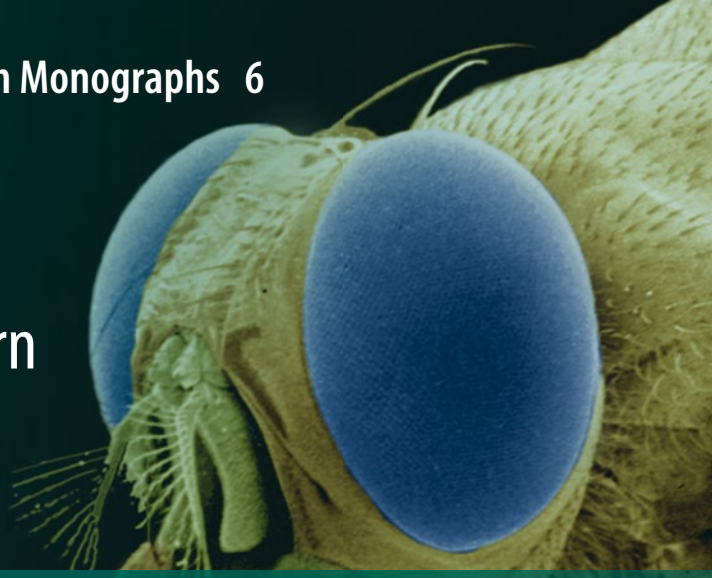
Parasitology Research Monographs 6



Heinz Mehlhorn
Zhongdao Wu
Bin Ye *Editors*

Treatment of Human Parasitosis in Traditional Chinese Medicine

Springer

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Volume 6

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Preface

Parasites of humans and their house or game animals threaten mankind since its beginning at the early steps of human evolution. Many parasites like the thread worm *Ascaris lumbricoides* or the head louse *Pediculus humanus capitis* had become even specialists for humans and were distributed by wandering groups of the late *Homo sapiens* into the farthest spots on earth. Thus, it is not astonishing that humans became aware of this unwanted neighborhood and started the search for means to get rid of these aggressors. This occurred especially everywhere on the world where mankind reached the status of "high cultures," e.g., the Assyrians, Babylonians, the Egyptians, the early Chinese, the Romans, the Mayas, Incas, or even as the Europeans in the so-called dark centuries of the Medieval.

Everywhere it was noted that plants or their fruits had an influence on the health of humans and their farmed animals. These plant effects were diagnosed as being lethal, bad, inefficient, or useful. This knowledge became at first transmitted orally from generation to generation and later was written down in many high cultures (e.g., Egyptians, Greek, Romans, Chinese, Europeans) and used to treat sick humans and suffering animals. Of course, plants were always in the focus of interest and were used as natural medicines, raw, in extracts, or as powders.

In modern times when chemotherapy had been propagated starting by scientists like the German Nobel Prize winners Paul Ehrlich (*1854 †1915) and Gerhard Johannes Paul Domagk (*1895 †1964) or by the English Nobel Prize Winner Alexander Fleming (*1881 †1955), a lot of the basic knowledge on plant effects was forgotten or neglected. However, in recent times where resistances of agents of diseases against chemotherapeuticals increase at high speed, it is worthwhile to consider again the efficacy of plant-derived products.

Thus, this book focuses on the effects of the *Traditional Chinese Medicine*, which has been used for at least 1,000 years and is still used. Thus, this book considers especially plant-derived effects on parasites, which become more and

more important in times where the number of humans on earth is enormously enlarged and where chemotherapeuticals are on the way to lose their efficacy. Of course, the contributors of this book always compared the effects of the plants on diseases to the effects of existing chemotherapeuticals. So it is always important to look into the past and in the future in order to be prepared.

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The quick, careful, and attractive publication of so many data based on own research and/or being selected from papers of a very broad spectrum of international authors is not possible without the help of many persons. At first we thank all contributors for their in-time delivery of their manuscripts, so that latest research aspects could have been included. Then we are indebted to the efforts of all coworkers of each group who gave text and figures their final shape. Our thanks are also directed to Mrs. Anette Lindqvist from Springer and the production team of SPi Global. Their duly and competent efforts made it possible to present these comprehensive insights into the present knowledge of plant-derived medicines, the importance of which is surely still underestimated despite its common and worldwide presence.

Düsseldorf, Germany — Heinz Mehlhorn
Guangzhou, China — Zhongdao Wu
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July 2013

About the Editors

Prof. Dr. Heinz Mehlhorn, Düsseldorf, Germany. He has investigated the transmission pathways of human and animal parasites for over 40 years at German and international universities and he and his university spin-off company Alpha-Biocare have developed many antiparasitic medical products based on more than 20 patents—several in cooperation with big international companies. He has published 25 books and more than 250 original papers, and he has served as Managing Editor of the journal Parasitology Research since 1981. Many renowned international scientists did their Ph.D. work in his laboratory and remain still today interconnected as a large group of lovers of parasitology.

