* (*Cysticercus viscerotropica*) infect pig liver and other viscera. The new taeniid shows characteristic geographical distribution, epidemiology, genomics, and immunodiagnosis (Eom and Rim 1993; Eom et al. 2009).

7.2 The Agent

Regarding morphology, the cestode life cycle includes three distinct stages: adult, egg, and larva (metacestode or cysticercus). The adult tapeworm is flat, ribbon shaped, and hermaphrodite; *T. solium* is 2–4 meters (m) in length, although it can reach up to 8 m, while *T. saginata* has an average size of 5 m and can measure up to 16 m. The adult consists of three parts: scolex, neck, and body or strobila. The scolex is a "head" at the anterior end, with globular form and 1 mm average diameter; the scolex of *T. solium* has four suckers, a rostellum, and a double crown of hooks (22–32); in contrast *T. saginata* scolex also presents four suckers but does not have either hooks or rostellum. These organs are used to maintain the position of the parasite in the host gut (Náquira 1999).

The neck is short, 5–10 mm, and slender and contains germinal cells that apparently are responsible for giving rise to proglottids (strobilation). The strobila is large, measuring several meters and consisting of hundreds of proglottids or segments; they are classified according to reproductive system development as immature, mature, and gravid. Mature proglottids are slightly wider than long, while immature proglottids are narrower (6 mm). Mature proglottids have genital organs consisting of about 150–400 testes, ovary, and a genital pore. Gravid proglottids are longer than wide, arranged in the last fifth of the worm, and have eggs in lateral uterine branches. *T. solium* and *T. saginata* differ in the number of primary lateral uterine branches: *T. solium* contains 7–15 lateral branches and *T. saginata* 15–30 lateral branches. Proglottids of *T. asiatica* are similar to those of *T. saginata* and possess more than 15 primary uterine branches (Pawlowski 2002).

The eggs pass out with feces of *Taenia* carriers, in either gravid or free proglottids. Importantly, *T. saginata* proglottids are able to migrate out of the anus. The eggs are

spherical, 30–50 μm, with an outer embryophore that is very thick and riddled, which protects the hexacanth embryo (oncosphere), and are fully embryonated when eliminated. The egg morphologies of human taeniids are identical, making diagnosis of species impossible on this character alone (Pawlowski 2002).

The cysticercus or metacestode is the larval stage of this type of taeniids. The cysticerci of *T. solium* (*C. cellulosae*) are rounded or oval vesicles, 6–15 mm in diameter, whitish, and fluid filled, with an invaginated scolex (hooks and four suckers) which can be seen as a small eccentric and solid granule. Occasionally, racemose cysticercus could develop, being a large, irregular, fluid-filled, and lobulated vesicle, similar to a bunch of grapes. The cysticerci of *T. saginata* (*C. bovis*) are akin to the *T. solium* vesicular cysticercus, although the scolex does not show the double row of hooks (Náquira 1999).

Finally, *T. asiatica* life stages are similar to *T. saginata*, although the adult tapeworm is smaller with a narrower scolex, which has 4 suckers and a rostellum, and gravid proglottids with many uterine twigs. The metacestodes are also smaller and covered by wart-like formations; some of them show rudimentary hooklets (Eom and Rim 1993; Eom et al. 2009).

Eggs, or gravid proglottids, are passed with feces of *Taenia* carriers; the eggs can survive for days to months in the environment, soil, and water. Cattle (*T. saginata*) and pigs (*T. solium* and *T. asiatica*) become infected by ingesting eggs or gravid proglottids. In the animal intestine, the oncospheres hatch, activate, invade the intestinal wall, and migrate to the tissues and some organs, where they develop into cysticerci. A cysticercus can survive for several years in the animal. Humans become infected by the ingestion of raw or undercooked infected meat, such as pork (*T. solium*/*T. asiatica*) and cattle (*T. saginata*). In the human intestine, the cysticercus becomes an adult tapeworm in 2 months; the adult can survive for years and transmit the infection by eggs. In contrast to *T. saginata*, *T. solium* eggs are able to infect human, invade the intestinal wall, and migrate to striated muscles, as well as the brain, liver, eye, and other tissues, where they develop into cysticerci. In humans, *T. solium* metacestodes can cause serious illness if they localize in the brain (Pawlowski 2002).

7.3 Epidemiology of Infection

Taeniosis and cysticercosis are endemic in some countries of Latin America, Asia, and Africa, especially rural areas, where socioeconomic status, sanitary conditions, and meat inspection infrastructure are insufficient. Importantly, human cysticercosis is identified as one of the most frequent parasitic diseases of the central nervous system (CNS), being considered to be related with the late-onset epilepsy cases in endemic regions (Carpio et al. 1998; Del Brutto and Garcia 2013). The World Health Organization (WHO) has estimated that neurocysticercosis (NCC) accounts for over 50,000 deaths per year and, for active epilepsy, many times this number of deaths, because more than 80 % of the world's 50 million people who are affected

by epilepsy live in low-income and lower-middle-income countries, many of which are endemic for *T. solium* infections. NCC is of great economic relevance, resulting from the cost of medical treatment, lost working days, as well as reduction in livestock industry profits. Very few studies have been conducted to evaluate the burden of NCC; therefore, the disability-adjusted life year (DALY) has not been yet estimated (Bhattarai et al. 2012). Regarding cattle and porcine cysticercosis, they are overlapped in many countries and cause costly condemnations and important economic losses as mentioned above (Murrell 1991).

The situation is different in high-resource countries such as the United States and Europe. In the United States, human cysticercosis has always been predominantly an imported disease. However, near 100 autochthonous cases have been reported (Sorvillo et al. 2011). In Europe, cysticercosis was practically controlled during the last century, but a significant increase has been detected in association with immigration in the last two decades (Zammarchi et al. 2013). Most of the imported cysticercosis patients have been diagnosed in Spain, France, Italy, and United Kingdom (Roca et al. 2003; Zammarchi et al. 2013). European autochthonous cases of human and porcine/cattle cysticercosis are infrequent (Allepuz et al. 2012; Fabiani and Bruschi 2013).

In Asia, the prevalence of the disease is variable according to different risk factors. So it is almost absent in countries like Japan and Singapore, with high standards of living, and in Islamic countries (Rajshekhar et al. 2003), while in others it is mainly endemic: India (Raghava et al. 2010), China (Ikejima et al. 2005), Indonesia (Wandra et al. 2011), and Vietnam (Trung et al. 2013). *T. saginata*, *T. asiatica*, and *T. solium* are overlapped in some Asiatic countries and, importantly, the Asiatic *T. solium* genotype differs from the African-American genotype (Ito et al. 2003; Sato et al. 2011).

In Africa, taeniosis and cysticercosis have been described in almost all regions except for the Muslim areas (Phiri et al. 2003; Mwape et al. 2013). In recent years, the number of studies on taeniosis/cysticercosis has increased in Africa, although most large-scale control programs have been carried out in Latin America and Asia (Assana et al. 2013).

In Latin America, several epidemiological studies demonstrated the relevance of the disease in Mexico (Fleury et al. 2010), Peru (García et al. 2003), and Brazil (Ishida et al. 2011). Other authors confirmed the significance of taeniosis/cysticercosis in Guatemala (García-Noval et al. 1996), Honduras (Sánchez et al. 1999), Bolivia (Carrique-Mas et al. 2001), Ecuador (Rodríguez-Hidalgo et al. 2006), Colombia (Sanzón et al. 2002), and Venezuela (Ferrer et al. 2003a; Cortez et al. 2010); more recently, a systematic review and meta-analysis of the literature found a consistent association between epilepsy and NCC in Latin American countries (Bruno et al. 2013).

Finally, it should be mentioned that taeniosis/cysticercosis complex is considered a neglected tropical disease (NTD) by the WHO, because it is not adequately addressed nationally and internationally in many endemic countries and affects the poorest populations, being not perceived as a significant burden on public health (WHO 2011).

7.4 The Host Response to the Parasite

There are few studies on the immune response in taeniosis, most of them about antibodies detection. However, as *Taenia* carriers can also suffer cysticercosis, it is difficult to determine whether the antibodies detected could be due to adult or cysticerci (Correa and Medina 1999). Recently, *T. solium* taeniosis experimental models in hamsters, gerbils, and chinchillas have been established, being chinchillas the most successful experimental definitive model since tapeworms with gravid proglottids were obtained (Flisser et al. 2010); new investigations on taeniosis immunology will be developed soon. By contrast, many studies have been made on cysticercosis to determine the mechanisms of immune response directed against the cysticerci of *T. solium*. This response has been evaluated in murine models (mouse/*T. crassiceps*), pigs, and humans and will be reviewed below.

7.4.1 Innate Immunity

In general, the innate immunity components are largely unknown. The Toll-like receptors (TLRs) appear to be important in recognizing these parasites and the induction of inflammatory responses. Dendritic cell (DC) unresponsiveness against parasite excretory/secretory (E/S) antigens could suggest that these antigens are immunosuppressive. In addition, the characteristic metacestode antigens could include pathogen-associated molecular patterns (PAMPs), mostly glycans in nature (Terrazas et al. 2012).

7.4.2 Adaptive Immunity

7.4.2.1 Humoral Responses

The humoral response in NCC patients has been mainly studied as a tool for immunodiagnosis. Most infected individuals produce antibodies of different specificities that appear at different periods of infection in response to changes in antigen release during parasite development (Flisser et al. 1980; Dorny et al. 2003). Anticysticerci IgG antibodies have been detected in serum, CSF, and saliva, along the infection; also, IgM, IgA, and IgE antibodies have been identified, but they are less common and present during inactive stages (Bueno et al. 2000). 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. 1983b).

7.4.2.2 Cellular Responses

In general, along the different infection stages and types, a well-defined Th1 or Th2 profile is not clearly associated with NCC, and a more mixed Th1/Th2 response seems to be the most commonly observed result (Restrepo et al. 2001; Toenjes and Kuhn 2003; Terrazas et al. 2012).

Cysticerci can 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 without, or with, a slight inflammatory reaction (Carpio 2002). This situation 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). In contrast, symptomatic NCC is significantly associated with the development of granulomas and degenerating cysts that are important components of the neuropathology leading to neurological symptoms; the initiation of granulomas has been related with a robust Th1 response; degenerating or dead parasites trigger an intense antigen-specific cellular proliferation and a strong proinflammatory response (TNF-alpha, IL-12, IL-18, IFN-gamma) (Terrazas et al. 2012).

Although there are some controversies about inhibition of T-cell proliferation in vitro and in NCC patients, it has been demonstrated that the parasite has developed several immunomodulatory activities to evade the immune response mechanisms. Immunomodulation through the production of alternately activated macrophages (AAMs) that lead to the production of downregulatory cytokines and activation of the alternative arginase 1 pathway has also been suggested as an immunoprotective mechanism of the parasite (Rodríguez-Sosa et al. 2006). It has been reported that the 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, in addition to blocking other immunological responses (Leid et al. 1987; Laclette et al. 1992). Also, a metacestode factor (MF), secreted by the parasite, inhibits inflammation, cytokine production, and lymphocyte proliferation induced in vitro (Arechavaleta et al. 1998). Furthermore, cysticerci are able to secrete molecules to induce apoptosis in immune cells, like cysteine proteases and annexin B1, or 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 parasite surface can adsorb host molecules (antibodies, complement units) and mimic the host repertoire (White et al. 1992; Spolski et al. 2002). Many of these mechanisms could support the local immunosuppression observed in the disease (Terrazas et al. 2012). All these evidences show that *T. solium* metacestodes influence on the host immune response to ensure their survival (Toenjes and Kuhn 2003). In the porcine model, apoptotic cells were observed in the inflammatory infiltrate, but not when the parasite was dead (Sikasunge et al. 2008).

In humans, some studies have noted variations in the immune response according to age and gender of patient, being stronger in females (Kelvin et al. 2009). It is hypothesized that population genetics (HLA polymorphisms) and parasite genotypes could be involved in the disease progression (Pal et al. 2000).

7.5 Immunopathological Processes

Several studies indicate that the symptom severity in NCC is associated with the intensity of the immune response, so symptomatic parenchymal disease occurs at the time of larval degeneration or death by cysticidal therapy. At first there is an asymptomatic period in which the immune response seems unable to resolve the infection, followed by a chronic hypersensitivity reaction, being inflammation the main response associated with the NCC pathology (Carpio et al. 2013).

Moreover, several studies showed that the specific humoral response is effective against oncosphere (Flisser et al. 1979; Molinari et al. 1983a, b; Correa and Medina 1999; Carpio 2002), but when the cysticercus is developed, it produces mechanisms (bioactive molecules) to evade the immune response, through apoptosis induction, antibodies breakdown, and complement system inactivation (Flisser et al. 1979; Terrazas 2008; Terrazas et al. 2012), as mentioned above.

Also, cysticerci can cause asymptomatic infection and persist for many years in the host without triggering an inflammatory response. Histological studies in pigs and humans have shown viable cysticerci without or with a slight inflammatory reaction, so it is believed that parasite secretion molecules have immunomodulatory activities (Carpio 2002; Terrazas 2008; Terrazas et al. 2012).

Several studies have revealed a general immunosuppression in patients with cysticercosis (Chavarria et al. 2006). However, it was also published that NCC patients had active peripheral immune response, both cellular and humoral, although in vitro studies showed reduced lymphocyte proliferation by parasite antigens, suggesting that this effect could be responsible for immunosuppression in the vicinity of cysts (Restrepo et al. 2001). This hypothesis is supported by a study in the *T. crassiceps*/mouse model, which observed T-cell anergy in the parasite vicinity (Villa and Kuhn 1996). Anyhow, other authors found a local active, and systemic, immune response in symptomatic and drug-treated patients (Alvarez et al. 2002; Pal et al. 2000).

7.6 Clinical Manifestations

7.6.1 Taeniosis

Taeniosis is usually asymptomatic; minimal lesions could be developed in the intestinal mucosa. Although some clinicians described abdominal pain, loss of weight, nausea, constipation, or diarrhea during taeniosis, others do not recognize specific symptoms associated with the infection. It is relevant to consider that *T. solium* taeniosis patients can also suffer from cysticercosis, in which the differential diagnosis of both infections before drug treatment is crucial (Bustos et al. 2012).

7.6.2 *Cysticercosis*

Most infections are asymptomatic. The symptomatic cases include several clinical forms depending on environmental factors, the individual (genetic background, age, gender), and the parasite (Pal et al. 2000; Chavarria et al. 2006). The disease prognosis is related with number, size, type, condition, and site of metacestodes, as well as immunological responses to cysticercosis (Sotelo 2011). *T. solium* cysticerci can invade the CNS, eye, skeletal muscle, and subcutaneous tissues, muscle and subcutaneous locations being the most benign forms of cysticercosis while ocular cysticercosis and NCC the most serious conditions. Subcutaneous forms are much more common in China than in Latin America or India, and serious NCC forms are more common in Latin America than in India.

Ocular cysticercosis can cause vision damage and produce blindness (Martínez et al. 1999). NCC may lead to death and shows wide variations of clinical manifestations that are a consequence of inflammation around a cyst(s), space occupation, and impedance to the flow of CSF, less commonly meningeal or vascular inflammation (Pal et al. 2000). Seizures (most common symptom), nausea, ataxia, confusion, hydrocephalus, vasculitis, stroke, altered mental state, paresthesia, headaches, and neurocognitive deficits have been reported (Del Brutto et al. 1996; Del Brutto and Garcia 2013).

NCC can be classified according to different criteria: (i) Cysticerci location. *Parenchymal:* parasites are in the brain parenchyma and it is the most common form. *Extraparenchymal:* the parasite occurs primarily in the subarachnoid space, ventricles, or spinal cord. (ii) Viability of cysticerci. *Active:* viable cysticerci. *Transitional:* degenerating cysticerci. *Inactive*: calcified cysticerci (Carpio 1999, 2002). (iii) Parenchymal lesions evolution states by neuroimaging techniques. *Viable or vesicular form:* small single or multiple lesions, hypodense rounded images, with a hyperdense nodule inside (scolex), lacking surrounding edema. *Colloidal:* lesions are surrounded by edema, represents the acute encephalitis phase in which the host's immune system is reacting against the parasite. *Nodular-granular:* hyperdense lesions surrounded by edema, referred to as "cysticercus granuloma." *Calcified lesions:* small hyperdense nodules without perilesional edema, being the most common finding in NCC (Garcia and Del Brutto 2003; Nash 2012).

In general *parenchymal cysticercosis* is the form that more frequently presents with seizures; differential diagnosis with cystic tumor should be done and in the case of solitary cysticercus granuloma, pyogenic abscess, tuberculoma, and metastatic brain tumors have to be excluded. *Extraparenchymal disease* varies in its symptoms according to the parasite locations, e.g., when cysticerci lodge within the ventricular system, life-threatening acute intracranial hypertension secondary to hydrocephalus may develop. *Subarachnoid* NCC is also characterized by hydrocephalus, a consequence of inflammatory occlusion, and ischemic cerebrovascular complications. *Ventricular* cysticercosis shows sometimes seizures or hydrocephalus, meningeal inflammation, nausea, vomiting, headache, ataxia, and confusion.

Spinal cysticercosis occurs with inflammatory and demyelinating changes, being characterized by radicular pain or paresthesia, or progressive cord compression. *Racemose* cysts usually occur in the basal cisterns and can cause an intense inflammatory reaction, fibrosis, and progressive thickening of the leptomeninges at the base of the brain; it causes usually an obstruction of the cerebrospinal fluid (CSF) circulation, resulting in hydrocephalus and intracranial hypertension as a consequence of the unrestricted growth of the racemose parasite (Pal et al. 2000; Del Brutto and Garcia 2013).

Computed tomography (CT) and magnetic resonance imaging (MRI) procedures, with disease diagnosis capability, have allowed to illustrate the heterogeneity of NCC already mentioned. Figure 7.1 includes several scans that show the diversity of this neurological pathology: (i) *Number of cysticerci*: there are cases with just one cyst (Fig. 7.1-1, 7.1-6) and others with tens of them (Fig. 7.1-2, 7.1-5, 7.1-7). (ii) *Cysticerci location*: parenchymal (Fig. 7.1-1, 7.1-2), ventricular (Fig. 7.1-3), and subarachnoid space (Fig. 7.1-4). (iii) *Cysticerci viability*: vesicular (Fig. 7.1-2, 7.1-6), colloidal (Fig. 7.1-8, 7.1-9), or calcified (Fig. 7.1-5, 7.1-9). (iv) *Inflammatory response*: weak (Fig. 7.1-10) and strong (Fig. 7.1-11).

It has not been demonstrated that *T. saginata* and *T. asiatica* produce human cysticercosis, although some authors suggest that the latter could do it (Galán-Puchades and Fuentes 2013).

7.7 Diagnosis

7.7.1 Taeniosis Diagnosis

It is important to note that *T. solium* adult tapeworm carriers could also have cysticercosis, situation to be considered at the time of diagnosis and treatment of the individual and their contacts. Furthermore, it is noteworthy that patients with *T. saginata* taeniosis are the source of cattle cysticercosis and those with *T. solium* are the origin of both human and porcine cysticercosis (Allan et al. 2003).

7.7.1.1 Parasitological Diagnosis

It is based on the finding and morphological differentiation of gravid proglottids. Proglottids with less than 15 uterine branches correspond to *T. solium*, while more than 15 are characteristic of *T. saginata*. Often proglottids are deformed, their species-specific morphological identification being very difficult (Mayta et al. 2000; Guezala et al. 2009). The microscopic observation of eggs in stools can only indicate taeniosis, but not determine whether the disease is caused by *T. solium* or *T. saginata*, since taeniid eggs are morphologically indistinguishable. These methods show low sensitivity (Allan et al. 2003; Guezala et al. 2009).

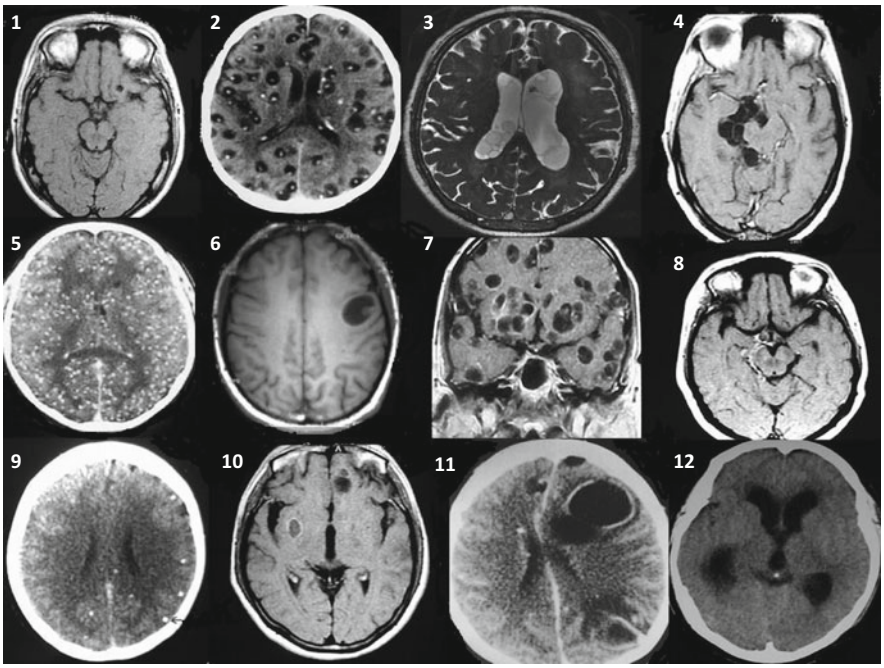

Fig. 7.1 Heterogeneity of NCC by MRI and CT scans (Images provided by Dr. Agnès Fleury, Instituto de Investigaciones Biomédicas, UNAM, Instituto Nacional de Neurología y Neurociencias, SS, Mexico City, Mexico)

7.7.1.2 Immunodiagnosis

Coproantigen detection was developed by the use of polyclonal antibodies (against adult crude extracts) in antigen-capture assays. The assay exhibited good sensitivity (85–100 %) and specificity to detect *Taenia* carriers although they could not distinguish between *T. solium* and *T. saginata* infections (Allan et al. 1990). It has been used to carry out epidemiological studies, determine the prevalence of taeniosis, and evaluate the efficacy of drug mass treatment campaigns in endemic regions (Bustos et al. 2012).

Also, rabbit polyclonal antibodies were prepared using E/S or surface antigens of adult tapeworms (Machnicka et al. 1996), but they did not allow the species-specific taeniid diagnosis. More recently, an ELISA to detect *T. solium*-specific coproantigens has been developed through a hybrid system, first antibody against *T. solium* adult crude extract and second antibody anti-E/S adult antigens (Guezala et al. 2009).

There are methods that employ monoclonal antibodies to identify *T. solium* eggs, with excellent sensitivity and specificity (Montenegro et al. 1996), or coproantigens (Praet et al. 2013).

On the other hand, various techniques have been developed for the antibody detection in sera of individuals with taeniosis. Of these, 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 baculovirus system, and they showed excellent sensitivities and specificities in EITB (Levine et al. 2004, 2007). TSES33, or ES33, was used in a magnetic immunochromatographic test to identify *T. solium Taenia* carriers with very good results (Handali et al. 2010). Also, an immunoblot has been developed to specifically diagnose *T. asiatica*-taeniosis (Jeon and Eom 2009). Finally, it is important to note that the antibody levels do not decrease after drug treatment (Allan et al. 2003).

7.7.1.3 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). Multiplex PCRs, PCR-RFLP (*PCR-restriction fragment length polymorphism*), and PCR sequencing have allowed the species-specific identification of *T solium*, *T. saginata*, and *T. asiatica* (Mayta et al. 2000; González et al. 2000, 2010; Jeon et al. 2009, 2011; Jeon and Eom 2013). Also, some molecular markers have permitted the differentiation between *T. saginata* and *T. asiatica* and the distinction of two genotypes within *T. solium*, 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). In addition, PCR *loop-mediated isothermal amplification protocol* (LAMP) has been established for the differential diagnosis of human taeniids (Nkouawa et al. 2010), and it is a really simple technique. Some molecular protocols have been used with infected stools, showing excellent sensitivity and specificity.

7.7.2 Cysticercosis Diagnosis

7.7.2.1 Parasitological Diagnosis

In NCC, parasitological diagnosis (direct visualization of parasite/lesion) is generally carried out during autopsies (*postmortem*) and by biopsies (Schantz et al. 1992). In ocular cysticercosis, the ophthalmologic examination is really useful. When cysticerci are located in muscle or subcutaneous tissue, palpation examination, biopsies, and fine needle aspiration cytology are employed, although differential diagnosis from other pathogens should be undertaken (Handa et al. 2008).

7.7.2.2 Neuroimaging

Computed axial tomography (CT) and magnetic resonance imaging (MRI) are crucial tools for NCC diagnosis, since they allow to know the number, size, evolutionary stage, and location of lesions, as well as the inflammation response (García and Del Brutto 2003). It is relevant to carry out a differential diagnosis from other neurological disorders and to consider that both neuroimaging cost and technical complexity hamper their use in some endemic areas (Del Brutto et al. 1996). Intramuscular cysticerci can also be identified by ultrasonography and MRI, detecting even a solitary intramuscular cysticercus (Tripathy et al. 2012). Recently, new imaging techniques have improved the detection of scolex and visualization of cysts in the extraparenchymal spaces (Carpio et al. 2013).

7.7.2.3 Immunodiagnosis

The immunodiagnostic techniques include detection of antibodies, as well as of antigens, in human serum and CSF samples. They are really important for NCC diagnosis (Del Brutto et al. 1996). These techniques are also used for cattle and porcine cysticercosis diagnosis.

Regarding antigen detection, Harrison et al. (1989) developed an antigen-capture immunodiagnostic system based on the use of HP10 monoclonal antibody (HP10-Ag-ELISA), specific for a repetitive glyco-residue secreted by *T. saginata* and other taeniid metacestodes. The assay has been used with both serum and CSF samples, showing the best sensitivity when patients have several alive cysticerci and severe NCC (Correa et al. 1989; García et al. 1998, 2002; Ferrer et al. 2003a; Fleury et al. 2007, 2013). Importantly, the Ag-ELISA permits the NCC treatment monitoring. Other authors have also 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 B158 monoclonal antibody, an anti-*T. saginata* reagent, in a new 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). Recently, these authors have confirmed the utility of urine samples for cysticercosis diagnosis (Castillo et al. 2009; Mwape et al. 2011).

In relation to NCC immunodiagnosis by antibody detection in serum, CSF, saliva, and urine samples, many methods and techniques have been used. Probably, they are the first choice for a routine microbiology laboratory, although it is worthy to consider that antibody detection indicates parasite exposure but not always active infection and works better for active NCC diagnosis than for inactive NCC (Harrison et al. 1989; García et al. 2001).

T. solium crude antigens, complete extract or vesicular fluid, have been used during many years (Diwan et al. 1982; Larralde et al. 1986). These techniques show a poor specificity, mainly due to cross-reactions with related helminth infections (Gottstein et al. 1987; Pammenter et al. 1992).

Also, heterologous antigenic extracts were employed. *T. saginata* (Harrison and Parkhouse 1989; Oliveira et al. 2010), *T. crassiceps* (Larralde et al. 1990; Espíndola et al. 2002; Suzuki et al. 2007), and *T. hydatigena* (Hayunga et al. 1991) have been used for detection of human cysticercosis.

More recently, purified antigens were introduced as diagnostic tools. Antigen B (AgB or paramyosin) and glycoproteins have been studied for NCC immunodiagnosis (Flisser et al. 1980, 1986; 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 or ELISA protocols, with serum or CSF samples. Although glycoproteins-EITB has 100 % specificity and an overall sensitivity of 98 %, major problems are that approximately 30 % of patients with a single brain parasite, or calcified lesions, may test negative (Wilson et al. 1991) and that the *T. solium* genotypes showed distinct glycoprotein patterns (Sato et al. 2006). Also, it is important to note the technique is very expensive, hampering its use in endemic areas (Villota et al. 2003; Suzuki and Rossi 2011).The EITB was recognized as the gold standard for NCC immunodiagnosis by Pan American Health Organization (PAHO) (Greene et al. 2000). Also, purified *T. solium* E/S antigens have been employed in ELISA and FAST-ELISA with very promising results in antibody detection of NCC (Ng and Ko 1994; Sahu et al. 2009; Atluri et al. 2009). From the metacestode secretion proteins, those that have low molecular weight, and especially glycoproteins, have showed the 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) have been described as the best candidates to prepare an antibody-detection system for NCC detection. Although these systems have worked properly, some difficulties (biochemical purification, big parasite amounts, reproducibility) restrict their uses.

Recombinant antigens: Biotechnological approaches have been used to solve the scarcity of *T. solium* parasitic material for the preparation and purification of diagnostic antigen candidates. The cloning and expression of *T. solium* metacestode genes relevant for diagnosis have allowed circumventing the limitations mentioned.

Many genes have been studied during the last decades. Paramyosin, sHSP, TSA18, F18, 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 have been checked in ELISA and Western blot, with good sensitivity and specificity for NCC diagnosis (Vazquez-Talavera et al. 2001; Ferrer et al. 2003b, 2005a, 2007a; Montero et al. 2003; Hancock et al. 2004). Even though most of them worked better with

active NCC samples, TSA18 expressed in baculovirus system showed the best sensitivity (60 %) for inactive NCC immunodetection. Also, some recombinant antigens have been used for animal cysticercosis identification.

Regarding recombinant products, one of the most promising NCC diagnostic antigens is the 8 kDa family. Their members are metacestode excretory/secretory glycoproteins (65–90 amino acid residues and 7–12 kDa), which invoke strong specific antibody reactions in the infected individuals, and appear to be expressed as variant arrays, with both sequence heterogeneity and homology in clusters of small domains that determine epitope differences among them (Ferrer et al. 2012). 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); the 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) recombinant antigens showed excellent sensitivity and specificity in NCC diagnosis.

Synthetic peptides: Synthetic peptides, derived from the cloned molecules, have been prepared as tools to be used in cysticercosis diagnosis. They have been employed in ELISA and Western blot, with good results in some cases, although the diagnostic properties of recombinant antigens were not improved.

Based on the *Taenia* 8 kDa family, sTS14 and sTS18 peptides, corresponding to TS14 and TS18 antigens, have been synthesized, showing excellent specificity but poor sensitivity (Greene et al. 2000). Also, peptides derived from *T. saginata* oncosphere molecules have been used for cysticercosis diagnosis and human and animal disease, with similar results to the ones reported above (Fleury et al. 2003; Ferrer et al. 2005b).

7.7.2.4 Molecular Diagnosis

In 2006, Almeida et al. demonstrated, for the first time, the presence of *T. solium* DNA in CSF from NCC patients. Such observation has opened the use of molecular techniques, PCRs, for NCC diagnosis. Both conventional and real-time PCR protocols have been developed, showing excellent sensitivity (70–95 %) for extraparenchymal case identification (Hernández et al. 2008; Michelet et al. 2011; Yera et al. 2011).