Prof. Dr. Zhongdao Wu (born 1962) is the head of the Department of Parasitology and Deputy Dean at the Zhongshan School of Medicine at the Sun Yat-sen University in Guangzhou, China. He got the Ph.D. degree of Medicine 1997 at the Nanjing Medical University. His research field is very broad reaching from malaria and schistosomes until nematodes like *Angiostrongylus*. As a research fellow he stayed at the Department of Tropical Public Health at Harvard School of Public Health, USA, with studies on epidemiology. He published more than 60 papers in renowned international journals supported by considerable research grants of high reputation. Besides international acceptance, his work was awarded by prizes launched by the Government of China and Guangdong Province.

Prof. Dr. Bin Ye (born 1963). He studied Biology and Internal Medicine (Infectious Diseases) and was awarded with the Ph.D. in 1999. Since 2002 until now he is Professor for Parasitology at the Chongqing Medical University, China. He was Visiting Research Fellow in the USA and works in several fields of emerging parasitic diseases. He is chapter author of five monograph books and of seven textbooks as well as coeditor of seven textbooks for medical and biological students. Furthermore, he published more than 60 papers in renowned journals. His research was awarded by prizes of the Chinese Ministry of Education and of the Chongqing Commission of Education.

Contents

Chapter 1
Introduction: Plants and Their Use in History

Heinz Mehlhorn

Abstract Humans collected knowledge on efficacy of plants against diseases since many thousands of years. This knowledge was at first orally transmitted from generation to generation. In early high cultures this knowledge was also collected in written/designed documents and thus kept alive until the beginning of the age of chemotherapy around 1900. Since then many details had been lost, but some were preserved especially in the Traditional Chinese Medicine and also in other countries and were used still today.

Keywords Plant extracts • Medicinal devices • Traditional medicine • Drugs • Antiparasitic remedies • Quinine • Artemisinin • Qinghaosu • Strychnine • Digitalis

It is about 100,000 years that the most recent precursors of the typical *Homo sapiens* of our days erected their heads in the "paradise" (i.e., in the Garden Eden within the Rift Valley in Central Africa) and that they were apparently forced by climate changes to leave this region of their offspring. As it had been proven by recent molecular genome studies, groups of these refugees apparently spread from there over all continents except for Antarctica during the last cold phases on earth, since at this time the sea level had fallen for about 90–100 m thus setting free "land bridges," which interconnected Europe/Asia with the Americas and South Asia with Australia for several thousands of years, until a new warm period overcame earth. The recent warm period allowed a considerable increase of the human population, since now huge amounts of plants and animals provide sufficient food. This increase of the human population made it necessary to start agriculture in order to prepare these sufficient amounts of food for survival, which was

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apparently not existing during the ice ages. This led to the extinction of the *Homo neanderthalensis*, who depended on hunting. However, the increase of human mankind led also to a closer contact of the different groups around the known world at this time. This phenomenon then increased the possibility of the transmission of agents of dangerous diseases, which had been existing since long among the human precursors and in animals belonging to the human food chain or which had been present in the huge armada of bloodsucking insects, ticks, and/or mites.

From the early beginnings, when humans ate plants—being collected or reared—wise women and men noted that some of these plants had positive effects on human health. These observations and the different modes of preparation of those useful plants were transmitted orally from one generation to the following one, so that within a rather short time a big bunch of plant medications were available among humans, the number of which grew considerably from century to century. And the search for new ones went on, since many treatments showed poor or even no effects.

However, this knowledge on the efficacy of plants or their fruits developed differently on each continent, which had been separated from each other during warm times as a follow-up of the constantly rising sea level and of course due to the fact that different plants had developed on each continent during the long periods of isolation.

On all continents humans created high cultures (e.g., the peoples of the Sumerers, Babylonians, Mongolians, Chinese, Egyptians, Mayas, Aztecs, Inkas, etc.). Plant medication had been established everywhere, was technically ameliorated from generation to generation, and the knowledge had been spread among early pharmacists and/or medicinal sophisticated people. Since this knowledge was mostly transmitted orally or since written documents (e.g., on dried/fired clay plates, papyrus, etc.) were lost during wars and/or at the fall of high cultures after centuries of excellency, only portions of all knowledge were retained until today. This knowledge is based on early heroes of science such as the unknown Egyptian author of the so-called **Ebers Papyrus** (~3000 BC), wherein many medications are described, the Greek physician **Hippocrates** (460–377 BC), who laid the basis of the Western medicine, **Theophrastus of Eresos** (371–287 BC), who worked in the sense of Hippocrates, **Galenus** (129–199 AC), who collected the medicinal knowledge of the ancient Greek–Roman world, the nun **Hildegard of Bingen** (1088–1173 AC) in Germany, who collected the knowledge of plant efficacy in her books, or finally **Paracelsus** (i.e., Philippus Aureolus Theophrastus Bombastus of Hohenheim, 1493–1541) who was the leading medicinal authority of the Middle Ages. Similar knowledge was very probable also present in other high cultures around the globe. Unfortunately, however, written documentations are extremely scarce, since many of these cultures fell during the attacks of cultureless crowds of military invaders (Bianchini et al. 1983; Bingen von 1148; List and Hörhammer 1969–1979; Weiss and Finkelmann 1999).

The knowledge and use of plants or plant extracts as medicinal devices was considerably reduced during the "dark" centuries of the European Medieval, since there were crazy beliefs (even stirred by the officials of the Catholic Church) that use of plants is witchwork. Thus, users were often condemned to death by burning on a stake

Fig. 1.1 Diagrammatic representation of an early pharmacist on peregrination from town to town in times of Renaissance in Central Europe (Germany/France). Reproduced from an untitled plant book at this period (~1700 AC)

and their knowledge was lost. However, as soon as the dark Medieval had passed and the Renaissance = Age of Reviveling had started (approximately fourteenth century), mankind was keen to find new methods to treat diseases. Therefore, intense investigations on the efficacy of plants started and the profession of pharmacists (Fig. 1.1) was developed as well as the physician as a specialist, who had its origin in former natural scientists (Figs. 1.2 and 1.3). Thus, the use of plants as medicaments increased daily, especially after the huge success of some plant drugs (e.g., Quinine against malaria) had been shown to cure millions of humans around the globe (Tables 1.1, 1.2, and 1.3) (Mehlhorn 2008; Pokert 1978; Neumeister et al. 2009).

This predomination of plant medication stopped suddenly or was drastically reduced when the "age of chemotherapy" started with the development of medicaments, which contained a chemically pure substance at an exactly dosed amount. The "father" of this until now reaching period of chemotherapy was the German **Paul Ehrlich** (1854–1915), who developed (together with the Japanese **Hata**) the first strongly effective drug (Salvarsan) against the agent of syphilis (the bacterium *Treponema pallidum*) and got the Nobel Prize for Medicine in 1908. This breakthrough and the following ones such as the discovery of the sulfonamides by the German **Gerhard Domagk** (1895–1964, Nobel Prize 1939), the finding of the antibiotic activity of *Penicillium* fungi by the English scientist **Alexander Fleming** (1881–1955, Nobel Prize 1945) or more recently the discovery to the anthelmintic and insecticidal activities of the avermectins by the Japanese **Satoshi Omura** (1935–) pushed the use of plants more and more in the background. As reasons

Fig. 1.2 Drawings of portions of the barks of several species of the so-called Red bark trees of the genus *Cinchona*, which in former times delivered the quinine, which is today produced using chemical pathways. Reproduction from a single plate of an old book (~1700 AC) sold in Paris page by page by "bouquinistes" along the banks of the river Seine

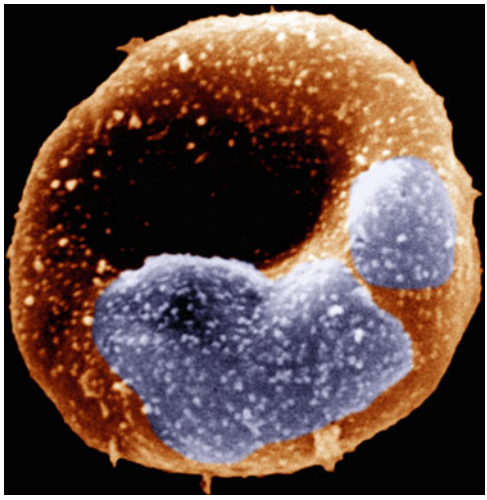

Fig. 1.3 Scanning electron micrograph of an infected erythrocyte containing two schizonts of *Plasmodium falciparum*, which is the agent of the so-called malaria tropica. Note the *white dots* at the surface (knobs). They are the sign that the two parasites (schizonts, *blue*, seen by protrusions at the surface) belong to *P. falciparum*

Table 1.1 Modes of preparations of plant extracts

Way of production	Obtained products
Alcoholic extracts	Tincturae (tinctures)
Alcoholic or alcoholic + aqueous extracts	Extracta fluids (fluid extracts)
Drying process after alcoholic and/or aqueous extracts	Extracta sicca Extracta spissa
Pulverization and drying	Granulates (thin, thick)—used for teas
Pressing	Succi (juices) Sirupi (syrups, molasses)
Squeezing	Pulpae (fluid jam, puree)
Heating	Essential oils
Mixture of plants with plant oils (e.g., olive, garlic, etc.)	Oilea medicate