To conclude the section, and considering the NCC complexity and diagnosis handicaps, the disease identification can be undertaken by compilation of laboratory diagnostic results, as well as clinical-epidemiological data, following Del Brutto criteria (Del Brutto et al. 2001). Although this last effort was important, it has not been validated yet.

7.8 Treatment

7.8.1 Taeniosis

It is treated using either praziquantel or niclosamide. Niclosamide is the drug of choice, 2 g orally in a single dose is recommended for adult patients. Praziquantel is used in a sole dose, orally, at 5–10 mg/kg. With praziquantel there is a risk of provoking neurological symptoms if latent NCC is present in the same individual. Both compounds are difficult to find in most of the markets.

7.8.2 Cysticercosis

Treatment of NCC is complex and should be individualized. Management of the disease involves the use of cysticidal therapy, symptomatic therapy, and sometimes surgery, being recommended to tailor the treatment to the type of NCC (location, number, and viability of the parasites) under medical surveillance.

Cysticidal therapy. The cysticidal therapy is carried out with praziquantel and albendazole. *Albendazole* is used at a dose of 15 mg/kg/day (maximum 800 mg). It is employed usually for 28 days, although shorter durations of 8–14 days have also been used; side effects depend on the dose and duration of therapy. *Praziquantel* is used at a dose of 50 mg/kg/day. The usual duration of therapy is for a period of 15 days; side effects are dose related, though they are uncommon (Singhi 2011). Praziquantel and albendazole have been used together with interesting results (Garcia et al. 2011). These drugs are mainly employed for parenchymal viable cysts. In general, the cysticidal therapy has been a matter of debate since its implementation, regarding both advantages of cyst destruction and real improvement of the clinical outcome. Most publications have reported "reduction of the number of lesions" to measure anthelmintic drug effectiveness, which is misleading; possibly, the evaluation of cyst disappearance could be a more appropriate approximation.

Summarizing, based on double-blind, placebo-controlled trials, and comparing the effect of albendazole and praziquantel, it is generally accepted, with few discrepancies, that both drugs are effective in destroying viable cysts, while their use in cases with enhancing lesions has been debated as these lesions are considered to represent degenerating cysts, many of which resolve spontaneously (Carpio et al. 2008; Thussu et al. 2008; Chaurasia et al. 2010). In relation to the role of cysticidal therapy in control seizures secondary to NCC, also there are some controversial results; some authors found an improved seizure control in adults with vesicular lesions, as well as enhancing lesions, with the use of cysticidal drugs (Garcia et al. 2004; Del Brutto et al. 2006), while others did not find any significant improvement (Carpio et al. 2008; Abba et al. 2010). In conclusion, cysticidal

therapy seems to be effective in reducing the number of lesions, but its role in improving long-term seizure control needs further larger studies.

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 these NCC types need to be managed with exceptional caution. However, cysticidal therapy should not be used in cases with markedly elevated intracranial pressure and in ophthalmic (intraocular) NCC or massive infections; steroids alone are used in these situations. Also, cysticidal therapy is of no use for calcified lesion(s). To sum up, there is no universally agreed single protocol for the treatment of NCC, and consensus guidelines recommend an individualized approach (Nash et al. 2006).

Symptomatic therapy. Seizures usually respond very well to first-line antiepileptic drugs (AEDs). The recurrence rate after AED withdrawal is low in NCC cases with single lesion. Recurrence of seizures after AED withdrawal is correlated with the presence of multiple lesions prior to starting cysticidal therapy and persistence or calcification of lesions after therapy (Talukdar et al. 2002; Goel et al. 2010).

Corticosteroids. Oral corticosteroids are administrated generally a couple of days before and a few days along with anticysticercal therapy so as to prevent any adverse reactions that may occur due to the host inflammatory response. Usually oral prednisolone, 1–2 mg/kg, is used; intravenous dexamethasone may be used if there are features of raised intracranial pressure. In cases with disseminated lesions and extensive cerebral edema, steroids may be required for a prolonged period (Singhi 2011).

Surgery. Surgical intervention is required in some cases, particularly in intraventricular and subarachnoid NCC. A ventriculoperitoneal shunt is needed for hydrocephalus; 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 and is therefore the procedure of choice. Excision of giant cysts that fail to respond to medical therapy may be required (Goel et al. 2008; Suri et al. 2008; Singhi 2011).

7.9 Prognosis

Striated muscle and subcutaneous cysticercosis have good prognosis. Ocular cysticercosis could end in blindness if the eye parasite is not diagnosed and cysticidal treatment is used for a concomitant NCC (Sundar et al. 2010). NCC cases with single lesions generally have a good prognosis, seizures are usually well controlled, and lesions disappear within 6 months in over 60 % of cases. Patients with multiple lesions and those with calcifications often have frequent seizure recurrences. Cysticercus encephalitis and extraparenchymal NCC have a cautious prognosis (Singhi 2011).

7.10 Prevention and Control

Cysticercosis is an NTD that occurs in communities with low socioeconomic conditions and poor sanitation-hygienic practices. Globally, it can be prevented through improvements in health and education standards, treatment of *T. solium* carriers, improved pig-rearing management, as well as by treatment of infected animals (Flisser et al. 2003, 2004; Engels et al. 2003; Sarti and Rajshekhar 2003; González et al. 2003; Xiao et al. 2013). More importantly, it is possible to consider its eradication taking into account that human tapeworm carrier is the unique definitive host 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). Thus, potential intervention measures should include the following criteria:

Educational programs. Educational designs for endemic rural areas, taking into account culture and idiosyncrasy of the population. The designs will include basic and proper hygiene and sanitation measures, teaching the parasite biology and epidemiology, and apprising of taeniosis/cysticercosis symptoms and the ways to interrupt transmission, among other information.

Drug treatment of Taenia carriers. To decrease the source of infection, finding and treating tapeworm-infected individuals would be the intervention of choice. Once a *Taenia* carrier is identified, careful treatment and follow-up can ensure the cure of the patient and thus close the transmission. So far, mass treatment programs of the population to eliminate tapeworms with the use of niclosamide or with praziquantel (Sarti and Rajshekhar 2003) have been successful and have temporarily reduced the disease transmission, but the effect has not been sustainable.

Pig management. Slaughterhouse control is suggested as a key control component, but it is relevant to avoid the development and establishment of illegal markets for infected pork. Also, pig corralling is really important, although this option is opposed to the main reason of raising pigs in endemic regions as they roam free and do not need to be fed by their owners. Pig treatment with oxfendazole is another alternative (Gonzalez et al. 2012).

Pig vaccination. Vaccination has been proposed as a possibility to control the transmission of cysticercosis. Recently, the advances made in the vaccine area are really promising to be applied to the control of cysticercosis. In fact, several intervention programs have already included the use of vaccines to interrupt the parasite transmission, among other measures (Assana et al. 2013; Xiao et al. 2013). There are vaccines based on the use of crude extracts, recombinant antigens, peptides, and naked DNA.

Pioneer vaccination studies in cattle were carried out with both crude and E/S antigens from *T. saginata* and *T. hydatigena* (Rickard et al. 1981). Since then, different parasite extracts prepared from *T. solium* oncospheres, as well as metacestodes, have been used in porcine vaccination trials, and different protection levels have been reported (Molinari et al. 1983a, 1997; Pathak and Gaur 1990; Verastegui et al. 2002). Also, this kind of assays has also been developed in the

mouse/*T. crassiceps* model (Valdez et al. 1994; Sciutto et al. 1995), obtaining similar protection levels with both *T. solium* and *T. crassiceps* antigenic extracts.

Also recombinant antigens have been used as vaccines. For *T. saginata*/cattle system, most of these molecules have been derived from both 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) and *T. saginata* TSA9 and TSA18 (syn. HP6) recombinant proteins were used in cattle immunization assays, yielding the TSA18 candidate excellent results (Benítez et al. 1996; Lightowlers et al. 1996). It is interesting to note that all these purified recombinant proteins were demonstrated to function as adhesion molecules, a property which is probably pertinent to their potential as vaccines, as is the case for the HP6 molecule of *T. saginata* (Harrison and Parkhouse 1989; Benítez et al. 1996; Bonay et al. 2002; Harrison et al. 2005). In *T. solium*/porcine system, the genes homologous to the ones described above (Tsol18, Tsol45-1A, TSOL45-1B, TSOL16), and others (paramyosin, fatty acid-binding proteins (FABPs), KETc1, KETc4, KETc7, KETc11, KETc12), have been used in vaccination assays (Manoutcharian et al. 1996; Vazquez-Talavera et al. 2001; Gauci et al. 2012). Of all molecules, Tsol18 plasmid construction, expressed in *Escherichia coli* and purified, is the candidate that yielded best results, almost 100 % protection, and is being used as a vaccine in the control programs organized in different endemic regions (Flisser et al. 2004; Gauci et al. 2012; Assana et al. 2013).

Regarding peptides as vaccination tools, the most used have been KETc1, KETc12, KETc7, GK1, GK2, and GK3 (Manoutcharian et al. 2004; Toledo et al. 2001). Later, the combination GK1, KETc1, and KETc12, called S3Pvac, has been extensively employed in vaccination assays in *T. solium*/porcine, *T. crassiceps*/mouse, and *T. pisiformis*/rabbit models. In pigs, the S3Pvac produced till 98.7 % protection and showed therapeutic properties (de Aluja et al. 2005; Rassy et al. 2010; Sciutto et al. 2013a, 2013b). Finally, naked DNA vaccination has also been used in experimental studies; *T. saginata* and *T. solium* cDNAs already mentioned (KETc7, paramyosin, Tso 18, others) have been employed with promising protection (Manoutcharian et al. 1998; Cruz-Revilla et al. 2000; Rosas et al. 2002).

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Chapter 8
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 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 creatine phosphokinase (CPK) 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 glucocorticosteroids.

A section is devoted to control measures, including a possible vaccine for which several molecules are under investigation.

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8.1 Introduction

Trichinellosis is a worldwide zoonosis caused by the nematode *Trichinella* spp. At the world level, it is mostly transmitted by pork from backyard pigs. It can be a serious disease, particularly in older persons, where severe complications such as myocarditis or encephalitis can lead to death. These parasites are widespread in wildlife on all continents but Antarctica and in domestic pigs of many countries (Pozio and Murrell 2006). Infections occur in populations that eat raw or undercooked meat and meat products of different animal origins (e.g. pork, horse, game).

8.2 The Agent

8.2.1 Species and Genotypes

At present, nine species and three genotypes are recognised 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* and *T. patagoniensis* (Table 8.1). The parasites circulate in a variety of hosts, with carnivorous and omnivorous animals representing the most important reservoirs. 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).

8.2.2 Parasitic Cycle

The parasitic cycle (Fig. 8.1) can be divided into two phases: an intestinal (or enteral) phase and a systemic and muscular (parenteral) phase, which can coexist for a period lasting from a few days to weeks. Infection occurs after consumption of raw or undercooked meat containing coiled larvae 0.7–1.1 mm long. After the gastric digestion of the infected meat, the larvae are released in the stomach, and then they arrive to the small intestine where they penetrate the mucosa and mature into adult worms (approximately 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. Upon reaching sexual maturity, male and female worms mate, and 5 days later adult females shed 100 μm-long newborn larvae (NBL) into

Table 8.1 *Trichinella* species and genotypes and human infections

Trichinella species or genotype	Distribution	Usual hosts	Human cases reported	Source of infection	Countries where 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 Hol-arctic zone	Terrestrial or marine carnivores	Yes ++	Bear meat, walrus, dog	Nunavut, Nunavik, Russia, China
T3; *T. britovi*	Temperate areas of the Pale-arctic 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 Nearc-tic zone	Carnivores	Yes +	Horse Bear	France, USA
T6	Arctic or sub-arctic regions of Canada and USA	Terrestrial or marine carnivores	?		
T7; *T. nelsoni*	Ethiopic region	Carnivores	?		
T8	South Africa	Carnivores	?		
T9	Japan	Carnivores			
T10[a]; *T. papuae*	Southeast Asia	Mammals and reptiles	Yes +	Soft-shelled turtles, wild boar	Thailand, Taiwan, Korea
T11[a]; *T. zimbabwensis*	East Africa	Mammals and reptiles	?		
T12; *T. patagoniensis*	Argentina	Carnivores	?		

[a]Non-encapsulated species
? means unknown
+++ numerous cases
++ frequent cases
+ occasionnal cases

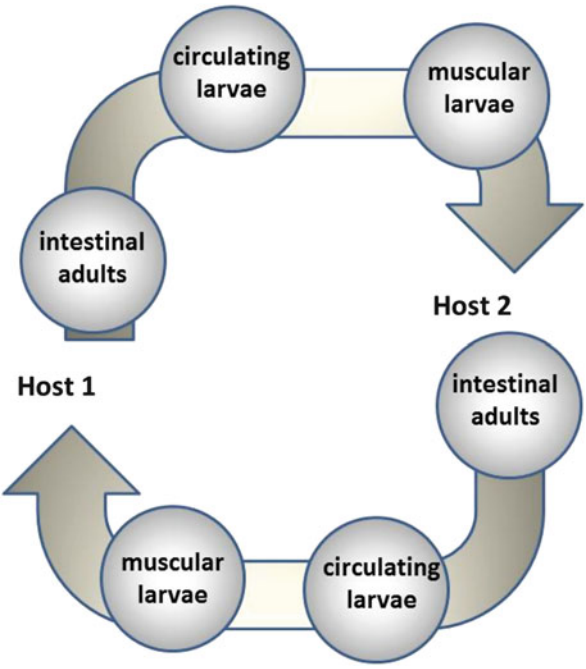

Fig. 8.1 Schematic biological cycle of *Trichinella*

the blood and lymphatic vessels. The NBL migrate in the general circulation to find their definitive niche: the musculoskeletal fibre. The circulating larvae induce in their host a parasitic vasculitis. After penetrating the muscle fibre, the larvae modify the metabolism of the muscle fibre 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 for another host. The larvae remain viable in the modified muscle fibre (called "nurse cell") for months or years. Mature females 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 Bruschi 2007). Female worms then die or are expelled by smooth muscle hypercontractility elicited by the immune response.

8.3 Epidemiology of Infection

8.3.1 Past Situation

Evaluating the worldwide prevalence of the disease is difficult because the definitive localisation 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 was infected (Stoll 1947). Serological surveys are possible but have several drawbacks: they are expensive, antibody titres fall quite rapidly though patients still harbour the parasite, and cross-reaction can occur, requiring the use of 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 serological 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 "localised 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, 1 million South Americans and 5 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 scrutinising the MEDLINE database (1965–1999) using the following query (*Trichinella* or trichinosis) and name of the considered country. Titles and abstracts were analysed to estimate the worldwide distribution of trichinellosis in humans and animals. These data confirmed that *Trichinella* has 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.

8.3.2 Present Situation

Trichinellosis remains an important zoonotic disease on a global basis. In an extensive review of published cases, Murrell and Pozio (2011) analysed outbreak report data for 1986–2009. Searches of six international databases yielded 494 reports. After applying strict criteria for relevance and reliability, they selected 261 reports for data extraction. From 1986 to 2009, there were 65,818 cases and 42 deaths reported from 41 countries. The World Health Organization 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. An analysis of all reports made on the ProMED-mail organisation from 1998 to 2013 yielded 58 outbreaks involving more than 2,400 cases and 15 deaths from 23 countries (see

Fig. 8.2). Pork was the source of the infection in 64 % of cases, horsemeat in 16 %, wild boars in 12 % and wild carnivores in 8 % (see Fig. 8.3). Of course, these reports were early warnings not fully analysed, reporting unusual vectors, imported cases or severe and lethal outbreaks; data from China are not reported through this media (Dupouy-Camet, unpublished). The usual sources of infection for humans are detailed in Table 8.1, the most frequent source being pork from domestic or wild pigs harbouring *T. spiralis*, *T. britovi* and sometimes *T. pseudospiralis*. Meats from wild carnivores (bears, dogs, badgers, etc.) are a source of small outbreaks amongst hunters (Schellenberg et al. 2003) 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 (tartare, carpaccio, etc.). A list of unusual vectors of human trichinellosis is also given in Table 8.2. It is not possible to give here details on the epidemiological situation 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).

8.3.3 An 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 disorganisation 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 (Greenland, Alaska or northern Canada) by individual or small groups of travellers or hunters (Ancelle et al. 2005; Houzé et al. 2009)). Isolated cases reported in travellers 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 and Laos). In addition, consumers from countries where the habit of eating raw meat is common will be at higher risk, particularly if they are backpackers, adventure travellers or hunters of exotic animals. Acquiring trichinellosis while travelling abroad is not a new phenomenon, as McAuley et al. (1991) reviewing "Trichinella infection in travellers" 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 2,497 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

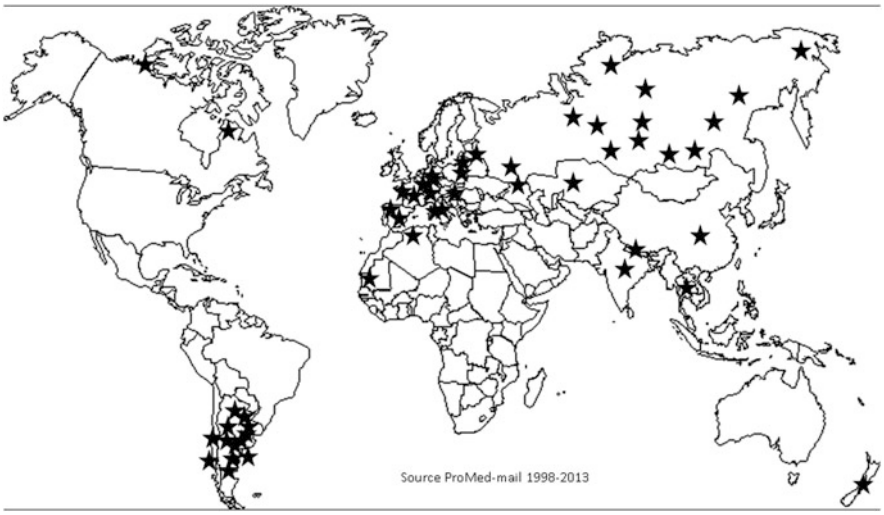

Fig. 8.2 Geographical locations of trichinellosis outbreaks reported in ProMed-mail (1998–2013)

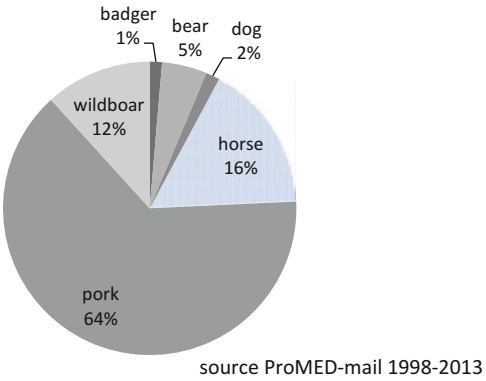

Fig. 8.3 Source of trichinellosis outbreaks reported in ProMed-mail (1998–2013)

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). The incidence of imported cases could have decreased as numbers of international travellers increased during that period. Imported cases diagnosed in France in the period 1975–1995 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

Table 8.2 Unusual sources of trichinellosis in humans

Unusual host	Countries	Number of cases	Species	References
Badger	Russia, Korea	+	*T. spiralis*, other?	Sohn et al. (2000), Suzdaltsev et al. (1999)
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)
Turtle	Taiwan, Korea, Thailand	Sporadic	*T. papuae*	Khamboonruang (1991), Lo et al. (2009), 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)

+ occasionnal cases
++ frequent cases

1990s (Angheben et al. 2008; Nöckler et al. 2007; Milne et al. 2001). Cases were also described in Asia after turtle consumption (Lo et al. 2009; Lee et al. 2013) and after travelling to a seaside resort in a neighbouring island of Singapore (Kurup et al. 2000). Travellers 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. As well, 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 (raw foodism, instinctive eating, Palaeolithic diet, etc.). Conversely, trichinellosis is practically never reported in Muslim countries or in Jewish communities, due to the proscription of eating 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 industrialised 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 (five outbreaks from 1975 to 1985, eight 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). Globalisation of international trade is also a risk factor: many countries where trichinellosis is endemic amongst 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 forests 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 industrialised 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 in the USA where reporting of human cases is required, but no surveillance programme per se is present; 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).

8.4 The Host Response to *Trichinella*

The host immune response to the parasite is regulated on a genetic basis, and the genes involved belong either to the major histocompatibility complex (MHC) loci or to non-MHC regions (Bell 1998). In Table 8.3 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).

Table 8.3 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, permission requested)

Parasite life cycle	Host response
Entry of infective muscle larvae Invasion of enterocytes Release of stichosomal antigen Exposure to surface antigen	Uptake, processing and recognition of antigens, initial Th1 response
Maturation of adults	**Antibody response**
Release of newborn larvae (NBL)	**Mast cell response**
Exposure to adult and NBL antigens	**Gut inflammation begins**
Expulsion of adult worms	**Antibody response**
Migration of NBL	**Mast cell response** Acute inflammation **Shift to Th2 response**
Invasion of muscles	**Antibody response**
Nurse cell formation	**Eosinophilia**
Release of stichocyte antigen	Gut inflammation subsides
Formation of capsule, when present	**Inflammation in muscle** **Consolidated Th2 response**

Processes described also in humans are written in boldface

8.4.1 Immune Response at Intestinal Level

The mechanisms regulating the intestinal immune response to a primary infection in humans are not well understood. In outbreaks involving the Inuit population in the Canadian Arctic, a prolonged diarrhoea was observed (Viallet et al. 1986; MacLean et al. 1989) suggesting the adult worm persistence in the intestine of people probably frequently exposed to infection. This might be ascribed to a possible downregulation of the intestinal immune response or of gut physiology, or to a premunition state, caused by the continuous antigen stimulation. 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). The immune response against *T. spiralis* at intestinal level depends on parasite-specific $CD4^+$ T cells (Grencis et al. 1985; Riedlinger et al. 1986), locally generated in the first 2–4 days of infection (Korenaga et al. 1989) and then migrating to Peyer's patches and to the mesenteric nodes and finally to the tissues (Bell 1998). The cytokine produced by the two Th subsets, Th1 and Th2 (Mucida and Cheroutre 2010), is involved in the adult worm expulsion process, a very 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 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 very early human infection, but later Th2 response lasts for all chronic phases of infection (Gomez-Morales et al. 2002). The Th2 response is essential to control infection at the intestinal level, by producing both IL-4 and IL-13 (this latter is produced also by natural killer cells (McDermott et al. 2005; Lawrence et al. 1998) through STAT6 activation (Urban et al. 2000)). IL-4 regulates the production of specific IgE (Finkelman et al. 1986), which transfers intestinal immunity (Ahmad et al. 1991), and stimulates also the uptake and transport of IgE in the intestine (Ramaswamy et al. 1994). The other cytokine produced by Th2 cells, IL-5, regulates the production of eosinophils (Watanabe et al. 2005). Contrasting results were obtained as regards IL-9; in fact if neutralised with vaccination or with a specific antibody, no significant effect on worm expulsion or muscle hypercontractility in *T. spiralis*-infected mice is observed, but its administration in vivo enhances jejunal muscle hypercontractility which follows infection and accelerates worm expulsion (Khan et al. 2003). The lack of IL-10 impairs a fruitful intestinal response to the parasite, as revealed by studies in IL-10 knockout (KO) mice or animals treated with a neutralising antibody anti-IL-10 receptor (Helmby and Grencis 2003a). IL-12 counteracts the effects of Th2 response, delaying worm expulsion and increasing worm burden at muscle level in an IFN-γ-independent way (Helmby and Grencis 2003b). Similar results were obtained in animals treated with a Th1 adjuvant, such as the *Helicobacter pylori* neutrophil-activating protein (Chiumiento et al. 2011), which increases serum IL-12 levels in *T. spiralis*-infected mice (Del Prete et al. 2008). 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 non-genetically modified mice do (Helmby and Grencis 2002).

8.4.2 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).

8.4.2.1 Mast Cells

Trichinellosis is characterised by a pronounced hyperplasia (mastocytosis) and activation of mucosal mast cells, not only in experimentally infected animals

(Woodbury et al. 1984; Miller 1996), but also in patients (Gustowska et al. 1983). These cells are produced and differentiate under the control of several cytokines produced by T cells (Garside et al. 1992) under the control of the transcription molecule STAT6 (Finkelman et al. 1986). Mast cells play a crucial role in worm expulsion (Suzuki et al. 2008). Bone marrow restoration of W/Wv mice, an animal strain naturally deficient in mast cells because of the mutation of the *c-kit*, a tyrosine kinase receptor for the stem cell factor, restaured 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) which makes the gut wall more permeable to antibodies, arriving easily to the parasite sites (Scudamore et al. 1995, 1998). Mast cells not only play an important role 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 90–99 % of *T. spiralis* L1 larvae from the intestine in a very short time (few hours) in a phenomenon called *rapid expulsion* (Bell 1998); this occurs only in rats, after a primary infection, while mice expel rapidly only a secondary infection (Bell 1992). If rats are infected bypassing the intestinal phase, mastocytosis does not occur, but after an oral challenge, the animals can again mount a rapid expulsion (Blum et al. 2009).

IgE-Independent Mast Cell Degranulation

Trichinella antigens, mainly those belonging to TSL-1 family (see below), activate mast cells directly, inducing the release of histamine, protein 5 and tumour necrosis factor (TNF)-α (Arizmendi et al. 1997) and increasing the expression of IL-4 and TNF-α, while depressing that of IFN-γ and IL-10, in a rat mast cell line (Arizmendi et al. 2001). The same antigens can also trigger histamine secretion from unsensitised rat mast cells, with no detectable changes in intracellular Ca^{2+} (Arizmendi-Puga et al. 2006). These effects on mast cells are not induced by NBL antigens (Yépez-Mulia et al. 2009).

8.4.3 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).

Differently 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).

8.4.3.1 Inflammatory Response at Intestinal Level

Enteritis renders the habitat hostile to the parasite, thus facilitating worm expulsion, regardless the age and consequently the status of the parasite (Bell 1998). Myeloid rather than lymphoid cells is involved in worm expulsion, as shown in chimeric mice selectively expressing the receptor α for IL-4 (IL4Rα) on bone marrow- or non-bone marrow-derived cells (Urban et al. 2001). Mice W/Wv deficient in mast cells (Ha et al. 1983) received bone marrow cells from wild-type animals or from KO mice for TNF-α or for IL-4, before infection with *T. spiralis*. Those receiving cells from KO for TNF-α or for IL-4 mice cleared worms more slowly and experienced a reduced enteritis differently from W/Wv mice reconstituted with normal bone marrow. Furthermore, mast cell responses were reduced in both types of KO mice, suggesting that autocrine production of TNF-α and IL-4 by mast cells is involved in the protective Th2 response as well as in inflammation associated to worm expulsion (Ierna et al. 2008). The proinflammatory 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). Recently, however, in transgenic mice for the transmembrane TNF-α (tmTNF-α) isoform (expressing only the non-cleavable TNF-α), it was observed that soluble TNF-α, but not the tmTNF-α, plays a role 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 TNF-α is required for the induction of Th2 immune responses typical of infections with intestinal helminths (Ierna et al. 2009). KO mice for inducible nitric oxide synthase (iNOS), 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).

8.4.3.2 Immune Response at Muscle Level

Trichinella is the only helminth which has a special relation with skeletal muscle cells; it is unique in inhabiting an intracellular localisation (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 characterised by a Th2 phenotype, according to ex vivo studies using cells collected from cervical lymph nodes or spleen cells of infected mice which, after stimulation with parasite antigen, were able to produce Th2-dependent cytokines, such as IL-5, IL-10 and IL-13 (but also IFN-γ) (Li and Ko 2001). Furthermore, increased levels of parasite-specific IgG1 and IgE during the chronic infection confirm such polarisation of immune response (Beiting et al. 2004, 2007; Fabre et al. 2009a). The host immune response to *Trichinella*, at the muscle level, is partially regulated by the enteric phase of infection; in orally infected animals, the myositis is higher compared to that observed in animals infected by intravenous injections of NBL, bypassing the intestine (Fabre et al. 2009a, b). The presence of parasites in the muscle fibres elicits, as already said, a strong inflammatory response which is not able to eliminate the parasite but 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 et al. 1985; Li and Ko 2001; Bruschi et al. 2009). At the muscle level, inflammatory cell infiltration is higher in BALB/c than in CBA/N mice and even lower in nude mice during infection with *T. spiralis*, whereas when animals are infected with *T. pseudospiralis*, cells are reduced in BALB/c mice and absent in both CBA/N and nude mice. Analysis of cytokines produced by popliteal lymphocytes recovered at different time points after injection of *T. spiralis* newborn larvae directly in the leg muscles showed a typical Th2 pattern (increase of IL-4 and IL-6, but not of IL-2 and IFN-γ) (Li and Ko 2001). In humans, cell-mediated immunity was studied at 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.

8.5 Immunopathology

8.5.1 Enteral Phase of Infection

During experimental infection with nematodes, a pronounced hyperplasia (mastocytosis) and activation of mucosal mast cells occur (Ha et al. 1983; Woodbury et al. 1984; Tuohy et al. 1990; Lawrence et al. 2004). This observation was confirmed in the jejunum of patients infected with *T. spiralis* (Gustowska et al. 1983).

8.5.2 Parenteral Phase of Infection

During this phase of infection allergic manifestations occur, which are caused by activation of sensitised 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 oedema (Dupouy-Camet and Bruschi 2007). Blood and tissue eosinophilia are characteristic of this phase (see below). A major question about the role of eosinophils in the parenteral phase is whether they are protective or not against *Trichinella*. In in vitro ADCC experiments with NBL as target, many cell populations such as eosinophils and neutrophils in both experimental animals and humans are able to kill the parasites, using different cytotoxic mechanisms, oxygen dependent (hydrogen peroxide, hypochlorite, etc.) and oxygen independent (major basic protein, eosinophil peroxidase, etc.). However, results in vivo are controversial, depending on the experimental model used (reviewed in Bruschi et al. 2008). Recently, it has been documented how eosinophils could support parasite growth and survival by promoting accumulation of Th2 cells and preventing induction of iNOS in macrophage and neutrophil NO-mediated killing (Fabre et al. 2009a, b; Gebreselassie et al. 2012). Whatever is the role of eosinophils in vivo versus helminthic parasites, they increase in the blood and tissues of infected hosts resulting in tissue damage to the skeletal muscle cells, myocardium, lungs and central nervous system as well (Bruschi et al. 2008). The parenteral or muscular phase is characterised by inflammatory and allergic responses to the invasion of the skeletal muscle cells by the migrating larvae. These can directly damage the muscle cells or indirectly induce the infiltration of inflammatory cells, primarily eosinophils. 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). In chronic trichinellosis, occurring some years from infection, skeletal muscle-specific antibodies recognising 28 and 41 kDa proteins in this tissue extract were detected in patient sera, suggesting that muscle damage is caused in the early phase of infection by the invasion process by the NBL but later is mediated by immunopathological processes (Pratesi et al. 2006).

8.5.3 Heart and CNS Involvement

Neurotrichinellosis represents a 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 re-entering 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 helminth infections are not yet fully elucidated. As already stated, 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-stage trichinellosis recognised several proteins present in human heart ventricle, not recognised by normal sera. In particular, when either tested against rat or human heart ventricle wall, a high proportion of sera (42 %) recognised 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 the 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 kidney, placenta and spleen (Pratesi et al. 2006).