Table 1.2 Main components of plant extracts and examples of use (compare Schönfelder and Schönfelder 2001)

Name	Chemical groups in plants	Examples of inclusions	Examples of plants
Essential oils	Monoterpenes and others	Valerian	*Valeriana officinalis*
Alkaloids	Bases, salts	Nicotine Atropine	*Nicotiana* sp. *Atropa belladonna*
Anthranoids	Chinones	Anthrachinones, fagopyrines	*Frangula alnus*
Bittern	Derivatives of terpenes, flavonoids	Gentiopicrin Absinthin Naringin	*Gentiana lutea* *Artemisia absinthium* *Citrus limon*
Coumarins	Lactones	Coumarin	*Galium odoratum*
Fatty oils	Triacylglycerides	Linolenic acid Linol acid Gamolenic acid	*Ricinus communis* *Arachis hypogaea* *Ribes nigrum*
Flavones	Flavonoid-phenylchromans	Apigenin Luteolin Rutin (rutosids)	*Solidago virgaurea* *Aesculus hippocastanum*
Tannins	Ester of gallus acids; catechins	Gallotannins Latechin	*Potentilla erecta* *Camellia sinensis*
Cardiac glycosides	Glycosides	Digitalis	*Digitalis purpurea*
Saponosides	Sapogenins	Saponines	*Saponaria officinalis* *Agave americana*
Plant mucilages	Homopolysaccharides Heteropolysaccharides and uronic acids	Glucomannans Galactomannanes Xylans	*Malva sylvestris* *Tilia europaea* *Verbascum densiflorum*
Starches	Polysaccharides	Amylose, glucose	*Zea mays* *Solanum tuberosum*

Table 1.3 Example of plan use in medication

Compound	Active ingredient	Plant of origin	Medicinal use
Strychnine	Alkaloid	*Strychnos nux-vomica*, Poison Nut	Analepticum Ayurvedic medicinal Homeopathy
Digitalis	Tropanalcaloids	*Atropa belladonna*, Deadly Nightshade	Atropine (enlargement of pupils) Antidote against E 605
Digotin Strophanthin	Cardiac glycosides, Cardenolides	*Atropa* species *Strophanthus* species *Clematis* species, liana	Intensification of heart beat Retardation of heart beat
Artemisinin (Qinghaosu)	Terpene lactones	*Artemisia annua*, Wormwood	Antimalarial drug
Quinine	Alkaloid	*Cinchona* species, red bark trees	Antimalarial drug Fever reduction Pain reduction
Essential oils, Flavonoids	Levomenol, Matrikines, Cumarines	*Matricaria recutita*, *M. chamomilla*, Chamomile	Tea against bacterial infections in the oral cavity, stomach
Fatty oils	Linol acids, Linolenic acids	*Linum catharticum*, flax *Arachis hypogaea*, peanut *Triticum aestivum*, wheat	Reduction of cholesterol levels
Tannins	Gallotannins, Catechins	*Potentilla erecta,* Common tormentil	Pain reduction Reduction of wound secretion
Tannins	Gallic acids, Triterpenes, Azadirachtin	*Azadirachta indica* (*Melia azadirachta*), neem plant	Insecticidal activity Anti-inflammatoric activity Skin diseases, etc.
Diterpenes	Fatty oils (Linolen), Flavonoids, Casticin	*Vitex agnus castus,* Monk's pepper	Insect, tick repellent Menstruation abnormalities

were seen the facts that plant extracts always were composed of several components, so that side effects could never be completely excluded and that therefore clinical trials always gave varying results. Furthermore, plant derivatives alone cannot cover easily the huge amount of active compounds being needed in the world of our days with a population of nearly seven billion of humans. All this led to a very restricted use of plant-derived materials during the last 70 years. However, times now change slowly, since nowadays many antibiotic-resistant bacterial strains (e.g., MRSA = methicillin-resistant *Staphylococcus aureus*) endanger health of humans and their number increases daily. Therefore, science switches and starts looking again (now with modern molecular biological methods) on plant effects. An exciting example of the successful look at plant effects is the development of the plant-derived antimalaria compound artemisinin (artemether), which is based on the plant *Artemisia annua* from China (known under the local name Qinghaosu, Fig. 1.4, Table 1.3), while extracts of European *Artemisia* species are used, e.g., to booster the production of the stomach fluid, etc.