8.6 Clinical Manifestations

The clinical aspects of trichinellosis have been extensively reviewed (Pawlowski 1983; Capo and Despommiers 1996; Kociecka 2000; Dupouy-Camet et al. 2002; Dupouy-Camet and Bruschi 2007; Gottstein et al. 2009) and are a summarised as follows.

8.6.1 Acute Phase

In most persons, the acute stage begins with the sudden appearance of general discomfort and severe headaches, an increasing fever, chills and 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 infected meat. The major syndrome of the acute stage consists of persistent fever, facial oedema (characteristically periorbital), muscle pain and severe asthenia, lasting for several weeks. Transient dizziness and nausea can also occur. Though less common, diarrhoea and conjunctival and subungual haemorrhages are also observed. This is the stage during which the adults and the migrating larvae provoke the signs and symptoms of the disease. The typical clinical signs (intestinal signs, fever, facial oedema and myalgia) must be searched for. The most common intestinal signs and symptoms are diarrhoea (from loose stools to as many as 10–15 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 one week. It has been observed that the shorter the duration between infection and the appearance of

diarrhoea and fever, the longer the duration of both fever and facial oedema (Dupouy-Camet et al. 1985). Fever is one of the earliest and most common signs of trichinellosis. Body temperature increases rapidly, usually stabilising at 39–40 °C. The fever usually lasts from 8–10 days, although it can persist for up to 3 weeks when the disease is severe. Symmetrical periorbital and facial oedema 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 5–7 d.p.i), particularly when glucocorticosteroids are used. In the severe form of trichinellosis, oedema extends to the upper and lower extremities. Myalgia or muscle pain affects 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 as a result of pronounced angiomyositis-type lesions and neuromuscular disturbances. The restriction of movement due to pain associated with exertion leads to contractions 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 haemorrhagic 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.

8.6.2 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, in persons who were improperly treated (including those for whom treatment was begun too late) and, particularly, in the elderly. A positive correlation has been reported between age and the frequency and severity of complications. Encephalitis and myocarditis, which are both life-threatening, are often simultaneously present (Fourestié et al. 1993).

8.6.2.1 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 5–20 % of all infected persons. The symptoms include pain in the heart region, tachycardia and electrocardiogram (ECG) abnormalities. The ECG disorder most frequently observed are non-specific

ventricular repolarisation disturbances (with ST-T wave changes), followed by bundle-branch conduction disturbances and sinus tachycardia. The 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, non-specific, ventricular repolarisation disturbance is the abnormality most commonly 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 hypokalaemia. Echography can identify myocardium functional anomalies (segmental 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. Sudden death may result from embolism of the pulmonary artery or from paroxysmal tachycardia. Echography can identify pericardial effusion or a transitory intracavitary thrombus.

8.6.2.2 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) 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 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, behavioural disturbances, transient hemiparesia or hemiplegia, oculomotor dysfunction, aphasia and cerebellar syndrome. Small hypodensities are seen with the CT scan or magnetic resonance imaging (MRI) (Feydy et al. 1996; De Graef et al. 2000; Gelal et al. 2005). CT scan can find nodular multifocal hypodensities which can be bilateral and of 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 ischaemic nature of the first, while the seconds are rather regarded as being of granulomatous origin. The imagery by MRI confirms these aspects. These images are not very 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.

8.6.2.3 Other Complications

Ocular lesions appear during the acute stage of the disease and result from disturbances in microcirculation. The typical traits are oedema 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 the early and late stages of trichinellosis. They consist of pneumonia, obstructive bronchitis or Löffler-type infiltrates or ventilation 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 hypoalbuminaemia and localised oedemas, acute intestinal necrosis or prolonged diarrhoea. In some outbreaks (Dupouy-Camet and Bruschi 2007), oedema of limbs was reported in 6–8 % of infected persons. As already mentionned above, a particular syndrome has been described in persons who regularly eat infected meat (i.e. Inuit populations). In these persons, trichinellosis manifests itself as a chronic diarrhoeal syndrome which is due to the strong intestinal immune reaction; this reaction consists of a rapid expulsion of adult worms from the intestine, thus preventing the muscular phase (Viallet et al. 1986; MacLean et al. 1989).

8.7 Clinical Forms

8.7.1 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 (the number of NBL shed by females differs by species); and the individual susceptibility which depends on ethnic factors as well as sex, age and the immune status of the host. There are no precise data defining the minimal infective dose able to exert clinical trichinellosis in an individual person. Murrell and Bruschi (1994), 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 has also been stated that meat containing at least one larva per gram is necessary to induce a clinical infection in man (Zimmermann 1983), which would correspond to an infective dose of approximately 150 larvae for the usual consumer (assuming a meat consumption of 150 g). Consequently, an infection with 1,000–3,000 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, specifically: the incubation period lasts approximately 1 week for the severe form, 2 weeks for the moderately severe form and at least 3–4 weeks for the benign and abortive forms. This is because clinical disease manifests itself as a result of the worm burden.

8.7.2 Species and Genotypes

Although clinical differences have been observed amongst persons infected with different species of *Trichinella* (Bruschi and Murrell 2002), it has not been possible to attribute these differences to the species 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 due to the fact that *T. britovi* females are less prolific in production of NBL (Pozio et al. 1993). *Trichinella pseudospiralis*, which is non-encapsulated, seems to provoke signs and symptoms that last longer (Jongwutiwes et al. 1998; Ranque et al. 2000).

8.7.3 Immunocompromised Patients

To our knowledge, only three cases of trichinellosis have been reported in immunocompromised persons. In a renal graft recipient, the infection was asymptomatic, even in the presence of 1,400 larvae/g in the deltoid muscle (Doby et al. 1984), and in an HIV-positive person, the clinical symptoms were not particularly severe (Louthrenoo et al. 1993). A very severe case was described in a person with chronic myeloid leukaemia (Jacobson and Jacobson 1977). Hypereosinophilia could be absent from these patients (Kociecka, personal communication).

8.7.4 Pregnancy and Childhood

In pregnant women, trichinellosis can cause abortion or premature delivery. 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). In children, the signs and symptoms of trichinellosis are the same as those found in adults, although myalgia and diarrhoea 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).

8.8 Diagnosis

8.8.1 Non-specific Laboratory Signs

8.8.1.1 Eosinophilia

Blood eosinophilia is a typical response to nematode infections, and this is particularly true in trichinellosis. This response depends on the parasite inoculum size and on T-cell activation, although a T-cell-independent eosinophilia has been described, according to the results obtained in experimental models, and is under the control of genetic factors (Bruschi et al. 2008). The control of eosinophil levels by T cells is exerted by activation of Th2 cells which, after activation, produce high amounts of IL-5, the cytokine that stimulates the production and differentiation of this granulocyte population in the bone marrow (Bruschi et al. 2008) and prevents their apoptosis as shown in *Trichinella*-infected rats, together with other factors, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) or IL-3, especially in early period of infection (Gon et al. 1997). Eosinophilia might be due then not only to an increased production but also to a reduced eosinophil apoptosis, favoured in both cases by increased levels of IL-5 and other protective cytokines such as IL-3, which would determine an accumulation of these cells in the blood and tissues (Simon and Blaser 1995).

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 2nd and the 5th week of infection. Eosinophilia occurs in various degrees: low (<1,000/μl or 1 G/l), moderate (1,000–3,000/μl or 1–3 G/l) and high (>3,000/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 three 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. The mechanism underlying this decrease has not been fully understood, though it could be related to modifications in the levels of certain

cytokines and to the massive exit of eosinophils from the vascular system, leading to huge tissue infiltrates.

8.8.1.2 Muscle Enzymes

The levels of all muscle enzymes increase in serum during the course of 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 2nd and the 5th 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).

8.8.1.3 Immunoglobulin Level Increase

Trichinellosis like other helminth infections is characterised by increased levels in serum immunoglobulins (hypergammaglobulinaemia), 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 characterises helminth infections (Bell 1998; Watanabe et al. 2005), and in part to the humoral-specific response against the parasite. It was long debated as to the possible protective role of parasite-specific IgE against *Trichinella*; in fact, the results in experimental models are contradictory (Watanabe et al. 2005). The most clear-cut results were obtained in KO mice for IgE gene (coding for ε chain) which harboured a twofold number of muscle larvae 28 days postinfection compared to wild-type mice (Gurish et al. 2004). In humans, it is not clear whether IgE is protective for the host, but certainly this immunoglobulin class mediates 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 helminth infections. However, this increase in total IgE levels is not a consistent phenomenon, and it is not possible to exclude trichinellosis on the basis 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 defence process (Watanabe et al. 2005). Clinical observations suggest that *Trichinella*-specific IgE is responsible for allergic manifestations typical of the clinical picture of trichinellosis, such as cutaneous rash or oedemas (Watanabe et al. 2005).

8.8.2 Immunodiagnosis

Immunodiagnosis of human trichinellosis is performed usually with the aim to (1) recognise the acute phase of infection to allow early anthelmintic treatment, (2) to make a retrospective diagnosis or (3) to acquire information to the epidemiology of an outbreak (Ljungström 1983). In the past many serological techniques have been used for detecting antibodies against *Trichinella* antigen, but now ELISA is the most frequently used technique as a screening test, in combination with immunoblotting (Western blot) to possibly confirm ELISA-positive samples. By using both techniques, it is also possible to make the diagnosis earlier (Costantino et al. 2001).

8.8.2.1 Antigens

The choice of the appropriate antigens represents the major challenge in setting up of a serology test. Different antigens can be used for serological diagnosis: (1) cryosections of infected muscles or isolated larvae (muscle-larva cuticle antigen), which are generally used for indirect immunofluorescence (IIF); (2) a crude antigen prepared from muscle larvae; (3) an excretory/secretory antigen (E/S antigen) produced in vitro after short-term culture of the muscle larvae; and (4) a 3,6-dideoxyhexose sugar (tyvelose), one of the major highly specific immunodominant epitopes of *Trichinella*. Tyvelose is highly specific, yet it is less sensitive than crude and E/S antigens; alternatively, use of crude and E/S antigens might result in cross-reactions with non-specific *Trichinella* antibodies (Morakote et al. 1991). The antigenic pattern is quite similar amongst 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. Recently an E/S antigen has been standardised and the procedure to prepare it validated (Gomez-Morales et al. 2008).

8.8.2.2 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 infecting dose of parasites and the isotype of the antibodies (Van Knapen et al. 1982; Ljungström 1983; Bruschi et al. 1990). For example, IgE-class antibodies are typical of the acute stage of the disease, but they are seldomly detected because of the shortness of their half-life in serum, and only an amplified ELISA or the use of tyvelose antigen can greatly increase the sensitivity of the search (Bruschi et al. 2001). During the first days of the febrile phase, there are frequently 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, even in benign or asymptomatic cases (Harms et al. 1993). Serology can help greatly in diagnosis, but not in prognosis since 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, the time being 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 by *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 amongst 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; this allows follow-up of 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). As in many other parasitological infections, serological screening techniques are represented by IIF and ELISA. IIF can be carried out alternatively using antigen frozen sections of infected animal tissue (Ljungström 1983) or formalin-fixed whole larvae (Brzosko et al. 1965; Pozio et al. 1988), the former being more sensitive. Up to 100 % of sensitivity can be reached with IIF (Dupouy-Camet et al. 1988; Pozio et al. 1988). As regards specificity, in persons affected by autoimmune diseases, it is possible to observe false-positive reactions (Robert et al. 1996). Only sections with a uniform fluorescence along the cuticle should be considered as positive, and the operator should be aware of this. Many kits for ELISA are commercially available with sensitivities ranging from 80 to 90 % and specificities from 70 to 97 %. Only a 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 E/S antigens in an ELISA, absolute sensitivity (100 %) has been reached in humans infected with *T. spiralis* (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 is searched; in fact IgM was found even 15 years after infection (Pinelli et al. 2007). Lower sensitivity rates were obtained with serological tests measuring other antibody classes, such as IgA or IgE (Murrell and Bruschi 1994). The study of the humoral response against stage-specific antigens has not improved the diagnosis; however, the detection of NBL-specific IgA has shown promising results, especially in the early phase of infection when more than 80 % of infected persons tested positive after 3 weeks of infection (Mendez-Loredo et al. 2001). In asymptomatic individuals, the tyvelose antigen was able to detect infection (Owen et al. 2001). Antibody directed to tyvelose was found in late

periods, after 3–8 years of *T. spiralis* infection (Bruschi et al. 2005) as well as sporadically even after 15 years after a *T. britovi* outbreak (Piergili-Fioretti et al. 2005). Specificity depends primarily on the type of antigen used (better specificity for E/S antigen compared to crude extract) and the cut-off value established. Use of the synthetic tyvelose antigen in the ELISA resulted in improved specificity (Bruschi et al. 2001; Owen et al. 2001), with some exceptions (Dea-Ayuela et al. 2000). However, further experience on this synthetic antigen is required. A capture ELISA (cELISA) was set up using TSL-1 antigens (characterised by a high content of tyvelose-bearing epitopes) immobilised on the plates with the specific monoclonal antibody (MAb). It resulted in a reliable method for serological diagnosis of human trichinellosis with 100 % specificity and sensitivity at the patent stage of infection (Escalante et al. 2004). A glycan microarray approach has been used to select for synthetic glycan antigens that could be used for serodiagnosis of trichinellosis, in particular using a glycan array containing over 250 different glycan antigens. A GalNAcβ1–4(Fucα1–3) GlcNAc-R (LDNF) was identified as a glycan antigen that is recognised by antibodies from *Trichinella*-infected individuals. An ELISA-based test using a glycan represented by 5 LDNF molecules coupled to bovine serum albumin gave a 67 % specificity (false-positive results were mainly obtained with cysticercosis and strongyloidosis sera) and 96 % sensitivity (Aranzamendi et al. 2011).

Western blot (WB) can discriminate efficiently patients with trichinellosis from patients with other helminth infection (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 using E/S antigens its results are quite specific and useful for follow-up studies (Andrews et al. 1995). 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 the Dupouy-Camet and Bruschi algorithm (2007), reacted with a three-band-specific pattern ranging from 48 to 72 kDa in a WB assay performed with an E/S antigen. A distinctive pattern of 53–72 kDa for recognising *Trichinella* spp. infections in humans by WB was defined, obtaining a sensitivity and a specificity of 100 %. Even sera with a high OD 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 with a lower frequency also other proteins (Gomez-Morales et al. 2012). It is mandatory to consider the results on the basis of a cut-off 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, amongst

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 cut-off value be confirmed, every time the antigen, reagents or materials (e.g. type of ELISA plate) are modified or changed.

8.8.3 Muscle Biopsy

Muscle biopsy allows a parasitological diagnosis. Muscle tissue 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.

8.8.4 Trichinelloscopy

Trichinelloscopy 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 larva. It is extremely useful to identify the parasite at the level of species or genotype. 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 an oat 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×, or between two microscopy slides, and examined under a light microscope at a magnification of 50–100×. The larvae are easier to detect when the muscle biopsy is performed in the late stage of infection, which is characterised 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 non-encapsulated species, resulting in false-negative results.

8.8.5 Artificial Digestion

Digestion of muscle samples using 1 % pepsin and HCl digestion fluid is very useful to achieve a parameter for follow-up of the patient, i.e. the number of lpg of muscle tissue. Furthermore, after digestion it is possible to isolate larvae for molecular identification. A critical point is represented by the period of infection; in fact, if the muscle biopsy used in digestion 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 or longer p.i. are not destroyed by artificial digestion. Particular attention should be paid when a non-encapsulated species is suspected to be the aetiological agent since long periods of digestion might destroy the parasites. The procedure consists in cutting the muscular biopsy in small pieces and incubating at 41 °C for 30 min 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.

8.8.6 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 amongst the muscle fibres (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 fatty metamorphosis, hyaline or hydropic degeneration, or both, increased vascularity or small haemorrhages (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 very small and not easily distinguishable from the muscle fibres (Wranicz et al. 1998).

8.8.7 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). Recently, a PCR amplification of the mitochondrial large subunit ribosomal RNA (lsu-RNA) gene was coupled with a pyrosequencing technique to distinguish amongst *T. spiralis*, *T. pseudospiralis*, *T. papuae* and *T. zimbabwensis*, to analyse either larvae in infected mouse muscles or single larvae isolated by digestion from infected muscles (Sadaow et al. 2013).

8.9 Differential Diagnosis

Grouped cases or outbreaks are highly suggestive of the disease. Although a definitive diagnosis of trichinellosis can only be made with highly specific immunodiagnostic tests or by detecting larvae in a muscle biopsy, the infection can be suspected on clinical and epidemiological grounds. If two or more persons in the same household or a number of persons in the same community have high fever, periorbital or facial oedema and myalgia, trichinellosis can be suspected. When cases are sporadic or the clinical course is atypical, it is more difficult to suspect infection or less likely that the infection will be suspected. Once infection is suspected, information should be collected on the consumption of raw or undercooked meat or meat products, including the place and time of purchase and consumption.

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 diarrhoea 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, toxocariasis 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 oedema with fever should be differentiated from glomerulonephritis, serum sickness, allergic reactions to drugs or allergens, polymyositis, dermatomyositis and periarteritis nodosa. Intense headaches and stiff neck with confusion, drowsiness, irritability and neurological symptoms should be differentiated from infectious meningitis and encephalitis. Haemorrhages of the conjunctiva or haemorrhagic skin petechiae associated with fever should be differentiated from leptospirosis, bacterial endocarditis and typhus exanthematicus. Persons without periorbital oedema but with high fever and

Table 8.4 Algorithm for diagnosing the probability of being infected with acute *Trichinella* in humans

Group A	Group B	Group C	Group D
Fever	Diarrhoea	Eosinophilia (>1 G/l) and/or increased total IgE	Positive serology (with a highly specific test)
Eyelid and/or facial oedema	Neurological signs	Increased levels of muscular enzymes	Seroconversion
Myalgia	Cardiological signs		Positive muscular biopsy
	Conjunctivitis		
	Subungual haemorrhages		
	Cutaneous rash		

The diagnosis is:
Very unlikely: one A or one B or one C
Suspected: one A or two B and one C
Probable: three A and one C
Highly probable: three A and two C
Confirmed: three A, two C and one D; any of groups A or B and one C and one D

neurological symptoms may be misdiagnosed with typhoid fever. Table 8.4 gives an algorithm which can help for diagnosis (Dupouy-Camet and Bruschi 2007).

8.10 Treatment

8.10.1 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). The principal anthelmintics used for trichinellosis are mebendazole (Vermox®, Janssen) and albendazole (ZENTEL®, GlaxoSmithKline). To eliminate adult worms from the intestinal lumen, thus preventing the 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.

8.10.1.1 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 harbour 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 10–15 days. The whole treatment cycle may be repeated after 5 days. In some countries (e.g. Germany and Italy), higher doses are recommended (20–25 mg/kg/day administered in three doses for 10–14 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 7.5–15 g of mebendazole for 10–13 days started 1 month after infection, the treatment failed to kill muscle larvae (Pozio 2001).

8.10.1.2 Albendazole

Albendazole, an anthelmintic benzimidazole carbamate, is absorbed in the intestinal lumen relatively quickly. When the drug is administered with a fatty meal, a two- to fourfold increase in plasma concentration is observed, although large intra- 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; 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 10–15 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. 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. Klein et al. (1980) showed that steroids used in combination with mebendazole would significantly shorten the duration of fever.

8.10.2 Non-specific Treatment

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 sulphoxide by about 50 % (Jung et al. 1990). The most commonly 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 10–14 days.

8.10.2.1 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 hospitalisation 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. For severe infection, mebendazole could be administered under a physician's control. 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 in 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).

8.11 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 a large infective dose, in elderly patients and in patients with associated debilitating factors.

8.11.1 Lethality

Death is a rare consequence of trichinellosis. For example, of the more than 6,500 infections reported in the EU in the past 25 years, only five deaths have been observed, all of which were due to thromboembolic disease, in persons over 65 years of age. Twenty fatalities out of 10,030 cases were reported in a worldwide survey performed by the International Commission on Trichinellosis between January 1995 and June 1997 (Dupouy-Camet 2000). During a horsemeat outbreak in France, caused by the newly described *T. murrelli*, a 0.46 % mortality rate was observed (Ancelle et al. 1988). No deaths were reported in outbreaks caused by *T. britovi* (Bruschi and Murrell 2002). In 2005, three deaths were recorded in Serbia amongst 339 cases (Sofronic-Milosavljevic and Djordjevic, personal communication, 2005) and two deaths in Romania amongst 574 cases (Cretu, personal communication, 2005).

8.11.2 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 characterised 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 actually exists is still under debate, and chronic trichinellosis could be difficult to distinguish from sequelae of the acute phase. However, the existence of chronic trichinellosis 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 localisations during the acute phase of the disease. For the treatment of sequelae and of chronic trichinellosis, anthelmintics are useless; on the contrary, glucocorticosteroids or non-steroidal 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.

8.12 Perspectives of Control

Control of trichinellosis in humans is mainly based on animal inspection at the slaughterhouse as well as on adequate 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: (1) education of the consumer about the risk of consumption of raw or semiraw 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; (2) farming of pigs (the most important source of *Trichinella* infection for humans) in modern, industrialised, indoor pigsties under strict veterinary control and use of certified feedstuff (see below); and (3) control of all susceptible animals (both domestic and sylvatic) by a standardised artificial digestion method at slaughtering or after hunting (Gottstein et al. 2009). 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, does 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 of obtaining

Table 8.5 Levels of protection against trichinellosis, achieved by different vaccine formulations

Antigen	Adjuvant	Host species	Route of administration	Protection level at intestinal level	Protection level at muscle level (%)	Reference
Crude NBL extract	CFA	Pig	i.p.	Not estimated	78	Marti et al. (1987)
Ts39 fusion protein	CFA	BALB/c mice	i.p.	Not estimated	78	Sun et al. (1994)
Gp-43 30 mer	Cholera toxin B subunit	NIH mice	Intranasal	68.3 (fecundity at 6 d.p.i.)	Not done	McGuire et al. (2002)
rTs-87	CFA	BALB/c mice	s.c.	Not estimated	29	Yang et al. (2003)
DNA coding Ts-31	None	BALB/c mice	i.m.	Not estimated	36.9	Wang et al. (2006)
Ts87 (phage-displayed epitopes)	None	BALB/c mice	s.c.	Not estimated	28.7	Gu et al. (2008)
Ts-HPS 70	CFA	BALB/c mice	s.c.	Not estimated	37	Wang et al. (2009)
Ts-paramyosin	CFA	BALB/c mice	s.c.	Not estimated	36.7	Yang et al. (2010a)
Ts-paramyosin	Montanide ISA 720	BALB/c mice	s.c.	Not estimated	34.9	Yang et al. (2010a)
Ts-paramyosin	Montanide ISA 206	BALB/c mice	s.c.	Not estimated	33.7	Yang et al. (2010a)
DNA coding Ts87	*Salmonella typhimurium*	BALB/c mice	Oral	29.8 (at 4 d.p.i)	34.2	Yang et al. (2010b)
Gp-43 30 mer	*Salmonella typhimurium*	BALB/c mice	i.n.	61.8 (8 d.p.i. expulsion)	Not done	Pompa-Mera et al. (2011)

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, b), 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. 2010a, b) or *Salmonella enterica* by intranasal route (Pompa-Mera et al. 2011). All these different approaches have resulted in variable levels of protection which however at present do not yet guarantee the availability of a reliable vaccine (see Table 8.5).

8.13 Conclusions

If, at the world level, pigs are the main vectors of human trichinellosis, their role in a given country will depend on the mode of pork production. In large confinement production systems with rodent and garbage control programmes, 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 the 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.

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Chapter 9
Soil-Transmitted Helminthiasis

Albis Francesco Gabrielli, Antonio Montresor, and Lorenzo Savioli

Abstract Soil-transmitted helminthiasis (STH) is a group of infections caused by *Ascaris lumbricoides*, *Trichuris trichiura* and *Necator americanus* and *Ancylostoma duodenale*. The risk of acquiring STH is significantly influenced by climatic and socio-economic factors including temperature, rainfall, occupation and availability of safe water and sanitation facilities.

STH are transmitted through the parasites' eggs excreted in faeces of infected humans that enter the human body by ingestion or skin penetration.

More than a billion people living in tropical and subtropical countries are currently infected with STH, with over 300 million of them suffering from morbidity.

The epidemiology of STH is characterized by overdispersed distribution in the population; heavy-intensity infections are the major source of morbidity and these infections usually occur in children and women; treatment administered at regular intervals to these groups at risk will periodically decrease the worm burden of infected individuals and control morbidity.

Soil-transmitted helminths can affect the nutritional status of their host by feeding on the content of the host's intestine or on host tissues (e.g. blood), impairing digestion or absorption of nutrients, causing an inflammatory response that leads to the production of substances affecting appetite, intake, metabolism and storage of micronutrients and eliciting fever and immune response, thus increasing consumption of energy.

The burden of disease associated with STH is mainly attributable to their chronic and insidious impact on nutritional status, physical 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.

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Eggs of intestinal helminths are generally easy to identify because of their large size and distinctive morphology and their identification in stool specimens remains the most commonly used means of diagnosis, especially in developing countries. The Kato-Katz technique direct smears in saline or concentration techniques (such as formol-ether) can be alternatively used.

WHO-recommended treatment options include albendazole, mebendazole, levamisole and pyrantel. All the mentioned medicines are to be administered orally. These drugs are effective, safe and extremely low cost. For these reasons, in area of high endemicity WHO recommends preventive chemotherapy; this measure consists on the large-scale distribution of anthelmintic drugs to population groups at risk. It has been demonstrated that 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.

One of the risks of this approach is the development of resistance to anthelmintic drugs in targeted parasites; as of today, however, scientific investigations have not demonstrated any presence of resistance among helminths that infect humans.

To further increase cost efficacy of the control activities WHO supports and endorses the development of multi-disease national plans of action (e.g. addressing also the control of schistosomiasis and lymphatic filariasis) implemented in integrated way and complemented by other public health interventions such as the provision of health education and the improvement of the sanitation infrastructure.

WHO envisages a world free of childhood morbidity due to STH. The goal is to reduce morbidity from STH among preschool-age children (aged 1–4 years) and school-age children (5–14 years) to a level below which it would not be considered a public health problem, i.e. when the level of prevalence of STH infections of moderate and high intensity among school-age children is $<1\%$.

This target is achievable; in 2012 over 260 million of children have been treated with preventive chemotherapy for the control of STH and the large drug donations by pharmaceutical companies and the support provided by international donors are sustaining the efforts of endemic countries.

9.1 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 drugs for their treatment and public health control are neither widely available nor affordable by those in need. STH is estimated to infect more than one 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 childbearing 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 is proportionate to the number of worms hosted by an individual and can be controlled by regular treatment with anthelmintic medicines (preventive chemotherapy). 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 is the case for many neglected tropical diseases (NTDs), STH and socio-economic status are intimately linked, both within and between countries (de Silva et al. 2003). In countries where an improvement in sanitation levels has taken place as a natural component of the country's economic progress, a parallel progressive decline in the magnitude of the STH burden has invariably been observed.

9.2 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–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 2,000 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 5,000–10,000 eggs per day. Both hookworms are characterised 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–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 (1.22 billion), followed by trichuriasis (0.79 billion) and hookworm infections (0.74 billion) (de Silva et al. 2003). 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* (Beaumier et al. 2013). 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 are generally regarded as diseases predominantly occurring in rural areas, their prevalence can be extremely high in urban and periurban slums and informal settlements; this is especially true for ascariasis (Crompton and Savioli 1993; Gabrielli et al. 2005).

STH are transmitted through the parasites' eggs excreted in faeces of infected humans. It is estimated that severely infected individuals can discharge between two and five 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 years) 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.

9.3 Epidemiology of Infection

More than a billion people living in tropical and subtropical countries are currently infected with STH, with over 300 million of them suffering from morbidity. The global distribution of STH is shown in Fig. 9.1 that depicts the proportion of children in need of regular treatment through preventive chemotherapy in each country requiring this intervention. Estimates indicate that in 2011, 874.6 million preschool-age and school-age children required preventive chemotherapy (WHO 2013a), compared to 889.8 million 1 year before (WHO 2012b). Overall, the largest proportion of the infections occur in India where 27.3 % of the world's children in need of treatment live. Nigeria (6.8 %), Indonesia (6.6 %), China (5.9 %) and Bangladesh (5.4 %) are also prominent given the size of their resident populations. More than 100 countries comprise the remaining 48 % of children requiring preventive chemotherapy (Fig. 9.2) (WHO 2012a, b).

The latest calculations made by the WHO's Global Burden of Disease project (GBD; WHO, 2008) indicate that in 2004 STH was overall responsible for 6,000 deaths and over four million disability-adjusted life years (DALYs) lost; the revised estimates for 2010 carried out by the Institute for Health Metrics and Evaluation attribute to STH 5.184 million DALYs lost (Murray et al. 2012). Data indicate that in 2004 most of the burden laid in low-income countries (5,000 deaths and two-thirds of the DALYs lost) and in low-income countries (1,000 deaths and one-third of the DALYs lost), while only a small fraction (no deaths and less than 1 % of the DALYs lost) was found in high-income countries. Ascariasis was responsible for 50 % of the DALYs lost, while trichuriasis and hookworm infections for 25 % each; deaths were mainly attributable to ascariasis and trichuriasis. Gender analysis indicates that the overall burden, both in terms of deaths and DALYs lost, was equally distributed among males and females.