Extracts of the plant *Vitex agnus castus* (Fig. 1.5) were known since long to act as phytomedicine in cases of health problems before and during menstruations. To clear these problems, watery-alcoholic extracts were obtained from seeds (Fig. 1.5)

Fig. 1.4 Photomicrograph of a portion of the plant *Artemisia* sp. from China

Fig. 1.5 *Vitex agnus castus*. Micrographs of flowers (*left*) and seeds. The latter are also called Monk's pepper, since they taste like pepper. In the Medieval, these seeds were eaten instead of pepper

and ingested over periods of at least 3 months (Pschyrembel 2006). Mehlhorn et al. (2005) detected that extracts of seeds when obtained by pressing with an oil mill can be used as repellent against ticks, mites, mosquitoes, fleas, etc. This oily extract is entered in an alcoholic solution, sprayed on shoes, trousers, legs, and/or naked skin portions and then those above-listed nasty biters stay away from humans for up to 8 h. In the Medieval, the seeds had been eaten as pepper substitutes, since

Fig. 1.6 Micrograph of closed and open coconuts (*Cocos nucifera*) before preparation of the interior

pepper was very expensive at this time. The seeds were called "monk's pepper," since monks were known as experts in plants and also as "connoisseurs" of fine food (Mehlhorn 2011).

Recent investigations of Förster et al. (2012), Gestmann et al. (2012), and Mehlhorn (2012) showed that arthropods (especially flies and fleas) are widely underestimated agents of severe diseases. Thus, there is an urgent need to search effective plant-derived repellents directed against flies. Since flies like to sit on animal or human food, spraying of chemical repellents would contaminate them.

In long years of testing, the efficacy of different plant extracts for their use as anthelmintic remedies our group found that a mixture of the inner fruit layer of the coconut (Fig. 1.6) and minced bulbs of onions are highly effective against trematodes, cestodes, and nematodes of animals and humans (Mehlhorn 2011; Mehlhorn et al. 2011), if ingested daily for about 8–10 days at a dose of about 1 g/kg body weight.

A further excellent example of the efficacy of plant-derived extractions is the Neem tree (*Azadirachta indica*). Schmutterer (2002) showed in his book that extracts of leaves and/or seeds of this tree were used in India for more than thousand years and now even worldwide to cure many health problems. Of course, several of the listed efficacy claims are not or not fully proven in clinical tests; however, the general efficacy seems to be broad. Proven in many tests is definitively the efficacy of Neem seed extracts (Fig. 1.7) against red bird mites (*Dermanyssus gallinae*) by Locher et al. (2010a, b) and Semmler et al. (2009). Also, the efficacy of Neem extracts against head lice was clearly shown (Heukelbach et al. 2006; Abdel-Ghaffar and Semmler 2007). These findings led to safe and highly effective products of our university spin-off company (http://www.alphabiocare.de). This proves that intense research definitively will bring economic fruits.

However, it is worthwhile to consider not only extracts of plants. This is proven by our group together with scientists at the Max Planck Institute in Mülheim, Germany. We succeeded to obtain and to characterize an antibiotic extract from giant snails of the genera *Archachatina* and *Achatina*, which have stiff shells of up to 15 cm in length, live in Africa and Asia and are often eaten at meals by the local population in these countries. Its antibiotic effects even against resistant strains of

Fig. 1.7 Photograph of seeds of a so-called Neem tree (*Azadirachta indica*). If the green cover is striped off, they look similar to peanuts and also show a similar size

the bacterium *S. aureus* had been granted patents (US 6,982,247B1 of January 3rd, 2006).

Thus, the aim of the book is to look on the means of the traditional Chinese and Asian methods to cure human infections due to important parasites and to open this information to the Western world, which was cut off from such knowledge for centuries due to not only political but also language problems. In addition, also a glimpse is thrown on the recent successful chemotherapeutical remedies.

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Chapter 2
Treatment Methods of Traditional Chinese Medicines Against Intestinal Protozoan Infections

Changling Ma

Abstract Protozoa that parasitize the human intestine include *Entamoeba histolytica*, *Blastocystis hominis, Trichomonas hominis, Cryptosporidium parvum*, *Giardia lamblia*, *Balantidium coli*, *Isospora*, and other protozoa. Most intestinal protozoan infections can cause acute or chronic diarrhea in healthy individuals and may result in intractable, life-threatening illness in patients in immunosuppressive status. Collectively, they infect over one billion people each year. *E. histolytica* can invade the gut epithelium and subsequently other organs, where it forms abscesses. *E. histolytica* infection (amoebiasis) is the second leading cause of death from parasitic diseases. *Cryptosporidium* and *Giardia* are the most common parasitic causes of diarrheal illness worldwide, especially in the developed countries, where they were associated with waterborne outbreaks. In addition, *Cryptosporidium*, *Entamoeba*, and *Isospora* have been most frequently identified as the most commonly implicated pathogens which cause persistent diarrhea in patients with HIV, followed by *Giardia* and *Strongyloides*. Clinically, the nitroimidazoles class of agents including metronidazole, tinidazole, ornidazole, etc, is considered as the front line of defense against intestinal protozoan infections in the world. However, drug-resistant intestinal protozoa (such as *E. histolytica* and *Giardia*) have appeared in clinical and laboratory isolates regularly. Accordingly, resistant strains have been treated with longer repeat courses or higher doses of the original agent in clinic. Chinese medicine therapeutic approaches have been employed for intestinal disease caused by intestinal protozoa for a long time in China and Asian countries, with low side effects compared with western medicine. Several Chinese medicines including *Radix Pulsatillae*, *Fructus Bruceae*, *Allium sativum*, *Radix Sophorae Flavescentis*, *Fructus cnidii*, etc, are shown to be effective for anti-protozoa. This review presents the current advances in traditional Chinese medicines with a focus on effective treatment and control of intestinal protozoan infections.