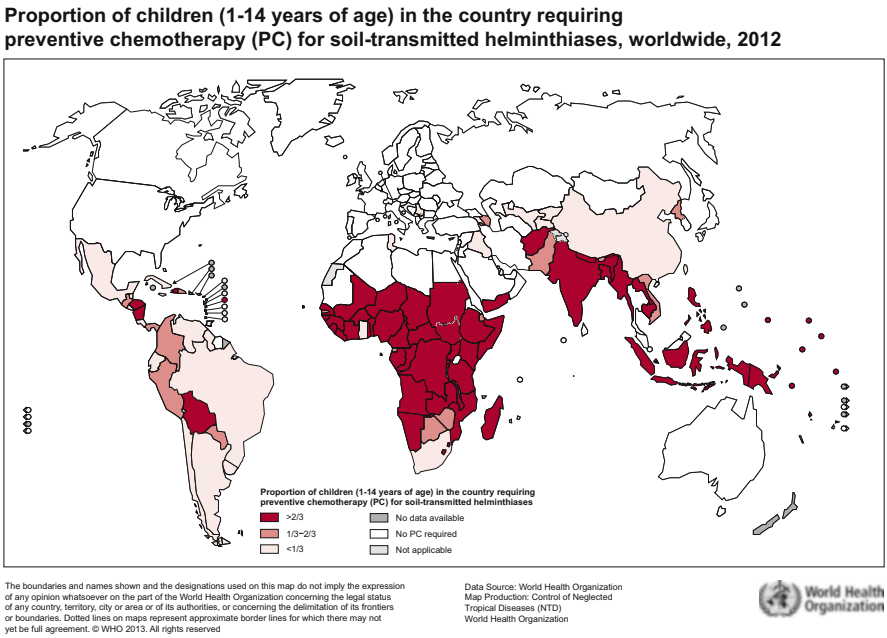

Fig. 9.1 Proportion of children aged 1–14 years requiring preventive chemotherapy for soil-transmitted helminthiasis, by country, as of 2009 (WHO 2012a)

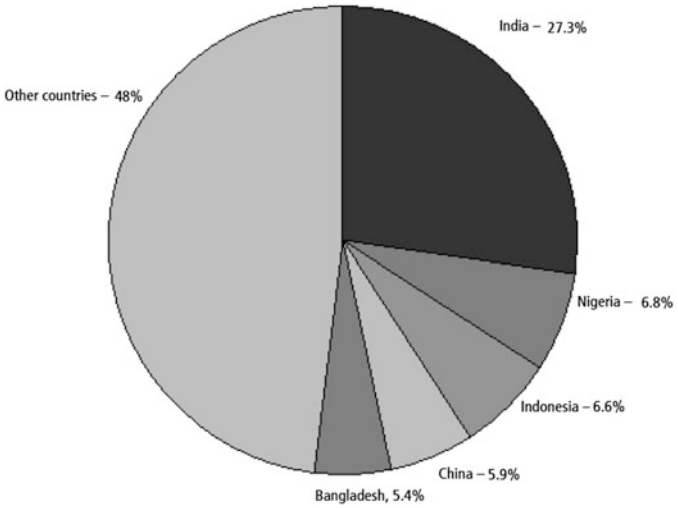

Fig. 9.2 The five countries with the highest absolute number of children requiring preventive chemotherapy for soil-transmitted helminthiasis, shown as proportions of the total number of children requiring preventive chemotherapy (WHO 2012b)

Epidemiology of STH is characterised by a few distinctive features:

1. STH does not uniformly affect a population, but is characterised 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.
2. Infections of heaviest intensity usually occur in children and women (Bundy et al. 1992); in addition these population groups are characterised by intense metabolism due to rapid physical growth, resulting in increased nutritional needs. Micronutrient deprivation associated with peak worm burdens and increased nutritional requirements explain why preschool-age children (1–4 years), school-age children (5–14 years) and women of child-bearing 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 compensated 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.

9.4 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.

9.5 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.

9.6 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 species 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 through 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. Generalised 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 colonised 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 non-responsive to diuretics.

9.7 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 direct smears in saline or concentration techniques (such as formol-ether) can be alternatively used (WHO 1994).

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 the number of eggs per gram of faeces examined, as shown in Table 9.1. 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 preventive chemotherapy intervention (Montresor et al. 2013).

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 minimising 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. 9.3; WHO 1994):

Table 9.1 Classes of intensity for STH (WHO 2002)

Organism	Light-intensity infections	Moderate-intensity infections	Heavy-intensity infections
A. lumbricoides	1–4,999 epg	5,000–49,999 epg	≥50,000 epg
T. trichiura	1–999 epg	1,000–9,999 epg	≥10,000 epg
Hookworms (*A. duodenale* and *N. americanus*)	1–1,999 epg	2,000–3,999 epg	≥4,000 epg

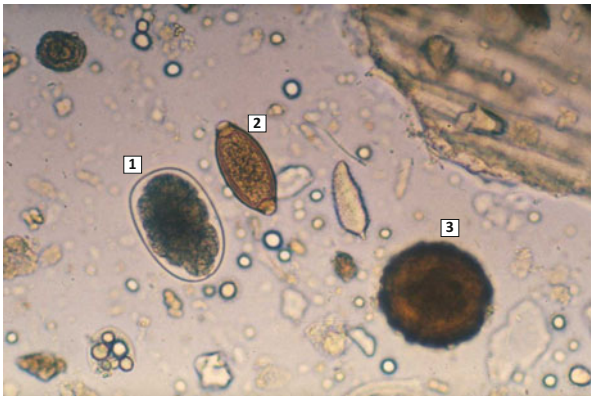

Fig. 9.3 Eggs of *Ascaris lumbricoides* (1, *right*), *Trichuris trichiura* (2, *centre*) and hookworm (3, *left*) in the same microscopic field, illustrating their relative sizes (WHO 1994)

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 organisation.

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 4- or 8-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.

9.8 Treatment

9.8.1 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 1st trimester of pregnancy.
- Levamisole is contraindicated in the 3rd trimester of pregnancy and during breastfeeding.

9.8.1.1 Ascariasis

Albendazole:

Adults and children over 2 years: albendazole 400 mg, single administration

Children 12 months–2years: 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

9.8.1.2 Trichuriasis

Albendazole:

Adults and children over 2 years: albendazole 400 mg, single administration (moderate infections), or 400 mg daily for 3 days (severe infections)

Children 12 months–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

9.8.1.3 Hookworm Infections

Albendazole:

Adults and children over 2 years: albendazole 400 mg, single administration

Children 12 months–2years: 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:

Adults 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

9.8.2 Preventive Chemotherapy

In preventive chemotherapy interventions, albendazole 400 mg, single administration, or mebendazole 500 mg, single administration, can be alternatively used against all STH species 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 1st year of life and pregnant women in the 1st trimester of pregnancy should be excluded from preventive chemotherapy interventions (WHO 2006).

9.8.3 Drug Efficacy and Its Assessment

Two parasitological indicators are used to assess the efficacy of an anthelmintic 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, public health interventions such as preventive chemotherapy aim 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 anthelmintic medicines in the context of preventive

chemotherapy. The reason is that its value 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 anthelmintic medicines (Montresor 2011).

The development of resistance to anthelmintic drugs in targeted parasites may be favoured by the fact that increasingly larger numbers of individuals are administered anthelmintic medicines, especially through preventive chemotherapy 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, 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 preventive chemotherapy interventions against STH (WHO 2013b).

9.9 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, anthelmintic 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.

9.10 Prevention and Control

WHO envisages a world free of childhood morbidity due to STH. The goal is to reduce morbidity from STH among preschool-age children (aged 1–4 years) and school-age children (5–14 years) to a level below which it would not be considered a public health problem, i.e. when the level of prevalence of STH infections of moderate and high intensity among school-age children is <1 % (WHO 2012a).

9.10.1 Preventive Chemotherapy

The mainstay of the WHO strategy to reduce morbidity due to STH is preventive chemotherapy. Preventive chemotherapy is “the use of anthelmintic 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, preventive chemotherapy is characterised 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 preventive chemotherapy from the clinical approach in which diagnosis is performed at the individual level prior to treatment.
- *Population-based treatment*
  In preventive chemotherapy, administration of anthelmintic drugs is not the outcome of a personalised, 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 child-bearing age) with delivery of single-administration medicines by both medical and non-medical personnel (teachers, volunteers or community drug distributors).
- *Implementation at regular intervals*
  Preventive chemotherapy is implemented at regular intervals of time (once a year or twice a year); the most appropriate re-treatment 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.

WHO recommends implementing preventive chemotherapy 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:

- Preschool-age children (aged 1–4 years)
- School-age children (aged 5–14 years)
- Women of child-bearing 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 9.2).

Table 9.2 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)

Implementing preventive chemotherapy 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 et al. 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.

9.10.2 Global Goals and Targets

Until the year 2000, deworming was implemented on a small scale and in a limited number of countries only. In 2001, following the adoption by the World Health Assembly of Resolution WHA54.19, more countries started implementing distribution of albendazole or mebendazole to preschool-age and school-age children. Resolution WHA54.19 requested 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).

The World Food Program (WFP) and UNICEF were the leading implementers in such early phase of control. The WFP carried out deworming interventions through its network of assistance focused on food supplementation to schools in fragile countries, while UNICEF started including anthelmintic treatment in its immunisation or micronutrient supplementation interventions targeting very young children. The gradual expansion of the Global Programme to Eliminate Lymphatic Filariasis (GPELF, established in 2000), whose strategy is based on the provision of a drug combination that includes albendazole to all individuals living in areas where lymphatic filariasis is transmitted, from the age of 2 years or from that of 5 years (depending on countries), also significantly contributed to scaling up STH control interventions.

Recent years have seen the birth of dedicated STH control programmes in many endemic countries, a fact that has contributed to increasing national ownership and

coordination with programmes targeting other helminth infections, not only lymphatic filariasis, but also schistosomiasis, a disease whose target population largely overlaps with that of STH.

In spite of all the mentioned efforts, however, the global goal endorsed in 2001 by the World Health Assembly through the adoption of resolution WHA54.19 was not reached. By that date, only a third of all children in need of deworming had received appropriate treatment (WHO 2012a).

Anticipating this problem, in 2007, WHO convened the first Global Partners' Meeting on NTDs. Some 200 participants attended the meeting, including representatives of WHO Member States, United Nations agencies, philanthropic foundations, universities, pharmaceutical companies, international nongovernmental organisations 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 neglected tropical diseases in general and STH in particular.

As a result of this global effort, the number of children receiving preventive chemotherapy for STH has progressively increased to exceed 300 million in 2011 (WHO 2013a), corresponding to a global 30 % coverage. A significant boost in the number of children treated and in coverage is expected over the upcoming years as an effect of recent and expanded drug donations: a quantity of 600 million tablets of albendazole 400 mg or mebendazole 500 mg for the treatment of school-age children in endemic countries will be made available each year. This unprecedented event has already generated a significant increase in requests for donated medicines submitted by endemic countries in 2012, compared to 2011 (+150 million tablets of albendazole or mebendazole).

Recently, WHO decided to update the global goals inherent in control of STH. All countries where STH is considered a public health problem should start national STH control programmes by 2015; and preventive chemotherapy interventions implemented in such countries should reach 75 % national coverage and 100 % geographical coverage by 2020 (WHO 2012a). WHO envisages a world free of childhood morbidity due to STH. The goal is to reduce STH morbidity among preschool-age children (aged 1–4 years) and school-age children (5–14 years) to a level below which it would not be considered a public health problem, i.e. when the level of prevalence of STH infections of moderate and high intensity among school-age children is <1 % (WHO 2012a).

Countries embarking in large-scale STH control should follow a stepwise approach as follows: (1) conduct a situation analysis aimed at assessing the magnitude of the STH burden; (2) classify the country in terms of level of risk, by implementation unit (usually, a homogeneous ecological zone); (3) conduct a pilot intervention in a geographically limited area; (4) scale up implementation and expand geographical coverage; (5) maintain high coverage ($\geq$75 % geographical coverage and 100 % national coverage) for at least 4–6 years; (6) conduct monitoring and evaluation assessments and adjust the treatment schedule accordingly; (7) institutionalise deworming and facilitate its progressive absorption by the

routine health services of the country; (8) maintain surveillance in order to ensure that no recrudescence in transmission occurs; (9) sustain benefits through the progressive implementation of complementary public health interventions (WHO 2012a).

A significant boost to control of STH is expected to be generated by the successful adoption of resolution WHA66.12, by the World Health Assembly on 27 May 2013. The resolution addresses all NTDs (its title is "Neglected Tropical Diseases") and calls for a renewed commitment by Member States to the fight against such diseases. In particular, countries should ensure that resources match national requirements; improve forecasting, procurement, custom clearance and management of quality-assured medicines and supply chains; and integrate NTD control programmes into primary health services.

9.10.3 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 optimise 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. utilising 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 intervention at different levels and between sectors to improve the overall health outcome.

Since 1997 countries have been developing programmes based on integrated approaches to disease control. Coordination of activities previously implemented in a disease-specific fashion as well as their integration within national public health systems has been encouraged; multi-disease national plans of action are increasingly drawn up by NTD programmes in a number of countries. WHO supports and endorses the process of development of such plans, 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 preventive chemotherapy, 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 anthelmintic drug donations since 2011.

STH are particularly suitable to integration as the approach to their 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 immunisation campaigns. The Global Programme to Eliminate Lymphatic Filariasis, based on regular treatment of communities with single-administration drugs such as ivermectin and albendazole, also effective against STH, creates 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 utilised mass treatment for soil-transmitted helminths 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 inter-sectoral collaboration and coordination between ministries at central level (e.g. Ministry of Health, Ministry of Education and Ministry of Infrastructure) and the intra-sectoral 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 anthelmintic medicines even without strict medical supervision; (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 anthelmintic tablets to the health package usually increases the overall coverage of the campaign; (2) health personnel are skilled in providing medicines or medical products to very young children.
- Deworming women of child-bearing 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 anthelmintic medicines into such packages makes sense as STH is known to adversely affect pregnancy and its outcomes.

9.10.4 Complementary Public Health Interventions

Health education and improved sanitation represent two public measures that can be implemented to complement and enhance the impact of preventive chemotherapy.

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 an innovative methodology that aims at mobilising 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.

9.10.5 Vaccinology

The only intestinal nematode currently targeted by a vaccine development programme is *N. americanus*. The US-based Sabin Vaccine Institute Product Development Partnership is working on a bivalent human hookworm vaccine consisting of two antigens derived from the adult stage of the worm. The two antigens have undergone preclinical essays in canine and murine models and are currently being tested in humans independently, as monovalent vaccines, before being combined into a single bivalent vaccine. The aim is to prevent moderate- to heavy-intensity infections (and thus control associated morbidity) in children younger than 10 years living in endemic areas; the immunisation schedule is based on a maximum of two doses. Further research is ongoing to establish the role of a vaccine as a disease control tool with regard to the other measures currently recommended, especially preventive chemotherapy (Beaumier et al. 2013).

9.10.6 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 control STH 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 is an essential component 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 preventive chemotherapy 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 preventive chemotherapy 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 preventive chemotherapy interventions in all countries affected by STH. This is considered an essential step to guarantee the protection of health among children and women.

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Chapter 10
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 the 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 the current status of strongyloidosis with special reference to biology, epidemiology, immunology, and vaccine development.

10.1 Introduction

Strongyloidosis is one of the chronic, soil-transmitted, intestinal helminth infections which affect the health of over one-third of the world population. 30–100 million people are estimated to be infected with *Strongyloides* spp. (CDC homepage). *Strongyloides stercoralis* is widespread, mainly in the tropics and subtropics and of species naturally infecting humans. Besides this species,

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S. fuelleborni infection in humans has been reported but restricted in Africa and the Southeast Asian country of Papua New Guinea. 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 (Olsen et al. 2009).

In this chapter, we focused mainly on human strongyloidosis together with recent advances of 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).

10.2 The Agent

10.2.1 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 sizes of the adult worms are 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. fuelleborni* are 2.9–4.2 (3.5) mm in length and 43.0–55.0 (51.0) μm in width. The ovaries of *S. 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. 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. 10.1.

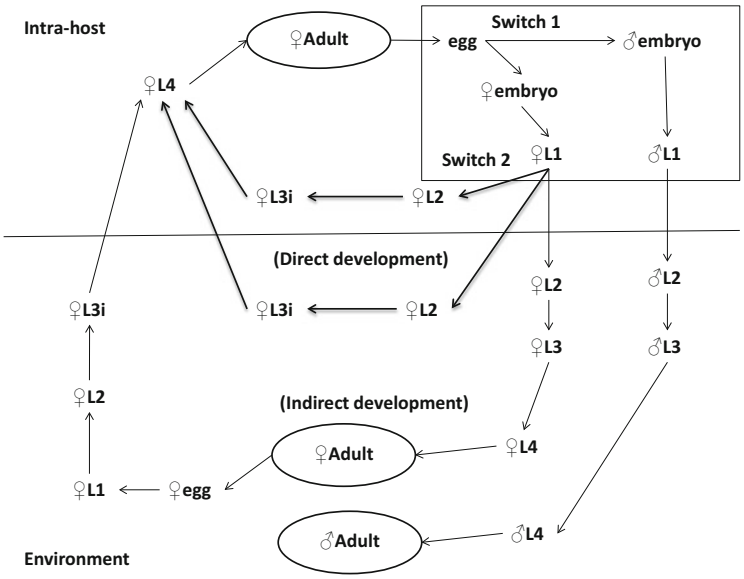

Fig. 10.1 The life cycle of Strongyloides stercoralis. The life cycle consists of direct (homogonic) and indirect (heterogonic) developments. Two developmental switches, a sex determination and a female-only developmental choice, have been demonstrated to control the development in *S. ratti* which is close phylogenetically to *S. stercoralis* (Nemetschke et al. 2010; Viney 2006). Such switches might be hypothesized in *S. stercoralis*

10.2.2 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 the lifetime of the hosts. Persistent infections lasting for 40 years 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).

Molecular biology and genomics of *Strongyloides* spp. are reviewed elsewhere (Charlesworth 2010; Nemetschke et al. 2010; Streit 2008; Viney 2006).

S. stercoralis L3i was shown to be strongly attracted to an extract of the mammalian skin. The active component in the skin extract was urocanic acid, which is abundant in the mammalian skin and skin secretions. The attractant activity of urocanic acid was inhibited by divalent metal ions. This suggests the 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 to 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). 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 phylogenetic and structural analysis (Gallego et al. 2005).

10.3 Epidemiology of Infection

Strongyloidosis occurs mostly in humid tropics and subtropics of more than 70 countries (Olsen et al. 2009). The number of people infected with *S. stercoralis* is estimated to be between 30 million and 100 million or higher (Siddiqui and Berk 2001). The precise number 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 10.1). 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.

Strongyloidosis poses a serious problem even in highly developed countries. 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

Table 10.1 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 %)[a]	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	4,695 Italian	2	0.04		2006–2008	Agar plate	Masucci et al. (2011)
A large teaching hospital	656 non-Italian	2	0.3				
Rural area, Brazil	ND[b]	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 preschool children	8	3.8	1.6–7.3			
	120 mothers	88	73.3	64.5–81.0		ELISA	
	225 preschool children	61	27.1	21.4–33.4			
Northeast Poland	120, 5 months–18 years old	7	5.83		2008–2009	Decantation	Żukiewicz et al. (2011)
Northern Laos	14 households × 6 villages	ND	8.9	7.4–10.4	2009	Formalin-ether concentration	Conlan et al. (2012)
	Household members >6 years old						
	Randomly selected						
41 GeoSentinel clinics in 19 countries	854 children (<18 years old)	40	4.7		1997–2009	ND	McCarthy et al. (2013)
	6,751 adult (>19 years old)	344	5.1				
	International migrants[c]						

(continued)

Table 10.1 (continued)

Regions and/or countries	No. of subjects surveyed	No. of positives	%	CI (95 %)[a]	Year of survey	Methods of survey	Reference
Flores Island, Indonesia, semi-urban area	675, 18–80 years old	5	0.7		2009	qPCR	Wiria et al. (2013)

[a]CI: confidence intervals

[b]ND: not described

[c]Diagnoses with strongyloidosis by region of migrant origin were of 7 % in Southeast Asia ($n = 1{,}200$), 3 % in South Asia ($n = 844$), 6 % in North Africa ($n = 503$), 4 % in East Africa ($n = 1{,}253$), 5 % in West Africa, and 5 % in South Africa ($n = 698$)

et al. 2013). Two cases with strongyloidosis were recorded on 1,046 kidney and 708 liver transplant recipients registered in four medical centers in Brazil from 2001 to 2006 (Batista et al. 2011). Expanded infectious disease screening program was done in the USA for Hispanic transplant candidates (recipients) between 2006 and 2008, minimizing the risk of posttransplant infectious complications. On 83 patients screened, most were from Mexico (74.7 %), and the 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).

10.4 The Host Response to the Parasite and Immunopathological Processes

Pathophysiological aspects in human strongyloidosis were reviewed extensively by Genta and Caymmi Gomes (1989). 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). Recently, 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 *Strongyloides 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).

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 minutes 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, retinoid-related orphan receptor γ^+ (RORγ^+) lymphoid tissue inducer-related cells, and Th2-type innate lymphocytes have distinct roles in innate immune responses, producing Th1, Th17, and Th2 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 Th2-type innate lymphocytes in the early phase of following Th2-type responses in murine helminthiasis models (Maizels et al. 2012). To date, however, a relation between Th2-type innate lymphocytes and immune responses to *S. stercoralis* remains obscure.

Toll-like receptors (TLRs) on dendritic cells and other various cells recognize invading pathogens through pathogen-associated molecular patterns (PAMPs) during both the innate and the 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 et al. (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 (Abraham's implantation method). This allowed to identify the different factors involved in protective immunity against *S. stercoralis* (Table 10.2). 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 as well as 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 to the adult stage of *S. stercoralis*, for 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) infection model in which the parasite can develop to the adult 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). We expect that a good model will be developed to clarify the interaction between adult worms of *S. stercoralis* and host immune mechanisms. More information regarding protective intestinal immunity to *Strongyloides* spp. is available in the papers written by Nawa (2003) and Iriemenam et al. (2010).

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 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

Table 10.2 Factors of protective immunity against larval *S. stercoralis*

Innate immunity	Adaptive immunity	References
	Granulocytes (neutrophils, eosinophils) Compliment (C3) IgM	Brigandi et al. (1996)
	Granulocytes, eosinophils	Rotman et al. (1996)
	Eosinophils IgM	Brigandi et al. (1997)
	CD4, IL-4, IL-5 rIL-12 (suppress immunity)	Rotman et al. (1997)
	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) Granulocytes (neutrophils) IL-5 (-)	Ligas et al. (2003)
	Human IgG, complement (C3) Granulocytes IgA + IgE + IgM (-) IL-5 + eosinophils (-) Ab-dependent cytotoxicity (-)	Kerepesi et al. (2004)
Eosinophils (CCR3) Neutrophils (CXCR2)	Neutrophils (CXCR2)	Galioto et al. (2006)
Eosinophils (Ag presenting)	Eosinophils (Ag presenting)	Padigel et al. (2006)
C3	C3, C3a	Kerepesi et al. (2006)
C5 (-)	C5 (-)	
TLR4 (-)	TLR4 PEC (neutrophils?)	Kerepesi et al. (2007)
Eosinophils (Ag presenting)	Eosinophils (Ag presenting)	Padigel et al. (2007a)
	Gαi2 protein signaling (neutrophil recruitment) Immune serum	Padigel et al. (2007b)
MPO (neutrophils) MBP (eosinophils)	MPO (neutrophils)	O'Connell et al. (2011a)
IL17A (-), IL17F (-) CXCR2 (neutrophil recruitment)	IL17A (-), IL17F (-) CXCR2 (neutrophil recruitment)	O'Connell et al. (2011b)

(-): not essential

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).

10.5 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-calories malnutrition, malignant conditions (carcinoma, lymphoma, leukemia, etc.), and chronic illnesses (tuberculosis, syphilis and 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 (Sheet 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).

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) (Lawn 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; Lawn 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 the 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).

10.6 Diagnosis (Inclusive Histopathology)

10.6.1 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. 10.2), fecal cultures can increase the sensitivity even if larvae are low in number in feces examined (Arakaki et al. 1990; Ines et al. 2011; Kaminsky 1993;

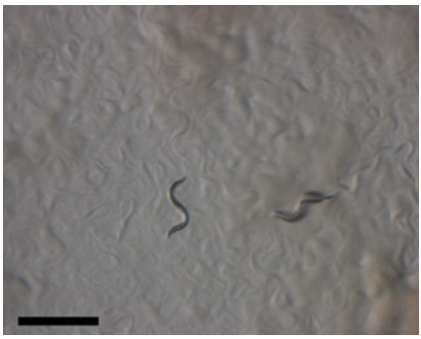

Fig. 10.2 Motile larvae of *Strongyloides venezuelensis* and furrows seen on agar plate (Bar indicating 0.5 mm)

Machicado et al. 2012; Salazar et al. 1995). When compared to 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 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 three 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 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 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. 10.3, 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,

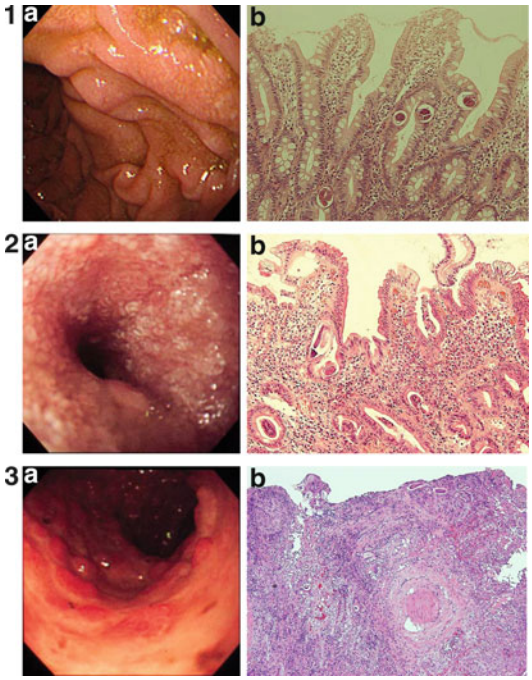

Fig. 10.3 Endscopic and histopathological observations on the duodenum of *Strongyloids stercoralis* hyperinfection. **1.** (**a**) Endoscopic image showing white villi and edematous mucosa in the second part of duodenum. (**b**) Biopsy specimen from the mucosa showing numerous larvae with villous atrophy and mild inflammatory cell infiltration (HE staining, ×200). **2.** (**a**) Endoscopic image showing white villi and stenosis in the second part of 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 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). Reference: Kishimoto K, Hokama A, Hirata T et al. (2008) World Journal of Gastroenterology 14(11): 1768–1773. The publisher and Hokama (correspondent author) gave us permission

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).

10.6.2 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 is slightly cross-reactive to Bancroftian filariasis as mentioned above, recombinant strongylastacin, a 40 kDa

metalloproteinase, does not cross-react with IgE antibodies from either patients 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).

10.6.3 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 that 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. However, 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). 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).

10.7 Treatment

According to 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:

10.7.1 First-Line Therapy

Ivermectin (Merck Sharp & Dohme Research Laboratories, NJ, USA)

200 μg/kg/day, 1 dose; repeat same dose after 2 weeks.

In case of immunosuppressive patients or disseminated patients, repeat totally 4 doses or more every 1–2 weeks. Follow-up stool examination should be done to verify eradication of worms.

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).

Refer to WHO recommendations:
http://whqlibdoc.who.int/publications/2006/9241547103_eng.pdf.

10.7.2 Alternative

Albendazole, 400 mg orally twice a day for 7 days.

Some patients complained of diarrhea and abdominal pain (Segarra-Newnham 2007).

Contraindications are 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).

10.8 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 semi-automated 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 the patients' serum IgG were candidates for vaccine. Successful immunization was done with plasmid containing DNA encoding Na^+-K^+ ATPase and plasmid containing DNA encoding granulomacrophage-colony stimulating factor (GM-CSF) (Kerepesi et al. 2005). Furthermore, a recombinant antigen SsIR that is highly immunogenic in humans generated protective immunity through an antibody-dependent manner, so that SsIR plus alum may have the potential to be used for a prophylactic vaccine in humans (Abraham et al. 2011).

10.9 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 repot 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.

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Chapter 11
Anisakiasis

Simonetta Mattiucci and Stefano D'Amelio

Abstract Anisakiasis refers to the zoonotic disease provoked in humans by the accidental ingestion of larvae of *Anisakis* spp. infecting fish or squid which is consumed raw and/or 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 present 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 our current knowledge of the *Anisakis* allergens involved in the human immunological response. Finally, current literature on possible control measures involving the inactivation of *Anisakis* larvae in fish fillets, thus reducing transmission to humans, is reported.

11.1 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 concern, due to their public health implications and their associated

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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 as the major etiological agents of human 'anisakiasis' (the term is related only to *Anisakis s*pp. as a causative agent) throughout the world. A recent review considering the pathogenetic aspects and occurrence of zoonoses related to the species of *Pseudoterranova* and *Contracaecum* has been published elsewhere (Mattiucci et al. 2013a).

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 (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 squids, with particular regard to those of commercial value that are commonly 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).

11.2 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; Mattiucci et al. 2005, 2009, 2014a). 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 based on the morphology and length of the glandular part of the oesophagus (i.e. the

'ventriculus') and the presence/absence of a caudal spine ('mucron'). Based on these differences, the Type I and the 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 aetiological 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 *A. simplex* (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, 2014a; 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 characterised 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) and 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, 2014a; Cavallero et al. 2011) have demonstrated that the genus *Anisakis* comprises at least nine distinct species. These are the three species included in the *A. simplex* (sensu *lato*) complex, i.e. *Anisakis simplex* (sensu stricto), *A. pegreffii* (= *A. simplex* A of Nascetti et al. 1986) and *A. berlandi* Mattiucci et al. (2014a) (= *A. simplex* C of Mattiucci et al. 1997); the two closely related taxa *A. ziphidarum* Paggi et al. 1998a and *A. nascettii* Mattiucci et al. 2009; the three closely related species *A. physeteris*, *A. brevispiculata*, and *A. paggiae* Mattiucci et al. 2005; and, finally, *A. typica*.

The existence of two major clades (Clade I and Clade II) has been demonstrated by different phylogenetic inferences (Valentini et al. 2006; Cavallero et al. 2011; Mattiucci et al. 2009, 2014a). Clade I comprises one subclade formed by ((*Anisakis simplex* (*s. s.*), *A. pegreffii*), *A. berlandi*) and a second one which encompasses the two species (*Anisakis ziphidarum* and *A. nascettii*), whereas three species currently belong to 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 the other species has also been demonstrated; its position in the phylogenetic tree, as representing a sister taxon to the other *Anisakis* species, has been discussed in recent phylogenetic analyses based on different genetic data sets (Cavallero et al. 2011; Mattiucci et al. 2014a).

Moreover, in recent years, both 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 the third taxon (*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 Atlantic equatorial area (Mattiucci et al. 2007; Garcia et al. 2011).

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 by means of morphological features but only by genetic/molecular markers.

However, despite the limited morphological characters available in adults of *Anisakis* spp., in the recent years, 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. 2008; Mattiucci et al. 2014a).

11.3 Current Methods Used for the Identification of *Anisakis* spp.

The limited value of morphological analyses makes the use of genetic and molecular methods absolutely necessary for the identification of species of *Anisakis*. The most used molecular/genetic methods are briefly reported below.

11.3.1 Multilocus Allozyme Electrophoresis

Multilocus allozyme electrophoresis (MAE) (19–24 enzyme loci) has 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).

11.3.2 PCR-RFLPs 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; Pontes et al. 2005) 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) provide useful approaches for the specific identification of species of *Anisakis* from different definitive and intermediate/paratenic hosts.

11.3.3 DNA Sequencing

Direct DNA sequencing of some genes has proved 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 recognised 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; Mattiucci et al. 2014a). 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 minimise 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) and the *rrnS* (the small subunit of the ribosomal DNA in the mitochondrial genome) (Nadler et al. 2005; Mattiucci et al. 2014a). These mitochondrial markers have been able to distinguish all of the taxa which have been characterised 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 the population genetics and phylogeography of *Anisakis* spp., as recently suggested for some species by Baldwin et al. (2011).

11.3.4 Multiplex and Species-Specific PCR

Umehara et al. (2008) have developed a method based on multiplex PCR which was able to recognise 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 characterised, 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.

11.4 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. 11.1). 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 faeces of their definitive host into the sea. According to some experimental studies, the eggs are embryonated, and the larvae moult 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 haemocoel. 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 and passes into the visceral body cavity and undergoes 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 moults and develop into a sexually mature adult nematode.

Since *Anisakis* larvae do not undergo any development or moult 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 their lifespan. 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 man, 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; Mattiucci et al. personal observation).

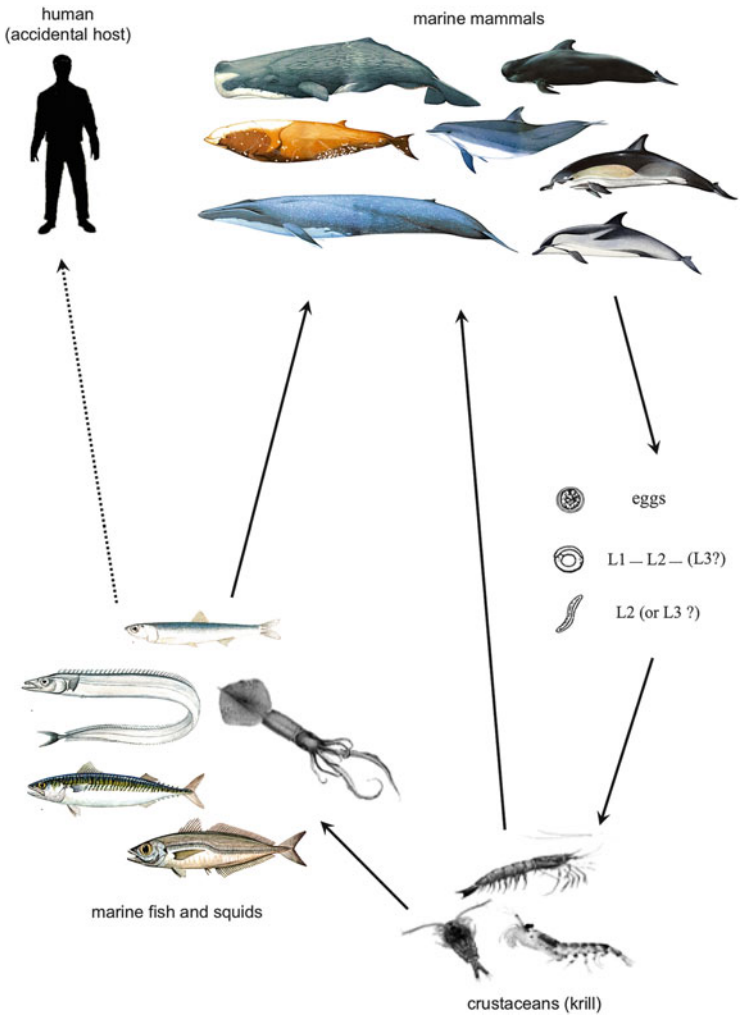

Fig. 11.1 Life cycle of *Anisakis* spp

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 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 1980). 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. 2014b) and bluefin tuna (Mattiucci personal observation). 'Red vent syndrome' (RVS, i.e. bleeding, swollen and haemorrhagic

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 characterised by haemorrhages 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 parasitised hosts, infected fish were generally in good overall condition.

11.4.1 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 11.1). *Anisakis* spp. larvae have been also detected in a variety of cephalopods and, rarely, in elasmobranchs. Among squids, they have been found mainly in the Ommastrephidae (Table 11.1). 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 are summarised in Table 11.1.

In the stomach of cetaceans, adults of *Anisakis* spp. (Table 11.2) 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 11.2), 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; Cavallero et al. 2011). Several oceanic dolphins of the Delphinidae, Arctic dolphins of the Monodontidae and porpoises of the Phocoenidae (Table 11.2) are hosts of the species *A. pegreffii*, *A. simplex* (*s. s.*) and *A. berlandi* (Mattiucci et al. 1997, 2014a; 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.

Beaked whales, *Ziphius cavirostris, Mesoplodon layardii, M. mirus, M. grayi*, *M. densirostris* and *M. europaeus,* are hosts of *A. ziphidarum* (Table 11.2) (Paggi et al. 1998a; Mattiucci et al 2009; Cavallero et al. 2011) and *A. nascettii* (Mattiucci et al. 2009, 2014a; Pontes et al. 2005) which are separated as a subclade and included in the main Clade I of the *Anisakis* phylogenetic tree. Thus, the

Table 11.1 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	–	–	–	–	–	–	–	–
Illex illecebrosus		WM							
Moroteuthis ingens									
Ommastrephes angolensis	–	SA	–	–	–	–	–	–	–
Ommastrephes sagittatus	IC	NAM	–	–	–	–	CM	–	–
Todarodes pacificus	KYS	KYS		KYS					
Todaropsis eblanae	IC, SA	IC, SA	–	–	–	–	–	–	–
Fishes									
Anguillidae									
Synaphobranchus kaupi	–	–	–	–	–	–	CSA	–	–
Anoplogastridae									
Anoplogaster cornuta	–	–	–	–	–	–	–	–	IRS
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	–	–	–	–	–

(continued)

Table 11.1 (continued)

	A. simplex (*s. s.*)	*A. pegreffii*	*A. berlandi*	*A. typica*	*A. ziphidarum*	*A. nascettii*	*A. physeteris*	*A. brevispiculata*	*A. paggiae*
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	–	–	–	–	–
Emmelichthyidae									
Emmelichthys nitidus nitidus	–	SA	–	–	–	–	–	–	–
Engraulidae									

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 aeglefinus	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	–	–	–	–	–	–	–	–

(continued)

Table 11.1 (continued)

	A. simplex (*s. s.*)	*A. pegreffii*	*A. berlandi*	*A. typica*	*A. ziphidarum*	*A. nascettii*	*A. physeteris*	*A. brevispiculata*	*A. paggiae*
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	–	–	–	–	–	–	–
Merlucciidae									
Merluccius capensis	–	SA	–	–	–	–	–	–	–
Merluccius hubbsi	–	FA	–	–	–	–	–	–	–
			–		MA, MOR	IC		MA	IC

Merluccius merluccius	NEA, IC, MA, NAM, MOR	CM, EM, WM, IC, NEA, MA, NAM, MOR		MA, EM, NAM, MOR			CM, MA, WM, IC, EM, NAM, MOR		
Myctophidae									
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	–	–	–	–	–
Nototheniidae									
Notothenia coriiceps	–	–	SHI	–	–	–	–	–	–
Notothenia rossii	–	–	SHI	–	–	–	–	–	–
Ophidiidae									
	–	SA	–	–	–	–	–	–	–

(continued)

Table 11.1 (continued)

	A. simplex (*s. s.*)	*A. pegreffii*	*A. berlandi*	*A. typica*	*A. ziphidarum*	*A. nascettii*	*A. physeteris*	*A. brevispiculata*	*A. paggiae*
Genypterus capensis									
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	–	–	–	–	–	–	–	–
Petromyzonthidae									
Petromyzon marinus	IC	–	–	–	–	–	–	–	–
Salmonidae									
Oncorhynchus gorbuscha	SI, ALA	–	–	–	–	–	–	–	–
Oncorhynchus keta	SI, KYS, ALA	–	–	–	–	–	–	–	–

Oncorhynchus nerka	ALA	–	–	–	–	–	–	–	–
Salmo salar	NWA	–	–	–	–	–	–	–	–
Scophtalmidae									
Lepidorhombus boscii	IC	IC, NAM	–	–	–	–	–	–	–
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							

(continued)

Table 11.1 (continued)

	A. simplex (*s. s.*)	*A. pegreffii*	*A. berlandi*	*A. typica*	*A. ziphidarum*	*A. nascettii*	*A. physeteris*	*A. brevispiculata*	*A. paggiae*
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	–	–	–	–	–	–	–
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, *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), Klimpel et al. (2007), Kuhn et al. (2011), Levsen and Karl (2013), 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, 2008), Serracca et al. (2013), Setyobudi et al. (2011), Shih et al. (2010)]

Table 11.2 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									
Balaenopteridae									
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	–	–	–	–	–
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	–	–	–	–	–	–	–	–
Neobalaenidae									
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	–	–	–

(continued)

Table 11.2 (continued)

	A. simplex (*s. s.*)	*A. pegreffii*	*A. berlandi*	*A. typica*	*A. ziphidarum*	*A. nascettii*	*A. physeteris*	*A. brevispiculata*	*A. paggiae*
Ziphius cavirostris	–	–	–	–	CM, SA, CS	NZ	–	–	–
Pinnipeds									
Phocidae									
Mirounga angustirostris	–	–	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: Garvalho 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), Mattiucci and Nascetti (2007), Mattiucci et al. (1986, 1997, 2001, 2002, 2004, 2005), Nascetti et al. (1986), Orecchia et al. (1986), Paggi et al. (1998a, 1998b, 1998c)]

phylogenetic relationships proposed elsewhere (Mattiucci and Nascetti 2008; Mattiucci et al. 2014a) for species of *Anisakis* mirror, in relation to several host–parasite associations, that proposed for their cetacean 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; Mattiucci et al. 2014a).

11.4.2 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. 11.5), 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 useful to summarise below the geographical distribution of all *Anisakis* species which have been detected genetically.

Anisakis simplex (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 recognised as occurring in several species of cetacean hosts (Table 11.1). Several squid and fish species have been found harbouring 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 recognised 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 been not so far been confirmed (Abollo et al. 2003). *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, 2014a; Paggi et al. 1998a).

Anisakis pegreffii 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). 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, 2014a). 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 et al. 2014a (= *A. simplex* C of Mattiucci et al. 1997) exhibits a discontinuous range, including the Canadian and Chilean Pacific coasts, New Zealand waters and the South African Atlantic coast (Mattiucci et al. 1997, unpublished observation; Nadler et al. 2005). This species has been identified at the adult stage in cetaceans as occurring syntopically with *A. pegreffii* and as a larva in some fish species (Table 11.1). 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 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 11.1 and 11.2). *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 recognised 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 et al. 1998a 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 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)

(Mattiucci et al. 2014a). 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 et al. (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 was first genetically characterised 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). Type II larvae of *A. physeteris* have been genetically identified in the swordfish *Xiphias gladius* from Mediterranean and Atlantic waters (Garcia et al. 2011).

Anisakis brevispiculata has been characterised 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 (Iberian coast). Type II *Anisakis* larvae corresponding to *A. brevispiculata* have been recognised using allozyme markers as 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 et al. (2005) was first genetically characterised 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).

11.5 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 the contraction of 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, 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 actually 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 characterised 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, 2013a).

11.5.1 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* larvae release proteolytic enzymes in order to invade the gastrointestinal mucosa. These antigenic proteins have been isolated and characterised as excretory/secretory antigens (E/S) (Moneo et al. 2000; Shimakura et al. 2004; Rodriguez-Perez et al. 2008; Cavallero et al. 2011; Kobayashi et al. 2011). Humoral and cellular responses are also involved in the infections with *Anisakis* larvae. Th2 cytokines production and the resulting mastocytosis, IgE response and eosinophilia characterise 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 which attracts these cells. Eosinophilic infiltration is the most effective process in the destruction of larvae at the local level (gastrointestinal tract). The presence of eosinophilic cells characterises a late stage of Type I immune hypersensitivity in response to *Anisakis* infection.

11.5.2 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 characterised 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', characterised by a further decrease in the presence of eosinophils, but with abundance of lymphocytes, giant cells and significant collagenisation (Kikuchi et al. 1990).

11.6 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 characterised by nausea, vomiting and epigastric pain. These symptoms appear 1–6 h after the ingestion of the infected fish. In IA, acute signs start to appear about 7 days after infection in the form of abdominal pain, nausea, vomiting, fever, diarrhoea and faecal occult blood. Several, rarely occurring, extragastrointestinal sites have been also documented (i.e. oropharyngeal, 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 submucosa layer of the gastric or intestinal wall.

11.6.1 Gastric Anisakiasis

Epigastric pain is the most frequent sign of acute gastric anisakiasis (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 localisation 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 oedematous

hypertrophic gastric folds, increase in gastric secretion and peristalsis and mucosal lesions, including oedema, redness, coagulation, haemorrhage and ulceration. Cases of GA described recently from Italy (Mattiucci et al. 2013a) were characterised clinically by epigastric pain after 2 h 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.

11.6.2 Intestinal Anisakiasis

Most of the cases of intestinal infection with *Anisakis* are characterised by symptoms such as nausea, vomiting and abdominal bulging, although numerous cases of asymptomatic IA have also been observed. The 'mild form' of IA is characterised by eosinophilic granulomas forming 'tumour-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 IA reported from Spain (Rosales et al. 1999) and from Italy (Moschella et al. 2004; Mattiucci et al. 2011) were characterised 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 the enzymes GOT and GPT, can be of help in suspected cases of IA (Ishikura and Kikuchi 1990). In radiography or ultrasonographic images, showing the thickness of the intestinal wall, a marked dilatation of the intestine, the so-called key board sign, and ascites pooling between the dilated intestines have been reported, characterising both 'mild' and 'fulminant' forms of IA (Ishikura and Kikuchi 1990).

11.6.3 Gastro-allergic Anisakiasis

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 characterised by urticaria, angioedema and anaphylaxis; it consists of an acute IgE-mediated, generalised reaction. In this type of anisakiasis, the allergic reactions take place starting from 2 or 3 h 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, characterised by urticaria and oedema of the oral mucosa, due to *A. pegreffii,* were recognised by molecular methods in Italian patients after they had consumed 'marinated anchovies' (Mattiucci et al. 2013b). In those cases, the endoscopical findings showed *Anisakis* larva invading the submucosa 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 11.3 and Sect. 11.6.4).

Table 11.3 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

E/S excretory/secretory products, *S* somatic, *(?)* indicates unknown location in the larva or molecular weight

11.6.4 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 five of 64 being hospitalised due to respiratory failure (Fernández de Corres et al. 2001). Although the usual signs of anisakiasis are characterised by urticaria, anaphylactic shock and respiratory failure due to oedema, 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, 12 *Anisakis (Ani s)* allergens have been characterised, numbered from *Ani s 1* to *Ani s 12* (Table 11.3), 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 11.3). Purified allergens have been proved to be useful in the diagnosis of *Anisakis* allergy, especially in combination (Moneo et al. 2007).

The *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 and Bruschi Personal Observation).

The major allergens of *Anisakis*, which are recognised at a high percentage level by IgE and IgG in serum samples of patients, are *Ani s1* (24 kDa) and *Ani s7* (139 kDa); they are located in excretory/secretory glands (ES) (Audicana and Kennedy 2008). *Ani s1* was recognised by IgE in sera of GAA patients (Moneo et al. 2000; Mattiucci et al. 2013b). *Ani s 1* has also been detected at a high percentage in patients from Morocco sensitised to *Anisakis* (Abattouy et al. 2012). *Ani s 1* has been recognised by both IgE and IgG in patients with *Anisakis* allergy (Mattiucci and Bruschi personal observation). *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 recognised 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 recognised 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 recognised 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 haemoglobin from *A. pegreffii* was recently characterised, 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 haemoglobins (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).

11.7 Diagnosis of Human Anisakiasis

11.7.1 Histological Diagnosis

Histological sections of haematoxylin–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 microscopical level, enable identification at the generic level:

> In transverse section: a thin cuticle lacking lateral alae; polymyarian muscle cells, separated into four quadrants by chords with two wing-like 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
>
> In sagittal section: the muscular part of the oesophagus followed by the glandular part (ventriculus) and absence of a ventricular appendix and/or intestinal caecum

These microscopical 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 ileocaecal 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. 11.7.2).

11.7.2 Molecular Diagnosis

The very limited specific diagnostic features of individual *Anisakis* spp. larvae available on the basis of morphological examination mean that it is impossible to identify them as aetiological 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 recognise the aetiological 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. (2007), who recognised a larva recovered from the oesophagus 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 the 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 aetiological agent, using the same method described by D'Amelio et al. (2000).

Finally, the first molecular identification of *A. pegreffii* in a paraffin-embedded granuloma as the aetiological agent of an IA case was performed by Mattiucci et al. (2011). In this technical advance, the PCR development allowed, for the first time, the molecular identification of an *Anisakis* larva in formalin-fixed and paraffin-embedded tissue.

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 can 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 apply.

11.7.3 Serodiagnosis

Currently, most serodiagnostic 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 sensitised 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 immunoblotting (WB) to differentiate, for instance, between anisakiasis or *Anisakis* allergy and asymptomatic *Anisakis* IgE-sensitised 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). Further analysis using more-refined methods and serodiagnostic tools are needed.

11.8 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 anthelmintic 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.

11.9 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. (2005) reported a low survival of live *Anisakis* larvae per fillet (0–3 %) after 6 h at −40 °C, but up to 30 % of them survived after 48 h 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 devitalise 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 *Anisakis simplex* larvae in raw fish using high hydrostatic pressure treatments, and the effects of microwave treatments has 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'. It should be emphasised that these control measures should be established in the future, when they can be based on data on the following: (1) there are infection levels of genetically identified *Anisakis* spp. larvae in food fish, (2) the fish are from a defined fishing ground, (3) the location and percentage of infection by larvae in fish fillets (the edible part) are known, (4) the pathogenicity to humans of different species of *Anisakis* is fully established and (5) the risk of *Anisakis* allergy due to antigenic proteins released by these parasites in fish products is fully clarified.

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Chapter 12
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 remains insufficient networks for disease control. In India, experts are encouraging the government to develop a functional public health infrastructure to manage diseases. India has 553 million people at risk for lymphatic filariasis (LF), one of the chronic filarial nematode infections that causes severe morbidity in humans. 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 LF and *Onchocerca volvulus* infections can lead to vision loss or skin pathology. Many 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. This chapter provides an overview about filarial agents drawing upon both their similarities and differences with regards 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.

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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

12.1 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. 12.1).

12.2 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. 12.2). 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 legs and a limestone relief on Queen Hatshepsut's temple at EL-Deir Bahari which

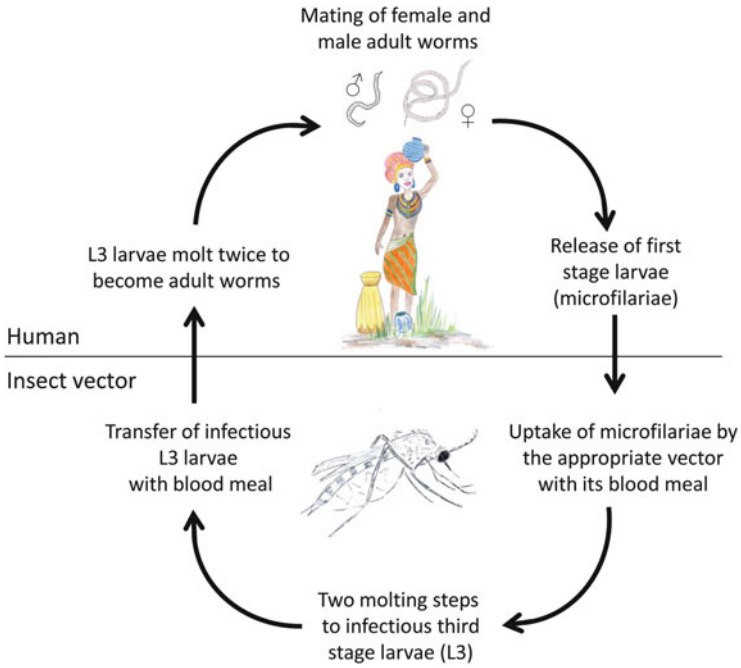

Fig. 12.1 Schematic life-cycle of filariae. Infectious third stage larvae (L3) are transmitted by a blood-feeding arthropod vector to the human host. During the next 6–12 months, L3 larvae molt twice into adult female and male worms. Following mating adult female worms release first-stage larvae (microfilariae), 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

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.

12.2.1 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 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

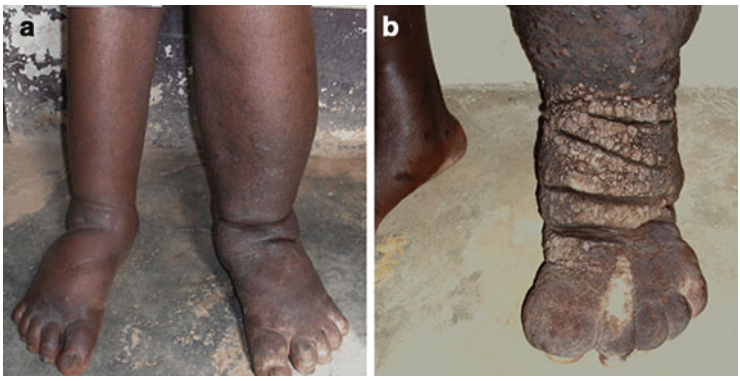

Fig. 12.2 Lymphatic filariasis. Lymphedema with (**a**), skin folds and knobs (stage 5) and (**b**), additional mossy lesions (stage 6)

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 and replication of these filarial nematodes occurs in both the mammalian and vector hosts (Fig. 12.1).

Perhaps one of the most important milestones in tropical medicine was Sir Patrick Manson's discovery that mosquitoes transmitted filariasis. Vector uptake of microfilaria (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 up to 3 weeks (Nutman and Kazura 2011) (Fig. 12.1). 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 p.m. and 2 a.m. 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.

12.3 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, 1.34 billion people live in endemic areas of Asia, Africa, Central and South America and the Pacific (Bockarie and Deb 2010; WHO 2010), and although mortality is uncommon, there is a considerable degree of morbidity. As mentioned above, *W. bancrofti* is the most widespread lymphatic filarial parasite affecting over 120 million people in 73 countries and a third of those are seriously incapacitated and disfigured by the disease (WHO 2012b). 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, the 12 million individuals infected with *B. malayi* are spread throughout Southern China, India, Indonesia, Thailand, Vietnam, Malaysia, the Philippines, and South Korea (Ramachandran 1981; Rebollo and Bockarie 2013).

12.3.1 The Host Response to the Parasite

To ensure long-term survival within the host, helminths have become masters of immune-regulation (Nutman et al. 1987a; King et al. 1993; Dreyer et al. 2000; Hoerauf et al. 2005). Therefore, although the socioeconomic impact of elephantiasis and hydrocele designates this infection as a major public health concern, only a fraction of the infected individuals present such severe symptoms (Keiser and Nutman 2002; Hoerauf et al. 2005). 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 (Turner

et al. 1993; Simonsen and Dunyo 1999). 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 traveling to the periphery and aid in the development of new prevention strategies (Arndts 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). The frequency and intensity of such responses have also been associated with symptoms and pathology (Dreyer et al. 2000; Pfarr et al. 2009: Hoerauf et al. 2011). 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; Martin et al. 2000; Volkmann et al. 2001, 2003; Specht et al. 2006), Th1 responses (Saeftel et al. 2001), $CD4^+$ T cells (Al-Qaoud et al. 1997), neutrophils (Al-Qaoud et al. 2000; Saeftel et al. 2001), B cells, and antibodies (Al-Qaoud et al. 1998). With regards 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 (Semnani et al. 2001, 2003, 2004; Babu and Nutman 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 regards 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). 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 $Foxp3^+$ regulatory T cells (Taylor et al. 2010). In fact, PBMCs 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. Such data provides a 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 increase Th17 production when compared to Mf^+ patients (Ottesen et al. 1977; Nutman et al. 1987a; Satapathy et al. 2006; Babu et al. 2009). These pro-inflammatory cytokines and their receptors are associated with the induction of vascular endothelial growth factors (VEGF) (Ristimaki et al. 1998; Numasaki et al. 2004) which has been further linked to lymphangiogenesis and vascular permeability (Debrah et al. 2006). In fact, investigations revealed that a single nucleotide polymorphism (SNPs) 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 SNPs for TGF-β than asymptomatic individuals indicating that genetic traits are also responsible for these overt reactions (Debrah et al. 2011a). Endemic normals (EN) are subjects who 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).

12.3.2 Immunopathological Processes and Disease

LF is a chronic and persistent disease and aside from lymphedema or the severe disfigurement to limbs (Fig. 12.2) 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 above mentioned ADL, TPE and in endemic areas DLA (dermatolymphangioadenitis) which presents as edematous inflammatory plaques, vesicles, ulcers, 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^- (King and

Nutman 1991). The development of TPE has been linked to the presence of gamma-glutaryl transpeptidase on L3 larvae since it is comparable to that present on 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 regards 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 (Shenoy 2008; Pfarr et al. 2009).

12.4 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 is usually a sign of filarial infection provided that 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 (3–5 μm 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. 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*. CFA even allows the identification of individuals with low parasitemia which could occur, for example, after treatment with microfilaricidal drugs. Although these methods are not applicable for brugian infections, serology assays can be performed by analyzing *Brugia*-specific Igs in ELISAs (Kurniawan et al. 1993; Haarbrink et al. 1999), and there is also a diagnostic dipstick test based on IgG4 serology. Should worms be available the different species can be identified by size and distinct traits (Table 12.1). 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 (Shenoy et al. 2000; Mand et al. 2006). A big improvement for filarial diagnostics may be polymerase chain reaction (PCR)-based assays which detect filarial DNA in blood samples (Pilotte et al. 2013). 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.

Table 12.1 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, Southeast Asia Caribbean, South America, Pacific Islands	120 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	DEC, Doxycycline, Albendazole, Ivermectin	*Culex*, *Anopheles*, *Mansonia*, and *Aedes* mosquitoes	No
Brugia malayi 1927	India, SE-Asia	13 million	Lymphangitis, elephantiasis	Blood, nocturnal, sheathed, 260 µm	Lymphatic vessels and lymph nodes	Males 2 cm, Females 4–5 cm	5–15 years	Yes	DEC, Doxycycline, Albendazole	*Mansonia, Anopheles* mosquitoes	Kra monkey, felines
B. 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	DEC, Doxycycline, Albendazole	*Anopheles barbirostris* mosquitoes	No
Loa loa 1770	Tropical forests in West and Central Africa	?	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 in Latin America	37 million	Blindness, dermatitis, sowda	Skin (upper dermis), unsheathed, 300 µm	Subcutaneous nodules	Males 2–5 cm, Females 35–70 cm	<14 years	Yes	Ivermectin, Doxycycline	*Simulium* black flies	No
Mansonella streptocerca 1922/1972	Sub-Saharan Africa	?	Mild dermatitis (mainly following treatment)	Skin (upper dermis), unsheathed, 180–240 µm	Dermal skin layer	Males 1.7 cm, Females 2.7 cm	?	?	DEC, Ivermectin	*Culicoides* midges	Chimpanzees
M. perstans 1890/1898	Sub-Saharan Africa, North coast of South America	30 million	Mainly asymptomatic	Blood, unsheathed, 200 µm	Peritoneal, pleural, and pericardial cavity	Males 3.5–4.5 cm, Females 7–8 cm	?	Regional differences	Doxycycline	*Culicoides* midges	Chimpanzees and gorillas
M. ozzardi 1897/1898	Latin America and 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

12.5 Tissue Filariasis

12.5.1 Loa loa

This disease has various names including Calabar swellings, fugitive swellings, 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 recently published and confirmed the lack of *Wolbachia* endosymbionts (Desjardins et al. 2013).

12.5.1.1 Life-cycle, Vectors, and Morphology

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 12.1), 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 larva that a single bite may result in infection (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.

12.5.2 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).

12.5.2.1 Life-cycle, Vectors, and Morphology

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 14 years (Hoerauf 2011). Again, adult females are longer than males and females can produce ~700 unsheathed Mf a day (Hoerauf 2011) (Table 12.1) 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 and hence the colloquial term for the infection "river blindness." In comparison with *W. bancrofti*, *O. volvulus* worms also harbor *Wolbachia* (Hoerauf et al. 2000).

12.5.3 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), whilst 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).

12.5.3.1 Life-cycle, Vectors, and Morphology

Although humans are the primary host, infections have been found in other primates and *M. ozzardi* has even been found in birds and amphibians (Peel 1946; Habermann and Menges 1968). Whereas *M. streptocerca* is specifically transmitted by *Culicoides grahamii* midges (Duke 1954), several species of *Culicoides* can transmit *M. perstans* and *M. ozzardi* larvae. In addition, the later can also be transmitted by the blackfly *Simulium 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 12.1). 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. 2009). With regards to *Wolbachia*, this endosymbiont has been identified in *M. ozzardi* (Casiraghi et al. 2001) and recently also in the Mf of *M. perstans* in Mali (Keiser et al. 2008), although at present this seems to be dependent on the geographical region since *M. perstans* in the Gabon (Grobusch et al. 2003) and probably Uganda (Büttner et al. 2003) were determined to be *Wolbachia*-free. It remains unclear whether *M. streptocerca* worms harbor *Wolbachia*.

12.6 Epidemiology of Infection

12.6.1 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 the 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, additional 15.2 million individuals are thought to be at intermediate risk (prevalence 20–40 %) (Zoure et al. 2011).

12.6.2 Onchocerca volvulus

Onchocerciasis is most prominent in sub-Saharan Africa where 99 % of all onchocerciasis cases occur. However, alongside these 27 sub-Saharan countries, additional transmission sites remain in Brazil and Venezuela and Western parts of Yemen (Basanez et al. 2006; MMWR 2013). In Africa, it is estimated that 37 million people are infected with *O. volvulus* with 90 million people at risk (Basanez et al. 2006). Of those, 270,000 individuals have become blind and an additional 500,000 suffer varying forms of visual impairment (Basanez et al. 2006). 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 *Simulium 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*.

12.6.3 Mansonella perstans, M. ozzardi, *and* M. streptocerca

An estimated 30 million people are infected with *M. perstans* in 33 sub-Saharan countries and along the Northern coast in South America (Stoll 1999). Minor foci also include Algeria and Tunisia and in highly endemic areas almost every infection develops into a patent form. *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). 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 Central 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).

12.7 The Host Response to the Parasite

12.7.1 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 (Garcia et al. 1999; Akue and Devaney 2002; Akue et al. 2002). Several studies have compared the immune responses of expatriates with EN and it was shown that the former are 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 (Nutman et al. 1988; Klion et al. 1991). 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).

12.7.2 Onchocerca volvulus

O. volvulus-infected individuals present a spectrum of disease symptoms with two polar forms, generalized onchocerciasis (GEO) 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* (Hoerauf et al. 2009), and even antihelmintich therapy. With regards 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; Satoguina et al. 2002; Korten et al. 2009). Such regulation was confirmed by blocking IL-10 or TGF-β signaling or microfilaricidal chemotherapy which also reversed immunosuppression (Gallin et al. 1988; Ward et al. 1988; Soboslay et al. 1992, 1999; Doetze

et al. 2000; Hoerauf and Brattig 2002). 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 (Soboslay et al. 1997; Hoerauf and Brattig 2002). 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 (Ottesen et al. 1985; Satoguina et al. 2005, 2008; Adjobimey et al. 2013). 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 regards to bystander stimuli, onchocerciasis patients have impaired responses to BCG and tetanus vaccination (Kilian and Nielsen 1989a, b; Cooper et al. 1998) 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 PBMC activation through the induction of IL-10 (Schonemeyer et al. 2001). Patients who present a sowda form of onchocerciasis do not display regulatory profiles; in fact they have a dominant Th2 milieu with high levels of total IgE (Hoerauf and Brattig 2002). 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).