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2.1 Introduction

E. histolytica is an intestinal protozoan that causes invasive amoebiasis in 40–50 million people, resulting in up to 100,000 deaths globally every year (Ali et al. 2008). *E. histolytica* infections occur worldwide but are more prevalent in the tropics. Recognized high-risk groups include travelers, immigrants from endemic areas, immunocompromised individuals, and men who have sex with men (MSM) (Weinke et al. 1990). About 90 % infections with *E histolytica* are asymptomatic, only 10 % of infected individuals develop symptoms including amoebic colitis and amoebic liver, lung or brain abscess. In China, it was estimated that ten million persons are infected by *E. histolytica* by fecal–oral transmission, particularly distributing in Xizang, Yunnan, Xinjiang, Guizhou, Gansu Provinces according to epidemic survey data about 1988–1991 (Jiang et al. 1997) (Table 2.1).

Nitroimidazoles, particularly metronidazole, has been the mainstay of therapy for acute and chronic invasive amoebiasis since the 1960s. After treatment with metronidazole and other nitroimidazoles (such as tinidazole, ornidazole, and secnidazole), luminal amebicides such as Diloxanide furoate, paromomycin, and iodoquinol are recommended to eradicate intestinal colonization and prevent relapse (Reed 2000). Noninvasive infections may be treated with paromomycin and Diloxanide furoate as first-line and second-line agent, respectively. However, Nitroimidazoles have many side effects, such as primarily gastrointestinal, disulfuram-like intolerance reaction, and neurotoxicity (Haque et al. 2003).

In addition, some other protozoa such as *Giardia lamblia*, *Cryptosporidium parvum*, *Trichomonas hominis, Blastocystis hominis*, and *Balantidium coli* are also prevalent in China. Nevertheless, the infection rate of intestinal protozoa declines apparently in the last 10 years. In Xinjiang Province, which is regarded as the province with higher infection rate of intestinal protozoa in China, the rate of decline of *G. lamblia* and *B. hominis* were 85.65 and 58.60 %, respectively, in 2003 (Maimaitijiang et al. 2010). There is no report about human coccidiosis in China. Metronidazole and other nitroimidazoles have been frequently used as the mainstay of chemotherapy of these intestinal protozoa diseases in China.

In China, traditional Chinese medicines have been employed to treat parasite disease for several thousand years. Here the review presents the current advances in traditional Chinese medicines with a focus on effective treatment and control of intestinal protozoa infections.

2.2 *Radix Pulsatillae*

Radix Pulsatillae, the dried root derived from *Pulsatilla chinensis* (*Bge*) *Reg*, was recorded to treat dysentery widely in Chinese classic medicine books. As early as the 1950s, it was used in the treatment of amoebiasis in China. Lab researches

Table 2.1 The infection rate (±SE) of *E. histolytica* in Province/Autonomous region/Municipality (Jiang et al. 1997)

Province	Infection rate (±SE), %	No. county examined	No. positive	Positive rate
Xizang	8.12 (1.86)	13	11	84.62
Yunnan	2.54 (0.50)	28	25	89.29
Xinjiang	2.37 (0.58)	23	21	91.30
Guizhou	2.25 (0.36)	24	23	95.83
Gansu	2.04 (0.56)	19	15	78.95
Hainan	1.58 (0.43)	5	4	80.00
Neimenggu	1.80 (0.42)	21	13	61.90
Tianjin	1.73 (0.36)	5	5	100.00
Zhejiang	1.50 (0.33)	29	24	82.76
Guangxi	1.49 (0.20)	20	19	95.00
Hebei	1.47 (0.32)	31	23	74.19
Shangdong	1.10 (0.10)	35	31	88.57
Jiangsu	0.89 (0.13)	33	28	84.85
Hunan	0.89 (0.15)	30	25	83.33
Jiangxi	0.88 (0.12)	23	20	86.96
Sichuan	0.83 (0.15)	45	39	86.67
Fujian	0.69 (0.13)	26	25	96.15
Anhui	0.59 (0.10)	22	20	90.91
Henan	0.57 (0.06)	39	30	76.92
Qinghai	0.48 (0.22)	23	9	39.13
Hubei	0.44 (0.08)	31	22	70.97
Shanxi	0.40 (0.08)	25	17	68.00
Liaoning	0.36 (0.11)	29	16	55.17
Guangdong	0.34 (0.05)	31	26	83.81
Beijing	0.29 (0.05)	9	8	88.89
Heilongjiang	0.17 (0.04)	26	12	46.15
Shanxi	0.15 (0.04)	26	11	42.31
Ningxia	0.10 (0.03)	20	1	5.00
Jilin	0.04 (0.03)	25	1	4.00
Shanghai	0.01 (0.004)	10	1	10.00
Total	0.95 (0.04)	726	525	72.31

demonstrated saponin extracted from *Radix Pulsatillae* and its decoction could inhibit the growth of trophozoite of *E. histolytica* in vitro and in animal model with low toxicity (Jiang et al. 1958; Lan et al. 1996) (Fig. 2.1).

The earlier clinical studies showed that the efficacy of *Radix Pulsatillae* against amoebiasis could reach up to above 90 %, administrated with 30 g orally, 10 days as one course. The high efficacy was ascribed to the powerful eradication of *Radix Pulsatillae* against trophozoite and cyst (206 Hospital 1960). Combined prescription is a method to combine several Chinese medicines guided by the idea of therapy with syndrome differentiation. *Radix Pulsatillae* decoction based on *Radix Pulsatillae*, consisting of *Radix Pulsatillae*, *Cortex Fraxini*, *Phellodendron Amurense Rupr*, *Rhizoma Coptidis Chinensis*, *Lonicera Japonica Thunb*, *Viola Yedoensis Mak*, and *Radixet Rhizoma Rhei*, was demonstrated to be an effective

Fig. 2.1 *Radix Pulsatillae* and *Pulsatilla chinensis* (*Bge*) *Reg*. *Radix Pulsatillae* is the dried root of *Pulsatilla chinensis* (*Bge*) *Reg*. of family Ranunculaceae, with slightly bitter and astringent taste. The best quality of *Radix Pulsatillae* is thick and long in regularity, *grayish yellow* of superficial, *white* villus at the top of it. The cold property of the herb is good at removing the stagnated noxious heat in the intestine and is effective in cooling blood and detoxicating

combined prescription to treat intestinal amoebiasis by oral administration and coloclysis with 90 % efficacy (Cai 1995). Beyond that, *Radix Pulsatillae* is also active to inhibit the propagation of *B*. *hominis* in vitro, suggesting potential beneficial effects in *B*. *hominis* infections (Zhang et al. 1997).

2.3 *Fructus Bruceae*

Fructus Bruceae, the fruit of *Brucea javanica* (L.) Merr, is distributed in the south of China. It is a traditional Chinese medicine to treat dysentery and malaria. It contains many chemical compositions, such as Brucealin, Brucamarine, Ratanine, etc. Crude glycosides and Brucealin (a glycoside), extracted from *Fructus Bruceae*, are tested to be effective chemical components to inhibit the growth of trophozoite of *E*. *histolytica* and kill it directly in vitro (Quan 1948; Song 1949; Yang and Ou 2001) (Fig. 2.2).

The first published report of successful use of *Fructus Bruceae* against human amoebiasis was in 1937 (Liu 1937). Unique in that it is effective both in acute and chronic amebic infection, *Fructus Bruceae* has been reported to eradicate up to 54–90 % of intestinal infections (Liu 1941; Wu 1943). Meanwhile, clinical studies suggested it was more efficacious for the combination of coloclysis and oral administration with *Fructus Bruceae* than single oral administration (Feng 1957). Based on the main function of *Fructus Bruceae* against *E*. *histolytica*, several combined prescriptions are found to be highly effective with 95.8–100 % efficacy. This statement has support from studies 14 amoebiasis cases treated by combination with *Fructus Litseae* and 24 patients treated by Jiedushenghua Dan, which consists of *Fructus Litseae*, *Lonicera Japonica Thunb*, *Glycyrrhiza Uralensis*

Fig. 2.2 *Fructus Bruceae* and *Brucea javanica* (L.) Merr. *Fructus Bruceae* consists of the dried ripe fruits of *Brucea javanica* (L.) Merr. The bitter and cold herb has been used in clearing heat, expelling toxins, checking malaria, treating dysentery, etc.

Fisch, *Radix Notoginseng*, and *Radix Paeoniae Alba* (Jingzhou Infectious Hospital 1959; Chen 1987).