12.7.3 Mansonella perstans, M. ozzardi, and M. streptocerca

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 (Wiseman 1967; Meyers et al. 1972; Almaviva et al. 1984; Nutman et al. 1987b; Baird et al. 1988; McNeeley et al. 1989).

12.8 Immunopathological Processes and Disease

12.8.1 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 (Nutman et al. 1986; Klion et al. 1991). 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 (Nutman et al. 1986; Klion et al. 1991), 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 and urticaria and may be 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 (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 (Zuidema 1971; Nutman et al. 1986; Klion et al. 1991). Other more seldom pathologies include inflammation of the lymph glands (Paleologo et al. 1984), arthritis (Bouvet et al. 1977), scrotal swellings (Fain 1978), eosinophilic lung infiltrates (Klion et al. 1992), and endomyocardial fibrosis (Brockington et al. 1967; Nutman et al. 1986).

12.8.2 Onchocerca volvulus

Classical symptoms of onchocerciasis are dermatitis, keratitis and chorioretinitis. Adult worms 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 (Parkhouse et al. 1985; Wildenburg et al. 1998; Brattig et al. 2001). 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 (Murdoch et al. 1993; Kipp and Bamhuhiiga 2002). Chronic skin inflammation may induce

dermatological changes including depigmentation of the skin (Leopard skin, Fig. 12.3b) 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. 12.3a). 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 punctate keratitis or iridocyclitis. However, permanent exposure 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; Saint Andre et al. 2002; Pearlman and Gillette-Ferguson 2007; Tamarozzi et al. 2011).

12.8.3 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 (Gelfand and Wessels 1964; Dukes et al. 1968), meningoencephalitis, neurological disorders (Adolph et al. 1962; Dukes et al. 1968), and ocular pathology (Baird et al. 1988; Bregani et al. 2002).

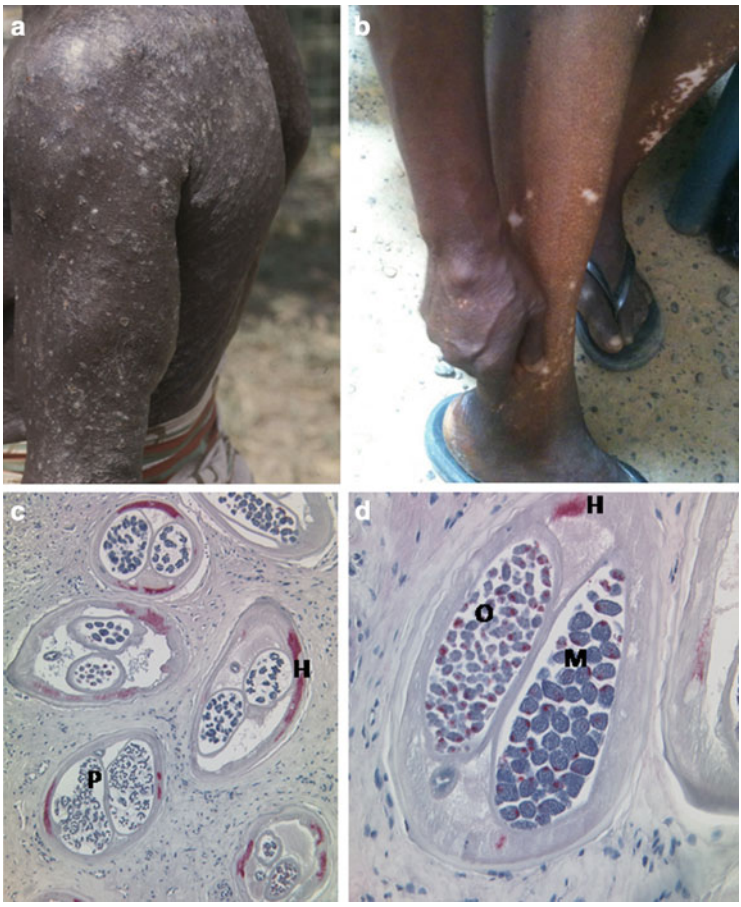

Fig. 12.3 *O. volvulus*. (**a**) Chronic onchodermatitis at shoulder and upper arm. (**b**) Depigmentation (Leopard skin) at legs and hand. (**c**, **d**) Histological sections of one female adult *O. volvulus* worm with embryonic stages in the uterus. Presence of *Wolbachia* (*red dots*) in hypoepidermis (H) and embryonic stages (*O* oocytes, *M* morulae, *P* pretzel stage) within the uterus (*Wolbachia* surface protein staining)

12.9 Diagnosis (Inclusive of Histopathology)

12.9.1 Loa loa

Diagnosis in endemic areas relies mainly on the detection and identification of the sheathed Mf which are diurnal in nature (Table 12.1). Therefore, sampling should be coordinated with their highest activity (10 a.m.–2 p.m.) but should also be based on information from the endemic regions. Adult worms can be surgically removed from either subcutaneous tissue or the eye and can be differentiated by their size

and characteristics (Table 12.1). Laboratory tests include serological assays detecting *L. loa*-derived Ll-SXP-1 antigen-specific IgG4 antibodies, but such tests cannot separate active infections from previous exposures or infections (Klion et al. 2003). Additional serology-based tests employ antigens from other species (Ambroise-Thomas 1974; Ottesen et al. 1982), but these are not specific and may lead to misdiagnosis (Akue et al. 1994). Obtaining sufficient amounts of *L. loa* adult worms may circumvent this problem and a recent study has shown that infection is feasible in rodents (Tendongfor et al. 2012). Upcoming PCR-based assays are a future perspective since they are highly sensitive and can differentiate between active and prior infections (Fink et al. 2011).

12.9.2 Onchocerca volvulus

The traditional way to diagnose infection is a clinical examination of the entire body to detect any signs of dermatitis and subcutaneous onchocercomas which occur in areas that correspond to the biting preferences of vectors. In Africa nodules are mainly located on 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 or 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 skin snip biopsies taken with a corneoscleral punch or razor and restricted to the upper dermis since deeper punctures do not increase diagnostic sensitivity and increase the risk of bleeding and contamination with other Mf 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, 6–24 h, motile Mf can be assessed using a dissecting microscope (Nutman et al. 1996; Mand et al. 2005). 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 min will allow the Mf to migrate to the visible part of the eye (Hoerauf 2011).

Serological tests using highly sensitive (100 %) filarial-specific Igs lack specificity due to cross-reactivity and are therefore only useful for non-endemic patients who were likely to be initially seronegative or to detect alterations in IgG levels after chemotherapeutic interventions. A rapid-format antibody card test is also available for onchocerciasis and invaluable for screening in the field since it is fast and inexpensive (Weil et al. 2000). Patients who 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, a PCR has been developed that detects O-150 DNA from skin snips or scrapings (Boatin et al. 2002), and recently, a new noninvasive diagnostic tool was developed that enables the detection of a secreted metabolite (*N*-acetyltyramine-O,β-glucuronide, NATOG) from *O. volvulus* in urine (Globisch et al. 2013). The test does not seem to cross-react with metabolites in the urine of LF patients; further specificity tests are however needed before its value can be finally determined.

12.9.3 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, unequivocal diagnosis can only be ascertained by identifying Mf in skin biopsies (*M. streptocerca* and *M. ozzardi*) or blood (*M. perstans* and *M. ozzardi*) (Table 12.1). *M. streptocerca* Mf are easy to distinguish since they have a unique hook-structured tail called the "shepherd's crook" (Orihel 1984; Eberhard and Lammie 1991). Detection of blood dwelling Mf may require concentration techniques similar to those described for *W. bancrofti*. Again, diagnostic PCRs on skin snip samples can specifically identify each *Mansonella* species (Morales-Hojas et al. 2001).

12.10 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 occur before filariasis. All filarial infections drive strong $CD4^+$ T-cell responses and the chronic regulatory phases of infection are thought to influence responses to bystander antigens. As seen with other helminth 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 bacillus 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 is 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 (Rougemont et al. 1977; Lipner et al. 2006). With regards to malaria, both vector-borne diseases thrive together in varying prevalence and LF are even transmitted by the same vector in areas of West Africa and Papua New Guinea (Chadee et al. 2003). In the animal studies of filaria and malaria coinfections, some have shown dampened malaria pathology (Fernandez Ruiz et al. 2009; Specht et al. 2010), whereas others 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 who 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 ruled out that this was due to an unspecific drug effect. 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.

12.11 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). 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; Mackenzie et al. 2012; Coffeng et al. 2013). For example, although originally included in the Global Alliance to Eliminate Lymphatic Filariasis (GAELF), 17 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 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 (Taylor et al. 2009; Osei-Atweneboana et al. 2011). Indeed, suboptimal IVM responses have already been observed 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 regards to other filarial infections, surgical procedures are not feasible and treatment relies solely on drugs. Chemotherapeutically, loiasis is primarily treated using DEC since it has both micro- and macrofilaricidal effects. However, this agent has been shown to elicit serious adverse events (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. In practice, only patients with a Mf burden under 2,000 Mf/ml blood are recommended to receive DEC (Boussinesq 2012) since the rapid death of numerous Mf may lead to renal failure, shock, coma

and encephalitis (Gentilini and Carme 1981; Gardon et al. 1997). The drug is also administered in increasing doses (3–6 mg/day to 400 mg/day) over 3–4 weeks to lessen SAE, but up to 50 % usually require more than one round to resolve infection completely (Klion et al. 1994; Boussinesq 2012). Since SAE occur in 50 % of patients, therapy should be started under hospital surveillance so that corticosteroids or antihistamines are readily available to lessen rashes, joint pain, and fever (Nutman et al. 1986; Boussinesq 2012). Within the first 48 h after DEC treatment, adult worms are often found as subcutaneous eruptions and can be surgically removed (Nutman and Kradin 2002). In combination with ALB (400 mg twice daily for 3 weeks), 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 (Eberhard et al. 1997; de Kraker et al. 2006). However, SAE can occur in LF patients 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). DEC treatment is actually contraindicated for onchocerciasis patients because the rapid killing of Mf leads 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. 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, and joint and muscle pain which generally begin 1–2 days after treatment (Meyers et al. 1972, 1978).

M. streptocerca and loiasis can also be treated with ivermectin (Boussinesq 2012; Fischer et al. 1999) although this avermectin family member is generally employed against onchocerciasis and LF. Ivermectin 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 more recently, it was shown to block contractile activity of excretory/secretory vesicles (Moreno et al. 2010). Despite having a serum half-life of only 12 h, this agent is very effective, depleting Mf within a few days (Martin-Prevel et al. 1993; Boussinesq 2012). The effects are long lasting and Mf loads slowly begin to reappear after 3–4 months (Duke et al. 1991; Basanez et al. 2008) 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 (Steel et al. 1991, 1994; Soboslay et al. 1992) 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 (×4/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). Although GAELF supports the annual administration of IVM/ALB in Africa, implemented by the WHO, it is mainly the African countries which remain behind schedule. The reasons behind such delay include the weak, if any, adulticidal capacity of IVM/ALB to DEC/ALB which requires therefore more rounds of MDA (Addiss 2010; Hoerauf et al. 2011). In addition, this combination of treatment is due to the prevalence of *O. volvulus* and to some extent loiasis since DEC treatment may elicit the above-mentioned side effects. Mapping LF is an essential step in fighting LF in Africa. Of the 34 countries in which LF is endemic or suspected to be endemic, countries such as Angola, Ethiopia, and Nigeria are in the process of mapping, whereas Chad and Eritrea have not yet started. Moreover, several countries which remain unmapped or outside of MDA programs are endemic for both LF and loiasis, and although some regions have been mapped, for example, Angola, it is currently deemed futile to treat regions of high risk with IVM (WHO 2012a). 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 limbs, face, and lymph nodes (Pacque et al. 1991; Chijioke and Okonkwo 1992; Awadzi 2003). These effects can be alleviated with anti-histamines 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 Mf loads below 8,000 Mf/ml blood as a single 150 μg/kg dose (Boussinesq 2012) but which can lead to mild adverse reactions (Ducorps et al. 1995; Twum-Danso and Meredith 2003; Kamgno et al. 2009). In cases of high Mf densities (8,000–30,000), IVM is only administered in hospitals (Ducorps et al. 1995; Boussinesq 2012) due to SAE. Those patients and patients with even higher Mf loads can be initially treated with ALB (200 mg, twice daily, for 21 days) before continuing with IVM since the drug works more slowly and causes no severe clinical adverse effects (Klion et al. 1993; Boussinesq 2012). Another alternative is mebendazole, but the effects remain controversial as one study did not observe any efficacy while others reported reductions in Mf during prolonged treatment (Burchard and Kern 1987; Van Hoegaerden et al. 1987). Moreover, all loiasis patients still require therapy with DEC in order to completely clear the infection (Boussinesq 2012). Since *L. loa* does not harbour *Wolbachia* and is therefore immune to such therapies, there is a substantial need for new drugs that slowly reduce Mf loads in *L. loa* patients.

Successful field studies in endemic regions of *Onchocerca* and LF have now proven that anti-*Wolbachia* therapy has long-term sterilizing effects, is a safe macrofilaricidal system, and not only provides a superior therapeutic prognosis but also 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 agent against *M. perstans* (Coulibaly et al. 2009; Hoerauf 2009), and the presence of *Wolbachia* in *M. ozzardi* (Casiraghi et al. 2001) suggests a similar susceptibility since there, all other antihelmintic drugs and combinations thereof have proved ineffective [reviewed in Bartholomew et al. (1978), Hawking (1981), Chadee et al. (1995), Simonsen et al. (2011)]. 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 % and had a 50–70 % macrofilaricidal effect and sterilized the remaining female adult worms (Hoerauf et al. 2008, 2009). At that time IVM therapy was also given to eliminate Mf (Hoerauf 2008). 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 9. These hindrances prompted the formation of the Anti-*Wolbachia* consortium (A-WOL), funded by the Bill and Melinda Gates Foundation, and ongoing research attempts to find alternative tetracycline candidates that could be used in shorter regimens and allow treatment of children. Although a 3-week course of doxycycline with a single dose of IVM/ALB resulted in reduced fertility, it did not show macrofilaricidal effects (Turner et al. 2006). On the other hand, if administered in combination with a single dose of DEC, worms were eliminated demonstrating that different combinations with doxycycline do allow shorter regimens (Mand et al. 2009). Alternative strategies include a 2- to 4-week course of rifampicin (10 mg/kg/day) 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). Further advantages of doxycycline therapy are that it can be used to treat expatriates, individuals with persisting severe dermatitis despite several rounds of ivermectin, and eventually patients who 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).

12.12 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 permethrin-soaked clothing. One should also avoid potential habitats since as mentioned above deer flies are attracted to campfire smoke and travel fair distances to do so (Fain 1978). Although there are no vaccines and no specific prophylactic therapies, the CDC suggests that long-term travelers in endemic areas of *L. loa* take 300 mg DEC per week. Although unofficial, travellers to endemic regions of *Wolbachia*-containing nematodes could take doxycycline since this was shown in several animal models to prevent the moulting from infectious L3 larvae to adult worms (Hoerauf et al. 1999; Smith and Rajan 2000; Rao et al. 2002). Accordingly, travellers who 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 African countries (Molyneux et al. 2003; Boatin and Richards 2006), 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. 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 regards to immunity and vaccine development, EN residing in regions of *L. loa* do not develop symptoms or Mf suggesting a certain level of immunity to loiasis (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 is the lack of suitable small animal models that allow immunological studies and testing of experimental vaccines. However, with the recent study from Tendongfor et al. (2012), perhaps new investigations will open up into 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 (Lange et al. 1994; Abraham et al. 2002; Tchakoute et al. 2006; Allen et al. 2008). However, to date no recombinant antigens were identified that induced a similar degree of protection (Lustigman et al. 2002). Nevertheless, society should not stop trying to develop vaccines since the overall effectiveness of MDA in several regions is worrying due to noncompliance or potential drug resistance. Recent novel possibilities stem from the development of a multivalent DNA-based vaccine comprising different nematode-specific antigens of LF (Samykutty et al. 2010).

By 2011, GAELF had provided combinations of DEC/ALB or ALB/IVM to 538,6 million individuals in 53 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 some defined areas and treat other parasitic infections and already readjusted 32 million DALYs. In the poorest regions of the Americas, the so-called NTD traps, the MDA programs are on the verge of eliminating LF, onchocerciasis, and also trachoma (Hotez et al. 2013). India has 45 % of all LF cases and in some regions like Orissa State has implemented its own community-based lymphedema management program to try and reduce morbidity associated with lymphedema and elephantiasis which relies on improving hygiene, skin care, and exercise. This study showed that increased community health information ameliorated symptoms and reduced sickness leave from work (Budge et al. 2013).

In 1995, the African Programme 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 2010, more than 144,000 endemic communities in 20 African countries participated in the APOC program (WHO 2011). Ivermectin was donated by Merck and distributed on a community-directed annual treatment to 76 million people in 2010 (WHO 2011). APOC was estimated to prevent 8.2 million onchocerciasis-associated DAILYs between 1995 and 2010, and it is foreseen that the strategy will adjust 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 requires a platform that allows a coverage rate of ivermectin distribution above 80 % (WHO 2011). Whether elimination of onchocerciasis is feasible remains unclear since mathematical modeling, based on the original area and Mf loads after the first IVM round, simulated that repeated ivermectin treatment will probably not break onchocerciasis transmission in Western Africa (Borsboom et al. 2003; Dadzie et al. 2003). However, a more recent prognosis suggests that elimination will be achieved in some endemic foci (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). Doxycycline may be an alternative in those co-endemic areas as it has no impact on the *Wolbachia*-free *L. loa* worms. Strategies in South America are managed through the Onchocerciasis Elimination Program for 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 two foci that are located at the border areas of Venezuela and Brazil (MMWR 2013). In the framework of the Pan American Health Organization (PAHO), onchocerciasis is thought to be eliminated in the Americas by 2015 by continuing ivermectin regimes. In summary, it is obvious that 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.

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Chapter 13
Dirofilaria Infections in Humans and Other Zoonotic Filarioses

Claudio Genchi, Claudio Bandi, Laura Kramer, and Sara Epis

Abstract *Dirofilaria repens* and *Dirofilaria immitis,* the main filariae of domestic and wild carnivores, are responsible for most cases of human infections by zoonotic filariae. Other species of animal filariae that have 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 increase risk of infection for humans in temperate countries. Infection by *D. repens* is more frequent in Europe, where the documented infections by *D. immitis* appear rare, but the situation is different in other countries, e.g., in the USA, where human infections by *D. immitis* are 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) in endemic areas, increasing the risk of acquiring filarial infections and of importing the infection in non-endemic areas.

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13.1 The Main Agents of Zoonotic Filarioses: 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 main causative agent of subcutaneous filarial infections (McCall et al. 2008). 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 (cats are generally amicrofilaremic), which act as reservoir (McCall et al. 2008). In the USA, other 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 to the infective larval stage (L_3) which 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, a very efficient vector of *Dirofilaria* spp. is *Culex pipiens*; other species that might contribute to the transmission of these nematodes are *Aedes* (*Stegomyia*) *albopictus* and *Aedes caspius* (Genchi et al. 2011a). The 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 antigen parasites (up to about 30 %) in endemic areas of Spain and Italy, where these mosquito genera are widely diffused (Simón et al. 2005).

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 mainly in subcutaneous tissues, although the parasite can be found in the abdominal cavity and along connective muscular fasciae (Genchi et al. 2011a). According to Webber and Hawking (1955), Cancrini et al. (1989), and Genchi et al. (2013), the prepatent period is around 200 days. Circulating microfilariae are 300–370 μm in length.

The 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 (Genchi et al. 2011a). Severe infections with allergic reactions likely due to sensitization toward the microfilariae 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 dirofilarial infection is based on clinical findings and confirmed by testing for both microfilariae and circulating antigens (from adult female) in the bloodstream in the case of *D. immitis* infection and for circulating microfilariae in the case of suspected *D. repens* infection (McCall et al. 2008). Circulating microfilariae of the two species can be differentiated morphologically by microscopical observation after application of a modified Knott test (Knott 1939); histochemical staining or molecular methods can aid the identification (for more information, see Genchi et al. (2011b) and ESCCAP Guidelines No. 5).

13.2 *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). For a recently described case of infection caused by *D. immitis* in Italy, see 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 Simon 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 the number of cases in Europe (>1,400) is dramatically higher compared to the rest of the world, including the USA, where 110 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).

The increased number of human infections in Europe is most likely a consequence of (1) the changing climate, with increasing temperatures, which allows the survival and the expanding seasonal activity of mosquitoes and (2) the movement and relocation of microfilaremic dogs from the well-known endemic areas of southern Europe, such as Italy, toward the northern and eastern countries. The number of published human cases in Italy has increased from 4.5 per year from 1986 to 1998 to 15.6 per year in the last decade (1999–2009) (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; Simón et al. 2012; Kartashev et al. 2011; Masny et al. 2013) (Table 13.1). 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 mosquito vector bite, although at least three cases of microfilaremic zoonotic infections have been reported in Europe and one in Iran. Interestingly, in all the American individuals, who acquired the infection within the previous 8 months–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 1,400 reported cases, the parasite was localized in the ocular region (e.g., orbital region, eyelid, subconjunctival, and intravitreal) in about 23 % of cases (Table 13.1), probably as a consequence of the perception of a "foreign body" by the human body and possibly also for the easy 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).

Table 13.1 Human *Dirofilaria repens* infection in Europe and other countries of the Old World

Country	No. of cases	Ocular	Pulmonary	Other unusual and seriousness localization
Austria	>16			Intradural tumor like 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[a]	624[1]	54		
Ukraine[b]	51	18	2	
Turkey	22	12	3	
Other countries[c]	192	134	2	
Total	1,430	334	42	

[a]Sergiev et al. (2009) reported worms from 140 individuals identified as *D. repens*; such a figure has not been added to Russian cases because of incomplete description

[b]Masny et al. (2013) estimated about 900 cases; such a figure has not been added to Ukraine cases because of incomplete description

[c]Albania, Bulgaria, Croatia, former Yugoslavia, Georgia, Kazakhstan, Poland, Romania, Serbia, Montenegro, and Slovenia

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 easter Europe (Masny et al. 2013), and 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, 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).

13.3 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 a more easy 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 2007) estimates current global warming to be almost 0.8 °C above preindustrial levels and foresees a further rise of 1.1–6.4 °C by 2100 (IPCC 2007). 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) that 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. Dirofilariasis, 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 Far East and America, such as the case of Asian tiger mosquito *Ae. albopictus*, which was imported into Italy in 1990 and then spread throughout Europe as far as the Netherlands (Scholte et al. 2008). Furthermore, 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 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*.

13.4 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 recent outbreak of chikungunya virus epidemics in Italy (Rezza et al. 2007) and West Nile virus (Sambri et al. 2013) 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:

- The spread of the infection has increased in endemic areas.
- Areas formerly free from the infection are now endemic.
- In dogs untreated with preventive drugs, both the abundance and the incidence of *Dirofilaria* infections have increased (Genchi et al. 2007).
- *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.
- 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 recently 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.

13.5 Other Species of Filarial Nematodes Infecting Humans

The most comprehensive and recent review of zoonotic filarial infections is by Orihel and Eberhard (1998). Table 13.2 summarizes published case reports of other filarial nematodes that have been reported in humans (Orihel and Eberhard 1998). These other zoonotic filariae are all parasites 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.

Table 13.2 Summary of published reports of zoonotic infections with filarial worms (other than *Dirofilaria immitis* and *Dirofilaria repens*)

Parasite	Definitive hosts	Vector	Affected organs	Reference
Dirofilaria			Skin/	Beaver et al. (1987),
D. ursi	Bear	Mosquitoes	subcutaneous	Gutierrez (1990), Orihel
D. subdermata	Porcupine		tissue	and Isbey (1990)
D. striata	Wild felids			
Onchocerca spp.[a]	Cattle, equids, cervids, and others	*Simulium* flies	Skin/ subcutaneous tissue	Ali-Khan (1977), Azarova et al. (1965), Beaver et al. (1974, 1989), Siegenthaler and Gubler (1965), Takaoka et al. (1996)
Brugia spp.[a]	Raccoons, rabbits, monkeys, and others	Mosquitoes	Lymphatics	Baird and Neafie (1988), Menendez and Bouza, (1988), Orihel and Beaver (1989), Elenitoba-Johnson et al. (1996)
Molinema (*Dipetalonema*)		Mosquitoes		Beaver et al. (1980)
D. arbuta	Porcupine			
D. sprenti	Beaver			
Loaina (*Pelicitus*)				Botero et al. (1984)
L. uniformis	Rabbits	Mosquitoes	Eye	
L. scapiceps	Rabbits			
L. roemeri	Kangaroos			

[a]Unidentified at the species level

Worms of the genus *Onchocerca* can also cause zoonotic infections occasionally. *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 which infect 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.

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Chapter 14
Toxocariasis

Clare M. Hamilton, Ayako Yoshida, 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 amongst 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, with particular emphasis on neurotoxocariasis and the subsequent implications of this syndrome. 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.

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14.1 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. malaysiensis* of cats in Malaysia and China; *T. vitulorum* of cattle, buffalo and other 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, and 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 amongst the socioeconomically disadvantaged (Hotez and Wilkins 2009). Given the high prevalence of toxocariasis in areas of poor hygiene, and the lack of awareness amongst 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).

14.2 Life Cycle and Transmission Routes

The life cycle of *Toxocara canis* is complex, with numerous modes of transmission to the definitive host (Fig. 14.1). 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. 14.2). 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 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. 14.3). 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. 14.1). 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 post-infection. 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.

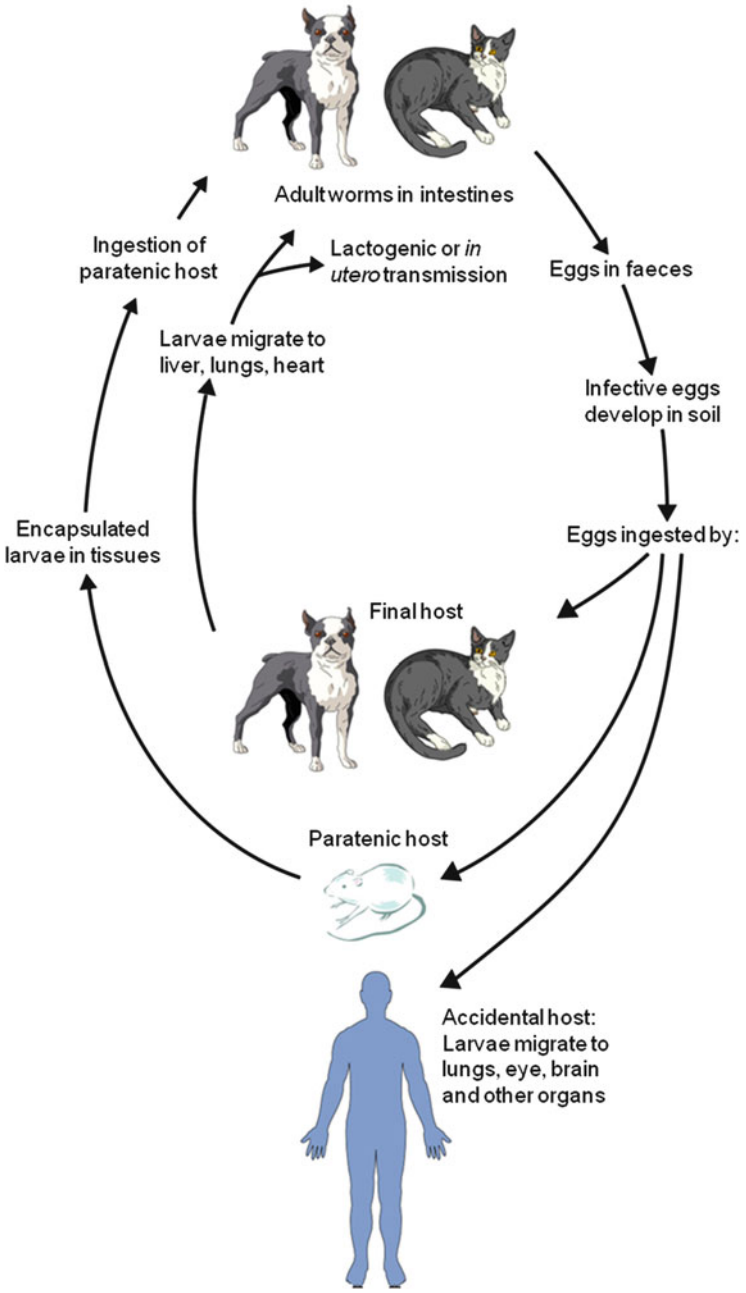

Fig. 14.1 Life cycle of *Toxocara* spp.

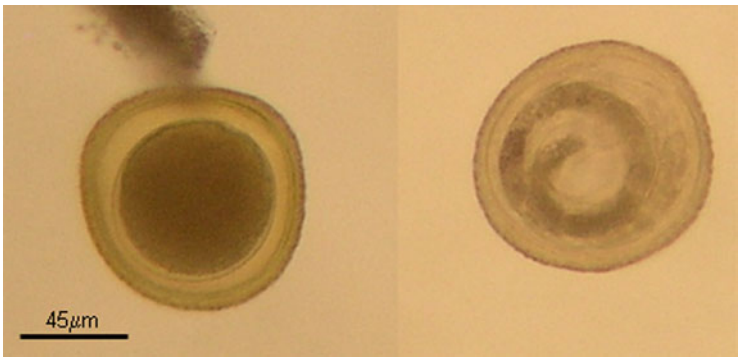

Fig. 14.2 Unembryonated (*left*) and embryonated (*right*) *Toxocara canis* eggs (90 × 75 µm)

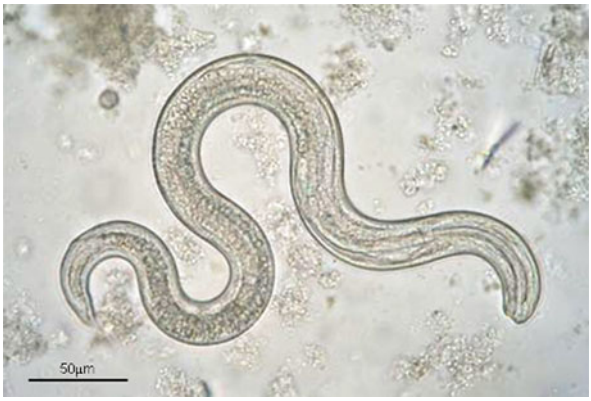

Fig. 14.3 *Toxocara canis* L3 larva (400 × 20 µm) isolated from the brain of an infected BALB/c mouse

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-for dogs poses a low risk of infection (Keegan and Holland 2013).

14.3 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).

14.3.1 Visceral Larval 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. 2001b; 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. 14.5). 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).

14.3.2 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 4 out of 5 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).

14.3.3 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).

14.3.4 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). Given the potential medical implications of cerebral infection and the increasing number of reports in recent years, this section will receive more focus than the other clinical syndromes which have been reviewed in detail elsewhere (Rubinsky-Elefant et al. 2010; Moore and McCarthy 2006).

Humans are known to carry *Toxocara* spp. larvae in their brains. Some of the earliest studies describe the presence of larvae within granulomas in the CNS, discovered accidentally at autopsy when the patient has died from another cause (Dent et al. 1956; Hill et al. 1985; Nelson et al. 1990). To our knowledge, there are

approximately 97 cases of neurological toxocariasis reported in the literature to date, as determined by the presence of *Toxocara* spp. larvae in the brain, seropositivity of CSF and/or amelioration of clinical and radiological symptoms upon anthelmintic treatment (list of references available from corresponding author). Although comparatively rare compared to the other clinical syndromes, 68 of these neurological cases have been reported since the year 2000 indicating an enhanced awareness for the clinical presentation and also improved diagnosis. Of the 88 presenting clinical cases (excluding 9 reported at autopsy), *Toxocara* spp. manifest itself as a number of neurological sequelae including eosinophilic meningitis (Keller et al. 2008), meningoencephalitis (Vidal et al. 2003), myelitis (Jabbour et al. 2011), encephalomyelitis (Marx et al. 2007), eosinophilic meningoencephalomyelitis (Ota et al. 1994), meningomyelitis (Eberhardt et al. 2005), meningoradiculitis (Robinson et al. 2002), arachnoidea (Villano et al. 1992), cerebral abscesses (Moiyadi et al. 2007), cerebral vasculitis (Lompo et al. 2012), cerebral lesions (Kincekova et al. 2008), optic neuritis (Komiyama et al. 1995) and cognitive dysfunction (depression, dementia, cognitive impairment) (Scheid et al. 2008; Salvador et al. 2010). Of note, over half of the cases describe some form of myelopathy which may explain the urinary retention and bowel dysfunction reported in a number of cases (Moreira-Silva et al. 2004; Umehara et al. 2006; Helsen et al. 2011). Interestingly, out of 82 cases where age was given, the average age of the patients was 33.3 years (range 1–79 years), indicating that NT may be a clinical syndrome more associated with adults rather than children.

The degree of neurological symptoms is likely to depend on the number and location of 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 (see Holland and Hamilton (2013) for a recent review), and studies have highlighted a number of issues that may have implications for human health: Varying numbers of larvae are recovered from the brains of genetically different mice infected with the same inoculum (Hamilton et al. 2006); 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 an inflammatory cerebral immune response (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 the first study, Worley and colleagues reported an association between *T. canis* seropositivity and poor reading achievement, distractibility and lower intelligence in kindergarten children; however, this was lost when they controlled for social class (Worley et al. 1984). A few years later, Marmor and colleagues reported deficits in neuropsychological tests carried out by *T. canis* seropositive children aged 1–15 years, compared to seronegative controls, which held up when adjusted for race, socioeconomic status and blood lead concentrations (Marmor

et al. 1987). A third study evaluated cognitive function in children aged 1–4 years and identified lower mental development scores and IQ levels in *T. canis* seropositive children (Nelson et al. 1996). However, the authors did not control for blood lead levels which have been linked with cognitive impairment (Lidsky and Schneider 2003), thus limiting the interpretation of their study. In a more recent study on 242 Polish school children aged 14–16, the authors demonstrated that seropositive boys had lower end-of-year grades than their seronegative counterparts but acknowledged that this may be explained by the amount of time boys spend outdoors (and potentially exposed to infection) and their lower level of personal hygiene (Jarosz et al. 2010).

Given the confounding variables of the above studies, their weight in the argument that *Toxocara* spp. infection can impact on cognitive function is somewhat limited. However, in a recent, more statistically robust study, Walsh and Haseeb (Walsh and Haseeb 2012) examined the differences in two cognitive tests between children with and without serological evidence of *Toxocara* spp. infection. By using data from the third National Health and Nutrition Examination Survey (NHANES III, 1988–1994), the authors were able to assess a large nationally representative sample of children (3,949 subjects) living in the USA. Socioeconomic and demographic data, cognitive testing and laboratory measures were analysed with *Toxocara* spp. antibody ELISA results and revealed that seropositive children scored significantly lower on both tests compared with seronegative children. Moreover, this relationship was independent of socioeconomic status, gender, ethnicity, rural residence and blood lead levels. While the authors admit that the cross-sectional design of the study imposes a limitation precluding a direct causal interpretation of the data, the results still suggest a strong and worrying association between *Toxocara* spp. seropositivity and diminished cognitive function in children.

Within the literature, there are few clinical reports of *Toxocara* spp. infection and cognitive deficits, although this is likely a vast underestimate given the lack of pathognomonic signs. Of those reported, researchers have described the following: 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) and 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, data from longitudinal studies are required (Walsh and Haseeb 2012).

Some reports have suggested an association between *Toxocara* spp. seropositivity and epilepsy (Woodruff et al. 1966; Arpino et al. 1990; Nicoletti et al. 2008), although the topic remains controversial. However, in a recent study, Quattrocchi and colleagues conducted a systematic review of the literature and a meta-analysis of available data to evaluate the association between epilepsy and toxocariasis (Quattrocchi et al. 2012). Of 7 studies analysed, they found the seroprevalence rate of *Toxocara* spp. antibodies to be higher in people with epilepsy than control

subjects. Moreover, the association between *Toxocara* spp. seropositivity and epilepsy was significant in four of the studies. Clearly, these results highlight the possibility of epilepsy being a clinical manifestation of *Toxocara* spp. infection and the need for further investigation.

14.4 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 the 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 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 2–6 months 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 main route of infection 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. The highest prevalence of 92.8 % was reported on the island of La Reunion (Indian Ocean) in a study of 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 Europe, 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 in children aged 6–16 years was recently reported as 13.4 %, and this differed between ethnic groups (African-Americans having more than twice the seroprevalence of Whites and Mexican-Americans) demonstrating a significant disparity in exposure (Walsh and Haseeb 2012).

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 14.1). Cat ownership is less frequently associated with seropositivity (Woodruff et al. 1982; Jarosz et al. 2010).

In a recent article, Fan and colleagues analysed a total of 368 reported cases of clinical toxocariasis from 290 papers published from 1990 to 2012 (Fan et al. 2013). Looking at geographical distribution (and irrespective of the variation in diagnostic methods used), the authors reported that Europe had the most clinical cases (169), followed by Asia (104 cases) and North America (62 cases). Latin America had 27 cases, Australia had 5 cases and only one case was reported from Africa. It is noteworthy that most of the cases reported were OLM with systemic symptoms.

Table 14.1 Risk factors for human toxocariasis

Risk factors	References
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)
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)
Rural residence	Holland et al. (1995), Zarnowska et al. (2008)
Ethnicity	Congdon and Lloyd (2011), Walsh and Haseeb (2012)

14.5 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 IL-4 and IL-5; increased levels of IgG1, IgM and IgE; and a marked eosinophilia (Kayes 2006). 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. 14.6).

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 over-expressing 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). 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). In a recent study, 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).

14.6 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.

14.6.1 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, CT and NT) 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 14.1) should be gathered as additional information for the diagnosis of toxocariasis.

14.6.2 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).

14.6.3 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. 2001b). These two findings along with chronic eosinophilia are usually considered as typical laboratory findings of toxocariasis (Magnaval et al. 2001b). However, patients with suspected toxocariasis should still be examined using a specific serodiagnostic test for toxocariasis with or without the findings mentioned above.

14.6.4 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.

14.6.4.1 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 14.2). 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. 2001b; Fillaux and Magnaval 2013; de Savigny et al. 1979).

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

Table 14.2 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
ELISA kit for Toxocara canis	Bordier Affinity Products SA, Switzerland	TES	IgG	91	86
ELISA Toxocara Ab	Cypress Diagnostic, Belgium	TES	IgG	100	98.4
RIDASCREEN Toxocara IgG	R-Biopharm AG, Germany	TES	IgG	100	98.4
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
Toxocara canis IgG Human ELISA kit	Abcam plc, UK	Not stated	IgG	>95	>95
Toxocara IgG CELISA	Cellabs Pty Ltd, Australia	TES	IgG	90	94
Toxocara Microwell Serum ELISA	Diagnostic Automation/ Cortez Diagnostics Inc, USA	TES	IgG	93	88
Toxocara Microwell Serum ELISA	Diamedix, USA	TES	IgG	87.5	93.3
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	Not stated

(24–32 kDa) are specific for *Toxocara* spp. infection only (Magnaval et al. 1991; Park et al. 2000). However, the WB is generally more expensive and labour-intensive than the 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 fluoro enzyme 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 of infection (Smith 1993). Measuring the avidity of *Toxocara*-specific IgG antibodies may aid in distinguishing between acute and chronic infections (Dziemian et al. 2008).

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). 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 %) than the IgG TES-based ELISA (Yamasaki et al. 2000). There were no cross-reactions with sera from ascariasis patients. In addition, there were only minimal cross-reactions with sera from gnathostomiasis, paragonimiasis and spirometriosis patients. The rTES-120 was also tested using IgG-ELISA. Fong et al. reported that rTES-120 had 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 the 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).

14.6.4.2 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.

14.6.5 Molecular Diagnostic Methods

A definitive diagnosis of human toxocariasis would be possible if the 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 the 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). 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 a tissue sample. 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.

14.6.6 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). 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). In addition, very sensitive and specific PCR methods were shown to detect *T. canis* DNA in liver tissues and BAL of experimentally infected mice (Ishiwata et al. 2004; Pinelli et al. 2013; Rai et al. 1997). These DNA detection techniques may offer a powerful approach for the identification and discrimination among *Toxocara* spp.

14.7 Need for Standardisation of Diagnostic Tools

14.7.1 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. 2001b; 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.

14.7.1.1 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 (1984), 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.

14.7.1.2 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 14.2). 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.

14.7.1.3 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 1,000. 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.

14.7.1.4 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.

14.7.2 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.

14.7.3 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). 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.

14.8 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 14.1. 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 amongst 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.

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. Thiabendazole, albendazole, mebendazole, diethylcarbamazine (DEC) and ivermectin have been examined as treatments of toxocariasis (Pawlowski 2001; Othman 2012; Magnaval et al. 2001a). However, experimental data on the efficacy of ivermectin in toxocariasis is still inefficient. DEC therapy is long known to provoke allergic reactions (Wiseman and Woodruff 1971), although it is accepted as one of the most effective in the treatment of toxocariasis. Thiabendazole has been used for a number of years at a dose of up to 50 mg/kg of body weight (kg bw)/day for 3–5 days, but the drug has been withdrawn from wider use due to its poor tolerability and potential side effects. Nowadays, albendazole is the main drug of choice and mebendazole can be used as an alternative, although optimal doses and duration of treatments are largely undefined. Oral administration of albendazole at 10–15 mg/kg bw/day in 2–3 divided doses for 5 days is recommended (Pawlowski 2001; Caumes 2003). Mebendazole can be given at 100–200 mg/day for 6 days (Wisniewska-Ligier et al. 2012). 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 titres, 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).

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 (ESCCAP 2010). 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). Coordinated 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 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). 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.

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Chapter 15
Angiostrongyloidosis

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Abstract Angiostrongyloidosis is an infection caused by helminth larvae of the genus *Angiostrongylus*. The two main species in the genus *Angiostrongylus* that can cause diseases in humans are *Angiostrongylus cantonensis* and *Angiostrongylus costaricensis*. Human infections are acquired by ingestion of raw or undercooked snails or slugs or contaminated vegetables that contain the infective larvae of the worm. This chapter describes the epidemiology, host response, immunopathology, clinical features, diagnosis, treatment, prognosis, and prevention of human angiostrongyloidosis and discusses how educating people in *Angiostrongylus* endemic areas about the dangers of eating raw or undercooked intermediate and paratenic hosts is essential for the prevention and control of this food-borne zoonotic disease.

15.1 Introduction

Angiostrongylus is a helminth of the phylum Nematoda, order Strongylida, superfamily Metastrongyloidea, and family Angiostrongylidae. The two organisms have different target organs; *Angiostrongylus cantonensis* is a neurotropic parasite, whereas *Angiostrongylus costaricensis* is an abdominal parasite that is located in the mesentery and causes gastrointestinal symptoms.

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15.1.1 Angiostrongylus cantonensis

15.1.1.1 The Agent

A. cantonensis is a type of neurotropic and food-borne parasite that occasionally causes human angiostrongyloidosis and clinically manifests mainly as eosinophilic meningitis, eosinophilic meningoencephalitis, or ocular angiostrongyloidosis. The parasite is acquired by ingestion of raw or undercooked snails or slugs, paratenic hosts such as prawns, or contaminated vegetables that contain infective larvae of the worm (Fig. 15.1). Adult *Angiostrongylus* nematodes are slender worms that can grow to be up to 20 mm long in male worms and 30 mm long in female worms. First-stage larvae are 250 μm long on the average, whereas third-stage larvae have a mean length of 500 μm (Fig. 15.2).

15.1.1.2 Epidemiology of Infection

Human angiostrongyloidosis is widely distributed worldwide where carrier rodents and marsupials are known to exist. It is endemic in South Asia, the Pacific Islands, Australia, and the Caribbean islands and has been reported throughout the world. Chen et al. reported in 1935 the earliest infection of *A. cantonensis* in the pulmonary arteries and hearts of domestic rats in Guangzhou (Canton), China (Chen 1935). In these areas where angiostrongyloidosis is endemic, people usually become infected by eating raw or undercooked food that is contaminated with *A. cantonensis* larvae. The first human case of angiostrongyloidosis was reported in Taiwan in 1945. In 1961, an outbreak of several hundred cases of eosinophilic meningitis, attributed to *A. cantonensis*, was reported in Tahiti, French Polynesia (Rosen et al. 1961). Thailand and Taiwan are the top two endemic areas for angiostrongyloidosis; however, the clinical profiles of patients from these two places are different. Most Taiwanese patients are children who become infected by playing with or eating giant African land snails (*Achatina fulica*) or slugs. By contrast, most Thai patients are adults who get infected by eating raw *Pila* snails. Additionally, epidemiological surveys indicate that most patients in these outbreaks have eaten raw or undercooked meat of an invasive freshwater snail, *Pomacea canaliculata*. This snail is native to South America, was imported into Taiwan in 1981 as a food source, and was subsequently introduced to mainland China (Wang et al. 2007).

15.1.1.3 The Host Response to *A. cantonensis* Infection

The life cycle of *A. cantonensis* involves rats as the definitive host, mollusks as intermediate hosts, and crustaceans (prawns and land crabs), predacious land planarians (flatworms in the genus *Platydemus*), frogs, and monitor lizards as

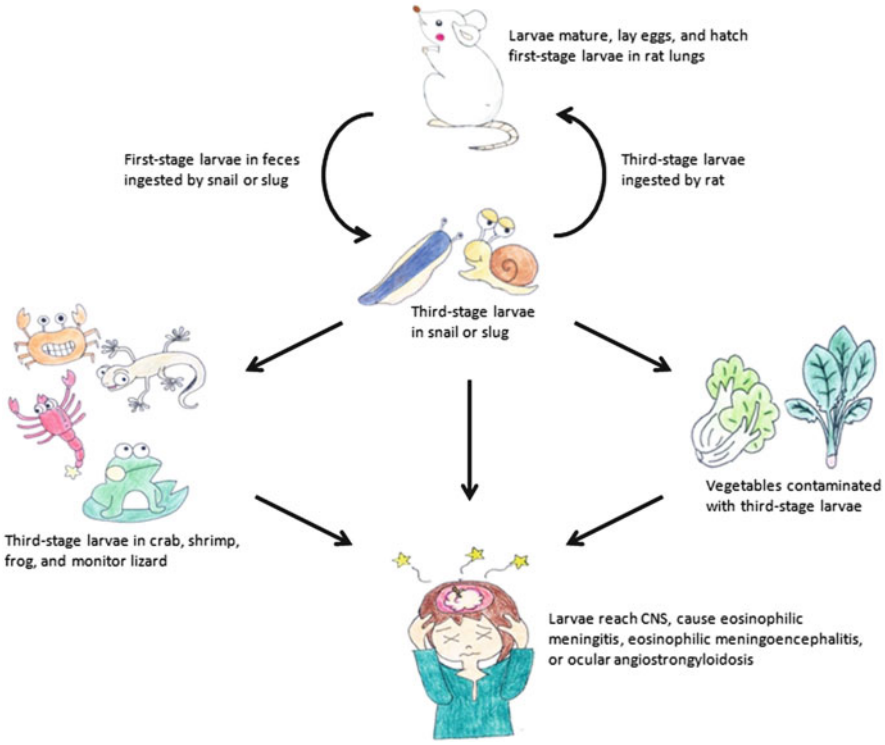

Fig. 15.1 Life cycle of *Angiostrongylus cantonensis*. Rats are definitive hosts. Adult worms develop to sexual maturity and then lay eggs in the pulmonary arteries. Eggs are hatched into first-stage larvae that migrate from the respiratory system to the pharynx, enter the gastrointestinal tract, and get excreted through feces. The infected feces are ingested by intermediate host mollusks (snails or slugs) and develop into third-stage larvae. Third-stage larvae are then transmitted to paratenic hosts, such as shrimps, land crabs, frogs, and monitor lizards. Humans, as accidental hosts, get infected by eating intermediate hosts, paratenic hosts, or vegetables, which contain infective larvae. The larvae are digested from tissues and enter the bloodstream through the intestine. The larvae migrate to the central nervous system, where they become fourth- and fifth-stage larvae via two molts, causing eosinophilic meningitis or encephalitis. Occasionally, the larvae move to the eye chamber and cause ocular angiostrongyloidosis

paratenic (transfer or transport) hosts (Fig. 15.1). Definitive hosts of *A. cantonensis* include wild rodents, especially *Rattus rattus* and *R. norvegicus*. Adult worms live in the pulmonary arteries of rats where they mate and release their eggs into the circulatory system. These eggs lodge as emboli in the lung capillaries, from which the first-stage larvae hatch. The larvae migrate from the pulmonary alveoli up to the trachea and into the esophagus and pass through the gastrointestinal tract and out in the feces. Intermediate hosts take in the larvae by ingesting infected feces. The life cycle is completed when rats eat the infected intermediate hosts. Subsequently, the larvae pass through the intestinal wall, migrate toward the portal system and right atrium, and finally reside in the meninges or brain tissue. The larvae become young

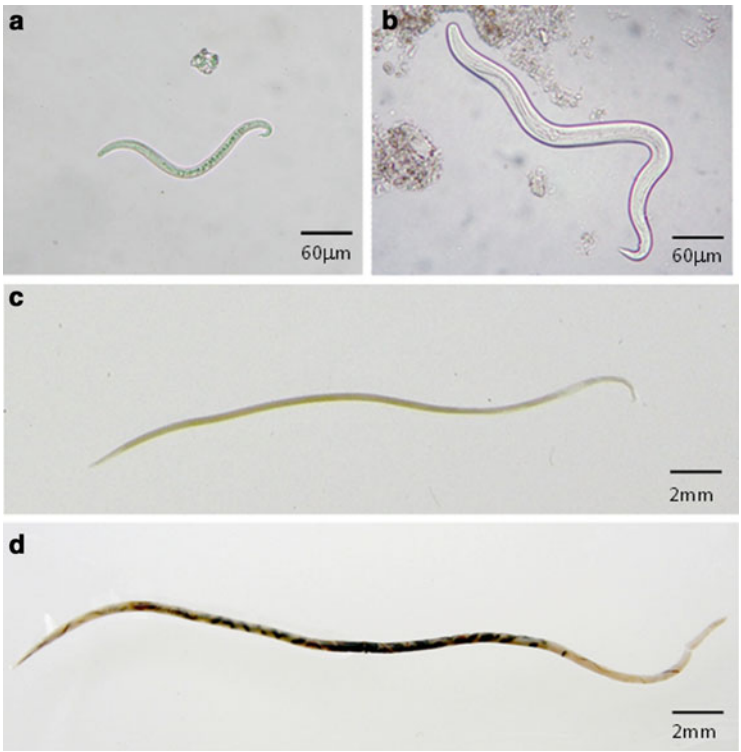

Fig. 15.2 *Angiostrongylus cantonensis*. First-stage larva is 250 μm long (**a**), third-stage larva is 500 μm long (**b**), adult male worm is 20 mm long (**c**), and adult female worm is 25 mm long (**d**)

adult worms in the brain, migrate back to the pulmonary arteries, and restart the life cycle by depositing eggs. Most species of mollusks are susceptible to and capable of transmitting *A. cantonensis*. Terrestrial and some aquatic snails are the primary intermediate hosts (Cross and Chen 2007; Lv et al. 2008). However, in certain regions, one or two species of snails or slugs act as the main intermediate host, and the intensity of infection in these hosts is usually very high. For example, the giant African snail, *Achatina fulica*, is the major source of infection worldwide. *A. cantonensis* was believed to have spread from its native origin in Africa and throughout the Pacific Islands and South Asia (Kliks and Palumbo 1992). The golden apple snail, *P. canaliculata*, is widely distributed in Asia and causes great damage to local agricultural systems. Unfortunately, this snail is also very susceptible to *A. cantonensis* and has become an important intermediate host in these regions (Wang et al. 2007). *Pila* spp. snails are frequently eaten by the Thai, causing human infection among them. However, *Pila* spp. are poor vectors and contain less infective *A. cantonensis*, so human infections have milder clinical signs than those caused by eating *P. canaliculata* (Cross and Chen 2007). *A. cantonensis* can be transmitted by paratenic (or transport) hosts, which subsequently facilitate the

transfer of the larvae from the snail to a human or rat host. Paratenic hosts of *A. cantonensis* carry only the parasite, and the parasite can only resume development if the host is ingested by a rat or human. Important paratenic hosts are freshwater and terrestrial crabs, freshwater shrimps, fish, shellfish, frogs, and monitor lizards. Humans are "dead-end" hosts, meaning the parasites do not reproduce in humans but either remain in the central nervous system (CNS) or move to the eye chamber where they cause ocular angiostrongyloidosis. They remain in the site until parasite death. Humans are not definitive hosts of *A cantonensis*, so the parasite is unable to complete its life cycle and eventually dies in the CNS.

15.1.1.4 Immunopathological Processes

Humans, as accidental hosts, get infected by eating intermediate or paratenic hosts or vegetables that contain the infective larvae of *A. cantonensis*. Once swallowed, the infective larvae are digested from these vectors; the parasites invade the intestinal tissue, cause human enteritis, and then pass through to the liver (Yii 1976). Cough, rhinorrhea, sore throat, malaise, and fever can develop when the worms move through the lungs (Cross 1978). The larvae reach the CNS after 2 weeks, causing eosinophilic meningitis and eosinophilic encephalitis. The major pathological changes caused by human angiostrongyloidosis occur in the brain. Infiltration of lymphocytes, plasma cells, and eosinophils is commonly reported in the meninges and around intracerebral vessels of infected patients (Sonakul 1978). The role for eosinophil is as both classic antiparasitic effector cells and as immune regulatory cells in eosinophilic meningitis caused by *A. cantonensis* (Gosnell and Kramer 2013). Eosinophils are specialized white blood cells of the granulocytic cell line which contain granules in their cytoplasm. These granules contain proteins that are toxic to parasites. When these granules degranulate or break down, chemicals that combat *A. cantonensis* are released (Yoshimura 1993). When the body is infected with *A. cantonensis,* eosinophils located throughout the body are guided by chemokines to sites of inflammation. Upon arrival, type 2 cytokines are released from helper T cells, communicating with eosinophils and signaling their activation. Once activated, eosinophils can begin the process of degranulation, releasing their toxic proteins in the fight against the foreign parasite (Yoshimura 1993; Yoshimura et al. 1994). Cellular infiltration around living worms is not prominent, but dead worms are usually surrounded by a granuloma, increased number of eosinophils, and sometimes Charcot–Leyden crystals. Physical lesions of tracks and microcavities caused by movement of the worms can be found in the brain and in the spinal cord. The larvae can also move to the eyes and cause ocular angiostrongyloidosis, with visual disturbance such as diplopia or strabismus, as reported in many patients (Sawanyawisuth et al. 2007).

The blood–brain barrier (BBB) functions through specialized structures, maintaining the environment of the brain in a steady state by regulating the entrance of substances in the blood to extracellular spaces in the CNS. Endothelial cells that

form the capillaries and venules in the CNS are connected by impermeable tight junctions, preventing all but a select few hydrophobic molecules and hormones from penetrating the brain from the blood–brain interface. Thus, pathogens circulating in the blood cannot enter the brain through these tight junctions. However, the protective effect of the BBB is lost during *A. cantonensis* infection. The primary mechanism of infection involves an increase in the permeability of the BBB and/or direct invasion of the brain by *A. cantonensis*. Some portions of the brain, such as the choroid plexus and preoptic recess, lack the BBB but usually employ other similar barriers, such as the blood–cerebrospinal fluid (CSF) barrier or the blood–retinal barrier. *A. cantonensis* burrowed in the neural tissue of the human CNS causes obvious complications. The following result from the damage of the CNS: (1) direct mechanical damage to the neural tissue from the worms' motion, (2) toxic by-products such as nitrogenous waste, and (3) release of antigens by dead and living parasites. In a mouse animal model of eosinophilic meningitis caused by *A. cantonensis* infection, researchers found that the brain barrier becomes impaired, as shown by the high concentrations of protein and albumin and high leukocyte counts detected in the CSF. When *A. cantonensis* larvae invade the host meninges or the brain, tissue-type plasminogen activator (tPA), urokinase-type PA (uPA), matrix metalloproteinase (MMP)-9, and MMP-12 increase in the CSF (Hou et al. 2004; Chen et al. 2006; Wei et al. 2011). These proteolytic enzymes are associated with brain barrier disruption and eosinophil infiltration. Overexpression of activated MMPs leads to immunopathological changes and contributes to the spread of *A. cantonensis*. Leukocytes are believed to be capable of crossing the blood–CSF barrier; they occupy the subarachnoid space during *A. cantonensis* infection. Activated leukocytes enter the subarachnoid space by extravasation through the wall of meningeal vessels that consist of endothelial cells and elastic tissue. Such parasite infection leads to the induction of MMP-12 from infiltrating leukocytes, causing elastin degradation in the meningeal vessels. These processes are associated with an inflammatory response in the subarachnoid space, as caused by *A. cantonensis* (Wei et al. 2011; Fig. 15.3). Increased blood–CSF barrier permeability is also associated with the disruption of tight junction proteins, as elicited by MMP-9 activation. In angiostrongyloidosis, claudin-5 protein degradation and blood–CSF barrier dysfunction in the brain are mediated by MMP-9 via the NF-κB/MMP-9 signaling pathway (Chiu and Lai 2013; Fig. 15.4).

15.1.1.5 Clinical Manifestations in Immunocompetent and Immunocompromised Patients

When immunocompetent patients become infected, *A. cantonensis* larvae and young adults migrate into the CNS. This infection usually involves the targeted attack of endothelial cells by the parasites to access the barrier structure. However, the worm is not able to grow to adult stage. The three main clinical forms of angiostrongyloidosis caused by *A. cantonensis* are eosinophilic meningitis, eosinophilic meningoencephalitis, and ocular angiostrongyloidosis. Incubation of this

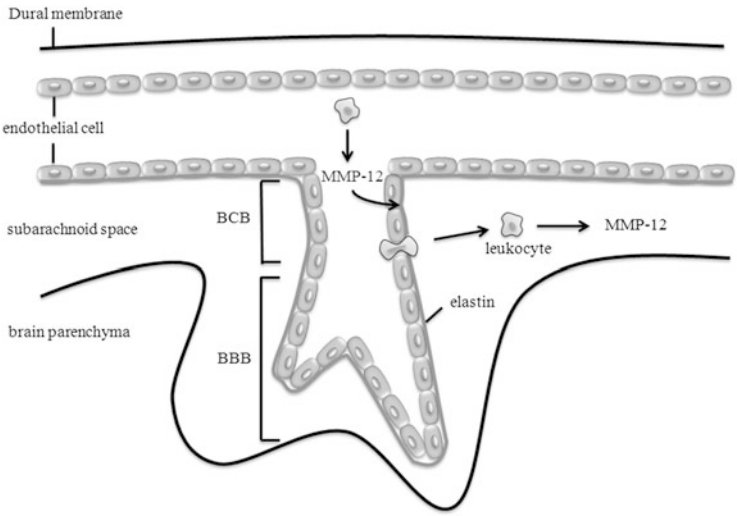

Fig. 15.3 Mechanisms of matrix metalloproteinase (MMP)-12 contribution to meningitis. Leukocytes cross the blood–CSF barrier and present in the subarachnoid space during *Angiostrongylus cantonensis* infection. Activated leukocytes enter the subarachnoid space by extravasation through the walls of the meningeal vessels that consist of endothelial cells and elastic tissue. Such parasite infection leads to the induction of MMP-12 from infiltrating leukocytes, causing elastin degradation in the meningeal vessels. These processes are associated with inflammatory response in the subarachnoid space caused by *A. cantonensis* infection. *BBB* blood–brain barrier, *BCB* blood–CSF barrier [Wei et al. (2011) Int J Parasitol 41:1175–1183]

disease is highly variable, ranging from 1 day to several months, depending on the number of parasites involved (Yii 1976). Infection initially presents with severe abdominal pain, nausea, vomiting, and weakness, which gradually lessen and progress to fever and further develop to CNS symptoms, severe headache, and neck stiffness. Headache mainly caused by increased intracranial pressure or direct damage of the larvae is intermittent, frequent, and severe at first but, after repeated lumbar puncture, becomes less frequent and less severe and is eventually resolved (Punyagupta et al. 1975). Neck stiffness is usually mild and lasts only for a short period. Nuchal rigidity is less common but has been reported in severe cases (Slom et al. 2002; Chau et al. 2003). Paraesthesia, defined by pain, numbness, itching, or a sensation of worms crawling under the skin, usually persists for less than 2 weeks and occurs in a variety of anatomical locations (usually in the extremities). Vomiting and nausea are probably related to the increase in intracranial pressure and usually disappear after the first lumbar puncture. Stiff neck and paraesthesia are less common in children than in adult patients, whereas reports of nausea and vomiting are higher in the former. Incidence of fever, somnolence, constipation, and abdominal pain is also higher in children than in adults (Yii 1976; Chotmongkol and Sawanyawisuth 2002). More than 90 % of cases develop eosinophilic meningitis, whereas less than 10 % progress into the fatal eosinophilic encephalitis. Although fatalities are rare in angiostrongyloidosis-derived

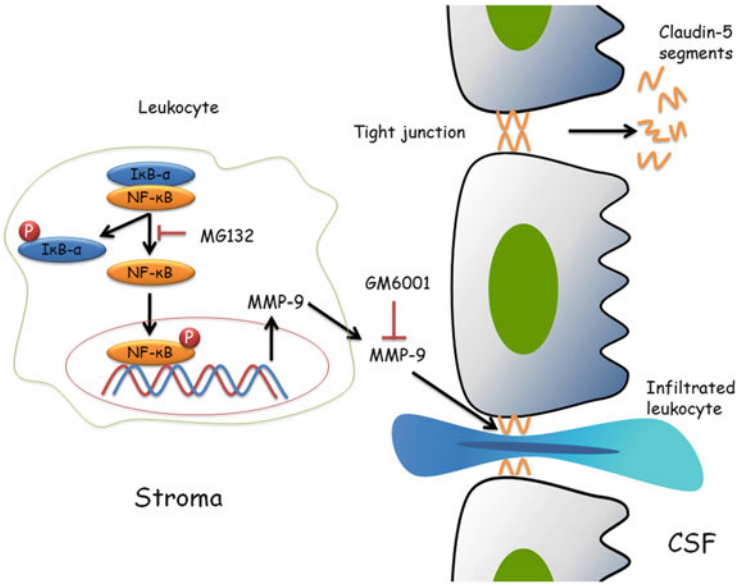

Fig. 15.4 Mechanisms of matrix metalloproteinase (MMP)-9 leading to claudin-5 degradation via the NF-κB pathway. The activation of NF-κB upregulates MMP-9 production in *Angiostrongylus cantonensis*-induced leukocytes. Blocking of MMP-9 activity can reduce claudin-5 degradation and blood–CSF barrier permeability during angiostrongyloidosis meningoencephalitis. Therefore, MMP-9 is suggested to cause claudin-5 degradation and that it promotes leukocyte infiltration into the CSF by the paracellular route during *A. cantonensis* infection in the mouse choroid plexus

eosinophilic meningitis, immunocompromised patients and young children are at risk of developing a fatal patent infection, such as when the larvae migrate to the heart and lungs, mate, and lay eggs, resulting in severe lung pathology. Angiostrongyloidosis is consequently another opportunistic infection that is a high risk to AIDS patients (Archer et al. 2011). Occasionally, patients present with cranial nerve palsies, usually in nerves 7 and 8. On rare occasions, larvae enter the ocular structures. The least common form of infection is ocular angiostrongyloidosis and is found in less than 1 % of infected patients (Sawanyawisuth et al. 2007). Symptoms of eye invasion include visual impairment, pain, keratitis, and retinal edema. Worms usually appear in the anterior chamber and vitreous humor and sometimes can be removed surgically.

15.1.1.6 Diagnosis

Confirmation of human angiostrongyloidosis is performed through the recovery of *A. cantonensis* from the CSF or the ocular chamber. However, the frequency of detecting these worms in patients is very low. Presumptive diagnosis of human angiostrongyloidosis based on clinical symptoms, medical history, laboratory

findings in blood and CSF, serological tests, and brain imaging results is conducted as an alternative. Angiostrongyloidosis caused by *A. cantonensis* is diagnosed mainly in the clinical setting. Symptoms normally occur within 2 weeks of exposure, but the incubation period ranges from 1 day to 3 months (Chotmongkol and Sawanyawisuth 2002). A history of eating intermediate hosts, paratenic hosts, or potentially contaminated vegetables is a crucial criterion for the diagnosis of *A. cantonensis* infection. A turbid or clear CSF is observed for infected patients, with the protein concentration in the CSF usually slightly raised and glucose concentration frequently lower than the normal range. During infection, the proportion of eosinophils reach up to at least 10 % of the white blood cell count in the CSF (100 eosinophils/μL to 1,000 eosinophils/μL; normal range in CSF <10 eosinophils/μL) (Kuberski and Wallace 1979; Slom et al. 2002), whereas in the peripheral blood, eosinophil count reaches up to 7–36 % of the white blood cell count (normal range 0.5–5 %) (Tsai et al. 2001). Identification of eosinophils in the CSF by more than 10 % is a crucial diagnostic test. The most effective strategy to identify CSF eosinophils is by Wright's or hematoxylin and eosin stain. Lumbar puncture should always be performed in suspected meningitis cases. In patients with elevated eosinophils, serology can be used to confirm a diagnosis of angiostrongyloidosis, as opposed to an infection with another parasite. A number of immunoassays aid in diagnosis; however, serological testing is available in only a few labs in endemic areas and is frequently too nonspecific. Some instances of cross-reactivity have been reported between *A. cantonensis* and trichinosis, making diagnosis less specific. Serological tests, including ELISA, have been developed to detect antigens of or antibodies against *A. cantonensis* in the serum or in the CSF. Various ELISA methods have been developed and shown to be effective. Several *A. cantonensis*-specific antigens, such as 29, 31, and 32 kDa, have been identified for immunodiagnosis (Li et al. 2005; Nuamtanong 1996; Maleewong et al. 2001). The 29 kDa antigen from female *A. cantonensis* worms is a potentially good marker for diagnosis (Intapan et al. 2003). A rapid dot immunogold filtration assay that uses purified 31 kDa glycoprotein is faster and easier to perform than traditional ELISA (Eamsobhana et al. 2013). Immuno-PCR can also detect serum antigens of *A. cantonensis* (Chye et al. 2004). Brain imaging by computed tomography (CT) reveals hyper-intensities in the basal ganglia or renders contrast enhancement of the meninges. CT scan of the brain can be normal or can show nonspecific findings, including cerebral edema, ventricular dilatation, and diffused meningeal-enhancing ring or disc lesions, which resemble tuberculoma (Chau et al. 2003; Tsai et al. 2003). T1-weighted magnetic resonance imaging (MRI) postcontrast administration often demonstrates leptomeningeal enhancement and thickening, increased signal in the basal ganglia, and small hemorrhages, as observed through gradient imaging. MRI findings include abnormal enhancing lesions in the brain, especially hyperintense T2 signal lesions, which are different from the hemorrhagic lesions observed in *Gnathostoma spinigerum* infections (Kanpittaya et al. 2000; Tsai et al. 2003). Brain lesions, with the invasion of both gray and white matter, can be observed on CT or MRI. CT and MRI have been used to reveal lesions in the CNS and are useful for differential diagnosis of the disease from other parasitic

diseases, such as cysticercosis, paragonimiasis, gnathostomiasis, and schistosomiasis (Jin et al. 2005).

Blurred vision, with or without eosinophilic meningitis, is a leading symptom of ocular angiostrongyloidosis. The identification of a living worm, usually a single worm in any part of the eye, is a diagnostic criterion. In patients with ocular involvement along with either eosinophilic meningitis or eosinophilic meningoencephalitis, the ocular manifestation will occur after the development of meningitis or meningoencephalitis (Sawanyawisuth et al. 2007). People experiencing clinical symptoms of severe headache, stiff neck, nausea, vomiting, and paraesthesia should be considered likely to be infected with *A. cantonensis*, and parasitological and serological tests must be done to confirm or rule out the tentative diagnosis. The most definitive diagnosis comes from the identification of larvae found in the CSF or the eye. However, due to rarity of detection by this method, a clinical diagnosis based on the above tests is a more likely alternative.

15.1.1.7 Treatment

Most cases of *A. cantonensis* infection are mild and self-limiting, but without prompt and proper treatment, death can occur in severe cases (Yii 1976; Chotmongkol and Sawanyawisuth 2002). Treatment of eosinophilic meningitis includes supportive treatment, anthelminthics therapy, corticosteroid therapy, and combined therapy with corticosteroids and anthelminthics. Supportive treatments for eosinophilic meningitis are repeated lumbar puncture and analgesics. Lumbar puncture must be done at first to confirm or exclude the diagnosis of eosinophilic meningitis. Lumbar puncture serves as a diagnostic tool as well as a temporary reliever and can be a clinical hinge. Patients suffered from remarkably less severe headaches after the spinal tap. Anthelminthics are often used to kill the worms; albendazole, mebendazole, ivermectin, and pyrantel are all commonly used anthelminthics. Anthelminthic drugs shorten the course of disease and relieve symptoms. Therefore, anthelminthics are generally recommended; however, in some cases, this medication causes patients' conditions to worsen due to toxins released by dying worms. Given that the severity of angiostrongyloidosis is believed to be secondary to the host inflammatory reaction, steroids have been studied for potential anti-inflammatory treatment. Corticosteroid was found to be an effective therapy for eosinophilic meningitis (Chotmongkol et al. 2000). Oral prednisolone-treated patients exhibited remarkable recovery from their infections, with shorter duration of headaches, as well as fewer repeated lumbar punctures and analgesic use without any serious drug reaction. In a study on mice, dexamethasone treatment did not decrease the number of larvae, but it did reduce IL-5 levels and eosinophil counts (Tu and Lai 2006). These studies indicate that corticosteroids inhibit inflammatory processes and eosinophil migration. Thus, eosinophils may be harmful to the human brain tissue regardless of the number of larvae. A combination therapy of anti-parasitics for killing the worms and steroids for limiting inflammation has also been suggested. Anti-helminthics should generally be paired

with anti-inflammatory drugs in severe infections to limit the inflammatory reaction by the host to dying parasites. In humans, combinations of corticosteroid and albendazole and corticosteroid and mebendazole (Chotmongkol et al. 2006) have been investigated. Studies suggest that a 2-week regimen of a combination of prednisolone and mebendazole significantly shortens the course of the disease and the duration of associated headaches without any harmful side effects. Other studies suggest that albendazole may be more favorable because it is less likely to incite an inflammatory reaction.

The current treatment regimen for ocular angiostrongyloidosis is not effective. Treatment options, such as laser therapy, surgical removal, or a combination of anthelminthics and corticosteroid, do not improve the visual acuity of patients. Corticosteroid was found to be involved in some inflammation-related conditions, such as retinitis and optic neuritis, and has also been observed in patients with eosinophilic meningitis. However, the final outcome depends on the initial visual acuity. Poor outcome involves permanent damage or retinal pigment epithelial defects. Surgery is required to remove worms from the eyes of patients with ocular angiostrongyloidosis (Kumar et al. 2005; Sawanyawisuth et al. 2007).

15.1.1.8 Prognosis

Prognosis of neurologic angiostrongyloidosis is usually good; however, fatal and chronic cases do occur. The infection frequently resolves itself without treatment or serious consequences, but in cases with heavy parasite load, infection can be so severe that even with treatment, chronic pain, death, or permanent damage to the CNS occurs. Ocular angiostrongyloidosis is a very rare form of the infection, which causes a permanent visual impairment and a wide range of ocular inflammation, depending on the route of the worm.

15.1.1.9 Prevention and Control

Given the large number of rats and mollusks that are highly susceptible to *A. cantonensis* worldwide, eradication of this parasite from the environment is difficult. However, blocking the transmission pathway of *A. cantonensis* to human beings can be made possible by educating high-risk populations to avoid eating raw or undercooked intermediate and paratenic hosts or potentially contaminated vegetables. The habit of eating raw snails and paratenic hosts should be strongly discouraged, although abandoning customs that have existed for generations is often difficult for people in endemic regions such as Taiwan, Thailand, and China, where snails are popularly eaten in various cuisine preparations. Preventive measures for *A. cantonensis* infection in endemic regions include the following: (1) educate people in endemic areas to abandon their habit of eating raw snails and paratenic hosts, and limit the spread of disease; (2) avoid consumption of uncooked vectors, such as snails, slugs, small mollusks, frogs, shrimps, land crabs, and

monitor lizards; (3) eradicate mollusks, planarians, and rodents; (4) prepare food appropriately and wash or cook all vegetables thoroughly, particularly the ones that are commonly infested by snails, such as lettuce; (5) avoid drinking water from open sources, which may be contaminated by vectors; and (6) prevent young children from playing with or eating live snails. Travelers heading to endemic regions must be made aware of the dangers of eating raw mollusks and vegetables from unknown sources and should avoid these dishes. Frequent washing of hands, particularly after gardening, is also strongly recommended (Wang et al. 2012).

15.1.2 Angiostrongylus costaricensis

15.1.2.1 The Agent

A. (*Parastrongylus*) *costaricensis* is a nematode (roundworm) that is the causal agent of abdominal or intestinal angiostrongyloidosis.

15.1.2.2 Epidemiology of Infection

Abdominal angiostrongyloidosis has been reported in Central and South America and occurs most commonly in young children. Humans are accidental hosts for these parasites. Nematode *A. costaricensis* was first described in 1971 as the etiological agent of human abdominal angiostrongyloidosis (Morera and Cespedes 1971).

15.1.2.3 The Host Response to *A. costaricensis* Infection

Rodents serve as definitive hosts of *A. costaricensis*. Natural infection has also been observed in coati (*Nasua narica*) and marmoset (*Saguinus mystax*) hosts. In rodents, *A. costaricensis* produces lesions that are located primarily in the cecum, focal or diffused edema of the subserosa, reduction in mesenteric fat, and swelling of the regional lymph nodes. In highly parasitized animals, eggs and larvae may be found in various viscera of the body. Humans can acquire the infection by eating raw or undercooked intermediate hosts (snails or slugs) infected with the parasite; infection may also be transmitted by the ingestion of raw produce contaminated with larva-containing slug secretions. Alternatively, infection can be transmitted by ingestion of infected paratenic animals, such as crabs or freshwater shrimps. In humans, worms migrate to the mesenteric arteries and release eggs in the intestinal tissues.

15.1.2.4 Immunopathological Processes

The life cycle of *A. costaricensis* is similar to that of *A. cantonensis*, except that *A. costaricensis* adult worms reside in the mesenteric arteries of the rodent definitive host. Females lay eggs that hatch in the ileum, yielding first-stage larvae, which migrate to the pharynx, are swallowed, and then excreted through the feces. The larvae penetrate, or are ingested by, an intermediate host (snail or slug) in which the larvae molt twice to become infective third-stage larvae. When the mollusk is ingested by the definitive host, the third-stage larvae migrate to the brain, where they develop into young adults. The young adults return to the venous system and to the arterioles of the ileocecal area, where they become sexually mature. Humans are accidental hosts for this zoonotic infection. Although the parasites reach an advanced degree of maturation in humans, even to development of adult worms, the eggs or larvae that are produced remain in the tissues and are incapable of reaching the intestinal lumen and being voided in the feces. Consequently, the parasite cycle is not completed in humans although the eggs and larvae in the tissue degenerate and may cause intense local inflammatory reactions. Tissue damage is common during migration, partly due to the host's inflammatory response to proteins secreted by the worm. Host immune status does not appear to affect disease severity.

15.1.2.5 Clinical Manifestations in Immunocompetent and Immunocompromised Patients

Most cases are asymptomatic or show mild symptoms. However, in abdominal angiostrongyloidosis cases showing severe symptoms, the main complaint is pain in the right iliac fossa and right flank. Symptoms include mild fever, anorexia, vomiting, abdominal stiffness, and pain on rectal examination. In chronic cases, patients may present with low-grade fever and occasional mild abdominal pain for several weeks. Sometimes, the intestinal inflammatory reaction becomes a palpable mass in the right iliac fossa. The clinical presentation can be intermittent. The patient may present with periods of remission followed by short symptomatic intervals; at this point, a sub-occlusive condition may occur. The distribution of parasites within the meso-appendix vessels also produces a clinical picture of terminal ileitis, which may mimic acute appendicitis. *A. costaricensis* adults reside in the arterioles of the ileocecal area, and eggs can be released into the intestinal tissues, resulting in local inflammation with abdominal pain, vomiting, and fever. The course of *A. costaricensis* infection is usually benign, but sometimes it leads to a much more severe form, causing intestinal obstruction, perforation, bleeding, and necrosis. Prolonged fever, anorexia, abdominal pain, and widespread tissue eosinophilia are the most evident signs and symptoms of the disease (Loria-Cortes and Lobo-Sanahuja 1980).

15.1.2.6 Diagnosis

Diagnosis of gastrointestinal infection caused by *A. costaricensis* is difficult because the human body is a nonpermissive host; therefore, the eggs and larvae are not passed in the feces, where they are easily observed. However, during surgery, the worms may be identified in intestinal tissues. Eggs and larvae in the blood vessels and eosinophilia in the blood and tissue can be observed in biopsy specimens. Serology is sometimes used to diagnose from a blood sample, and specific ELISA tests for detection are available (Ben et al. 2010). DNA probes using PCR-RFLP that differentiate species of the genus *Angiostrongylus* have been developed, and a diagnostic PCR test from a serum sample has been attempted. Despite the use of laboratory methods, detection of parasitic structures in examination of biopsies or surgical specimens is still the gold standard that allows definitive diagnosis. Imaging for the liver and intestine can also be helpful. A thickened intestinal wall accompanied by necrosis and perforation has been the most important macroscopic finding. Microscopic examination shows granulomas and heavy eosinophilic infiltration around the eggs and larvae of *A. costaricensis*.

15.1.2.7 Treatment

No specific treatment is indicated for *A. costaricensis* infections. Most infections resolve spontaneously, although sometimes surgical treatment is necessary to remove portions of inflamed intestine. Very minimal improvement results from chemotherapy, as the infection may probably be self-limited in most human cases. Experimental chemotherapy trials have been made with anthelminthics, including albendazole, mebendazole, thiabendazole, ivermectin, flubendazole, pyrantel, diethylcarbamazine, and levamisole.

15.1.2.8 Prognosis

Prognosis is generally good and serological follow-up of patients shows that abdominal angiostrongyloidosis is usually not a persistent infection but a self-limiting disease. This disease is usually benign, but it may cause intestinal obstruction or perforation requiring surgery.

15.1.2.9 Prevention and Control

The means of prevention of *A. costaricensis* infection is similar to that of *A. cantonensis*. Control of rodents and mollusks would limit disease. Scrupulous attention to personal hygiene and handwashing after outdoor work is recommended.

The most effective method for prevention is by persuading people not to eat raw or undercooked intermediate or paratenic hosts.

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Chapter 16
Can the Study of Helminths Be Fruitful for Human Diseases?

Justyna Rzepecka and William Harnett

Abstract Parasitic helminths have an inclination to be long-lived invaders with certain human parasites reported as surviving for in excess of a decade. Such longevity tends to be associated with an apparent lack of pathology and one contributor to this perhaps somewhat surprising situation is likely to be the secretion of anti-inflammatory immunomodulators by the worms. Such molecules act to dampen and effect the polarization of immune responses and this invariably potent immunomodulation frequently extends to responses to third party antigens, vaccines and other diseases. Relating to the latter, a particularly serendipitous consequence of worm infection that is being increasingly recognized, is its effect on human conditions that are associated with aberrant inflammation. For this reason, helminths have within the last decade attracted substantial attention in the research community as a potential source of novel therapies against allergic and autoimmune diseases. In this article we describe the effects of helminths on five such diseases—asthma, rheumatoid arthritis, multiple sclerosis, type I diabetes and inflammatory bowel disease. In particular, we consider the immunological mechanisms that underlie helminth-mediated protection against these diseases and in addition, highlight individual helminth molecules that may have therapeutic potential.

16.1 Introduction

Parasitic helminths such as nematodes, tapeworms, and flukes are large, multicellular invaders that are generally very well adapted to their hosts. One of the consequences of such adaptation was the development of a very particular 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

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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 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 diseases (Harnett and Harnett 2010). 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 could be found. 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, 2011). In addition, a protective association of prior hookworm infection with Crohn's disease was found 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 infection in an area where filariasis was endemic. Also recently, hookworm infection was found to be a protective factor against atopy as patients harboring the worm 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 have 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 to support 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 from the host is launched shortly after the parasite has entered its body and relies largely on the response of antigen presenting cells of the host such as dendritic cells (DCs) to molecules secreted by the worms. In the following steps of the immunological cascade, priming of a specific T-helper cell response takes place 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 the 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). Thus, worms are able to counteract the 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 the host immune responses can 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.

16.2 Worms and Asthma

Numerous helminth species have been reported to attenuate symptoms of experimentally induced allergic airway inflammation in mice (Table 16.1). Eosinophil influx into the lungs, and 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 aluminum hydroxide adjuvant (Alum) and a series of intranasal OVA challenges (Pacífico 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 (Pacífico 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 but not IL-10 are responsible for the ability of worm eggs to suppress asthma. In an attempt to further dissect the mechanism by which this helminth can ameliorate asthma, three of the *S. mansoni* antigens were tested in the OVA-induced airway inflammation model (Cardoso et al. 2010). All three proteins could 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 regulatory T cells, on the other hand, were 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 regulatory T cells 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, decreased 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.

Table 16.1 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)
Schistosoma japonicum	Infection	OVA-induced airway inflammation	Liu et al. (2010), Mo et al. (2008)
	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)
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)
	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)
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)

(continued)

Table 16.1 (continued)

Helminth species	Infection/Antigen/Cells	Disease model	Reference
	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 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)

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 shown 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 regulatory T cells in helminth-infected mice that were subjected to OVA-induced airway inflammation were also reported in the study published by Hartmann et al. (2009). Regulatory T cells 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 population that does not require IL-10 to exert its beneficial effect in the course of allergic lung inflammation, regulatory B cells 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).

Continuing on the *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 products-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 regulatory T cells in vitro and transfer of these cells into asthmatic mice suppressed allergic airway inflammation (Grainger et al. 2010).

Similar to the excretory-secretory products derived from *H. polygyrus*, products released from *Trichinella spiralis* could induce expansion of CD4+ CD25+ Foxp3 regulatory T cells in an in vitro assay (Aranzamendi et al. 2012). Expansion of regulatory T cells 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 from 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 regulatory T cells into the lungs and elevated levels of IL-10 and TGF-β (Park et al. 2011).

Worm extracts from the porcine parasite, *Ascaris suum*, were shown to suppress accumulation of eosinophils in the airways and decreased 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*, were reported to protect mice from asthma (Pinto et al. 2006), in agreement with the fact that infection with the same parasite also had potential to mediate beneficial effects on the course of disease (Pinto et al. 2004).

ES-62 is a native molecule purified from excretory–secretory products of a filarial nematode *Acanthocheilonema viteae*. This tetrameric protein with complex, immunologically active posttranslational modifications, in particular phosphorylcholine (PC) attachment to an N-type glycan, 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 signaling restored the number of infiltrating cells and the levels of OVA-specific IgE in the cystatin-treated asthmatic mice. Also 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 IFN-γ, as was reported earlier with ES-62, could promote helminth-induced regulation of asthma.

16.3 Worms and Arthritis

Several species of helminth have been shown to be able to attenuate symptoms of arthritis in a mouse model (Table 16.2). 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 (Song et al. 2011; He et al. 2010; Osada et al. 2009). 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

Table 16.2 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)
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)
Heligmosomoides polygyrus	Infection	MRL/lpr model	Salinas-Carmona et al. (2009)
Nippostrongylus brasiliensis	Infection	MRL/lpr model	Salinas-Carmona et al. (2009)
Ascaris suum	Extract	Zymosan-induced artrhritis; 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)

DBA/J1 mice with collagen-induced arthritis (CIA) diminished the severity and incidence of CIA 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 regulatory T cells and TGF-β.

Apart from flukes, a tapeworm species *Hymenolepis diminuta* exerted anti-arthritic 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 or the IL-4 receptor α chain (Shi et al. 2011).

In addition to Platyhelminthes that have been shown to reduce arthritis, two nematode species, namely, *H. polygyrus* and *Nippostrongylus 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).

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 signaling between dendritic cells and γ/δ or CD4+ T cells (Pineda et al. 2012). This recent paper confirms and expands 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 accordingly. Consistent with these findings, ES-62-treated DCs showed a reduced ability to skew naive OVA-specific T cells toward 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).

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).

16.4 Worms and Multiple Sclerosis

Similar to the studies discussed in the previous paragraphs, different *Schistosome* species and their products have been used intensely to study the impact of helminths on multiple sclerosis (Table 16.3). 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 the 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 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 Stat6-sufficient mice, which points toward the involvement of the Th2 environment in this process.

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 signaling.

Table 16.3 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)
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-Milosavljevic et al. (2013)
Trichinella pseudospiralis	Infection	Experimental autoimmune encephalomyelitis	Wu et al. (2010)
Strongyloides venezualensis	Infection	Experimental autoimmune encephalomyelitis	Chiuso-Minicucci et al. (2011)
Heligmosomoides polygyrus	Infection	Experimental autoimmune encephalomyelitis	Donskow-Łysoniewska et al. (2012a)
	B cells from helminth-infected mice	Experimental autoimmune encephalomyelitis	Wilson et al. (2010)

The immunomodulatory effect of helminth products on DC function and its importance in ameliorating EAE was also studied by Sofronic-Milosavljevic et al. (2013). They showed that DCs stimulated with excretory–secretory products 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. 2008). 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 the 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 the 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). 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).

Apart from different nematode species shown to improve the course of EAE, some *Platyhelminthes* have been shown to share this ability. 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). Also, *F. hepatica* infection 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.

16.5 Worms and Type 1 Diabetes

As early as 1999, Anne Cooke 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 16.4). 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 expand 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 induces Foxp3 T cells from NOD mouse naïve T cells (Zaccone et al. 2011). This raises a 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.

Recently another SEA-derived molecule, lacto-N-fucopentaose III (LNFPIII), a Lewis(X)-containing immunomodulatory glycan, was reported to improve glucose tolerance and insulin sensitivity in diet-induced obese mice (Bhargava et al. 2012).

In a model of multiple low-dose streptozotocin-induced diabetes, *T. crassiceps*-infected mice had lower blood glucose levels throughout the study, no insulitis, and normal insulin content in the pancreas. In terms of immunological parameters,

Table 16.4 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), Zaccone et al. (2010)
	Antigen: ω-1	Non-obese diabetic mice	Zaccone et al. (2011)
	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)
Trichinella spiralis	Infection	Non-obese diabetic mice	Saunders et al. (2007)
Litomosoides sigmodontis	Infection	Non-obese diabetic mice	Hübner et al. (2009), Hübner et al. (2012)
Strongyloides venezualensis	Infection	streptozotocin-induced diabetes	Peres et al. (2013)

helminth infection induced greater numbers of alternatively activated macrophages and IL-4 levels than found in uninfected mice, with no increase of regulatory T cells (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 the disease (Peres et al. 2013).

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 toward Th2/T regulatory-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 toward 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 mice, IL-4-deficient and non-manipulated, when infected with the helminth upregulated frequencies and numbers of regulatory T cells and continuous depletion of TGF-β but not IL-10, prevented the beneficial effect of *L. sigmodontis* in the diabetes model. In a similar fashion, neither the in vivo depletion of CD4+ CD25+ T cells nor blocking of IL-10 signaling affected *H. polygyrus*-induced protection

against type I 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 the development of type I 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 I diabetes, but not in 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 I diabetes development.

16.6 Worms and Inflammatory Bowel Disease

Reardon et al. in 2001 published one of the first reports describing beneficial effects of helminths on colitis (Table 16.5). In this study, mice were infected with *H. diminuta* and colitis was provoked by administration of DSS in the 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 of *H. diminuta* in this model depended on IL-10 and did not predispose to enhanced enteric sensitivity to a third-party antigen which suggested perhaps that there is minimal risk of side effects associated with potential application of helminths to colitic patients. More recently, *H. diminuta* was 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 pro-inflammatory 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).

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 was dependent on Stat-6 signaling. In a similar manner, injection of TNBS-treated mice with *S. japonicum* eggs reduced the inflammation in the colon and 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 regulatory T cells was shown to increase in the colitis-protected mice; however, their involvement in the parasite-induced

Table 16.5 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), Ruyssers et al. (2010)
Schistosoma japonicum	Injection of eggs	TNBS colitis	Mo et al. (2007), 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)
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-enterocilitis triggered by feeding with ovalbumin	Leung et al. (2012)
	Infection	DSS-induced colitis	Donskow-Łysoniewska et al. (2012b)
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)

(continued)

Table 16.5 (continued)

Helminth species	Infection/Antigen/Cells	Disease model	Reference
Anisakis simplex	Recombinant product: macrophage migration inhibitory factor-like protein	DSS-induced colitis	Cho et al. (2011)

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 more recent publication that showed that *S. japonicum* eggs maintained epithelial barrier function through increasing tight junction proteins, thus causing less exposure of NOD2 (intracellular pattern recognition receptors which recognize 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 make 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 motility disturbances during murine colitis (Ruyssers et al. 2010).

Prior infection with *T. spiralis* also reduced the severity of colitis together with a decreased mortality in mice and was correlated with a downregulation of MPO 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). 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 II MIF (As-MIF) from *Anisakis simplex* 3rd stage larvae was found to ameliorate DSS-induced colitis (Cho et al. 2011).

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.

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 *H. polygyrus* 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* also suppressed lamina propria mononuclear cell-derived IL-17 production, another pathogenic cytokine in this model (Elliott et al. 2008).

In a similar model of colitis, it was shown that the worm could reverse piroxicam-induced gut inflammation in Rag 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 signaling; however, their mechanism of action has not been addressed so far. 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). In this model, 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 plasmacytoid 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 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 the TNBS-treated mice by decreasing Th1 and increasing Th2 cytokines (Setiawan et al. 2007). Blocking of IL-10 signaling in vitro restored Th1 cytokine secretion from lamina propria mononuclear cells (LPMC), whereas in vivo intervention worsened colitis in *H. polygyrus*-infected mice.

16.7 Conclusions

As can be seen in this book chapter, there is an abundance of experimental data confirming the potential of helminths to treat asthma, rheumatoid arthritis, inflammatory bowel disease, type 1 diabetes, and multiple sclerosis. Interestingly, some

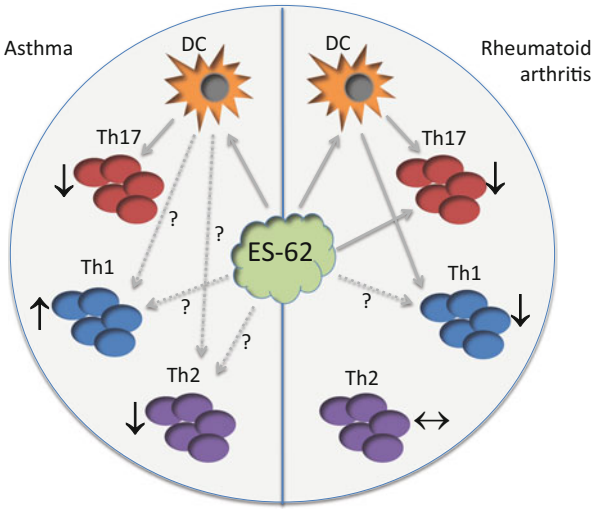

Fig. 16.1 ES-62-mediated immune regulation that confers protection of mice from experimentally induced asthma and rheumatoid arthritis. ES-62 alters the potential of dendritic cells (DCs) to prime naïve CD4+ T cells to differentiate into Th17 cells that are pathogenic in both disease models (IL-17 released 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 cells 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

species of helminths or their products can be effective against more than one disease. ES-62, for example, is effective against asthma and rheumatoid arthritis, two types of inflammatory disorders whose pathologies are shaped by different arms of immunity (Fig. 16.1). This implies that there might be one mechanism, not necessarily dependent on regulatory T cells as these are not upregulated by ES-62, 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 protect from a range of inflammatory disorders. Further work is needed in this area, but 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 axes.

Type I diabetes: symptoms of type 1 diabetes and appearance of pathogenic Th1 cells are reduced by helminths via induction of regulatory T lymphocytes.

IBD: helminths protect mice from experimentally induced IBD by decreasing the potential of DCs to prime Th1/Th17 responses and inducing Tregs. 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.

Considerable progress has 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 any of the 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 relieve 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 *T. suis* reduced symptoms of Crohn's disease and ulcerative colitis in the studied group of patients, and more recently, 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). In addition, more trials using *T. suis* eggs to treat different inflammatory disorders are planned or undergoing across the world (Weinstock 2012).

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