2.4 *Allium sativum* and Allicin

In addition to being a secondary ingredient for dish, *Allium sativum* (garlic) has traditionally been prescribed as a remedy for intestinal disorders (Adetumbi and Lau 1983). Precious clinical studies demonstrated that *Allium sativum* was efficacious against *E. histolytica* by oral administration and by coloclysis with fresh liquid of it. Meanwhile, it played the role in preventing amoebiasis (Ren et al. 1951; Zhang 1953). Allicin [*S*-(2-propenyl) 2-propene-1-sulfinothioate], which is one of the active principles of freshly crushed *Allium sativum* homogenates, has been shown to have a variety of antimicrobial and antitumor effects. Lab researches indicated Allicin could inhibit strongly the growth of trophozoite of *E. histolytica* by inhibiting its cysteine proteinases and alcohol dehydrogenase which are the significant contributors to amebic virulence and the enzyme required for the survival of the parasite (Mirelman et al. 1987; Ankri et al. 1997). In addition, Allicin was carried out against Cryptosporidium successfully in infant cases, showing its killing effect on Cryptosporidium and improving immune function effectively. Dosing is usually 80 mg four times a day for under 1-year-old infants, 90 mg for 1–2-year-old children, 120 mg for over 3-years-old children, 7 days as a course (Ge et al. 1991; Zheng et al. 2000) (Fig. 2.3).

2.5 *Radix Sophorae Flavescentis*

Radix Sophorae Flavescentis, a classic heat-clearing and dampness-eliminating herb, has widely been used as antimicrobial, antiparasitic, and antitumor medicine in China. It contains some chemical compositions, such as alkaloid, ethanol extract, and flavonoid which have anti-Giardia activity with the same efficacy in comparison with metronidazole in vitro. However, the mechanism of killing of Giardia is

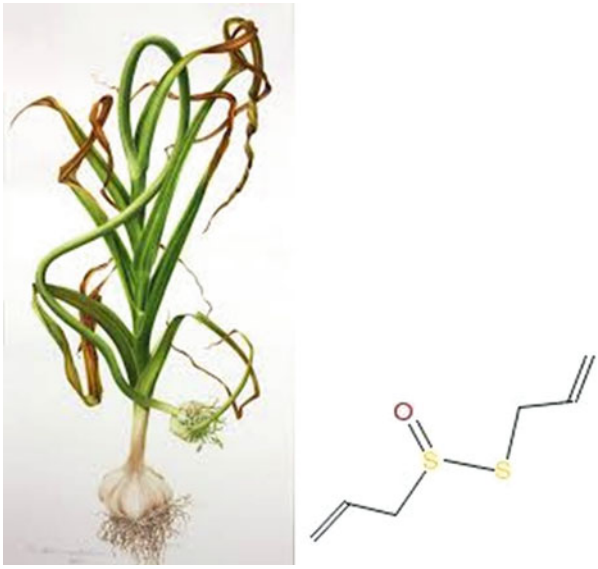

Fig. 2.3 *Allium sativum* (garlic) and formation of Allicin. *Allium sativum*, commonly known as garlic, is a species in the onion genus, *Allium*. It has been used for both culinary and medicinal purposes. It has anticholesterol, antibacterial, antiviral, antibiotics, anti-HIV, and antifungal activities. Allicin [*S*-(2-propenyl) 2-propene-1-sulfinothioate], which is one of the active principles of freshly crushed *Allium sativum* homogenates, has been shown to have a variety of antimicrobial and antitumor effects

not thoroughly studied. It is just found that morphological changes in the trophozoites are effected by *Radix Sophorae Flavescentis*, such as separation of adhesive disk from the cell body and appearance of pit in cell membrane (Lu et al. 1993; Wu et al. 1994). Clinical studies demonstrate that *Radix Sophorae Flavescentis* could treat giardiasis with 92 % successful rate (Chen et al. 1965) (Fig. 2.4).

Previous reports also showed that combination drug therapy of *Radix Sophorae Flavescentis* is more effective in treating Trichomonas intestinalis. For example, it could be combined with *Fructus cnidii*, *Dictamnus Dasycarpus Turcz*, and *Phellodendron Amurense Rupr* or combined with *Fructus cnidii*, *Cortex Fraxini*, *Radix Stemonae Sessilifoliae*, and Sijunzi decoction. The efficacy could reach as high as 95.65–100 % (Tang 1987; Qiu 2002). However, the therapeutic effect of *Radix Sophorae Flavescentis* alone on *T. hominis* is not confirmed.

Radix Sophorae Flavescentis combined with *Radix Astragali*, which is considered as an improving immune function Chinese medicine, was used to treat Cryptosporidiosis in Children. The two Chinese medicines may have repelling effect on the Cryptosporidium and be helpful to improve cellular immunity (Li et al. 1993). Matrine, a main active alkaloid extract from *Radix Sophorae Flavescentis*, has potential antineoplastic, antifibrotic, and anti-inflammatory activities. Lab studies clearly demonstrated that it could significantly reduce the number of Cryptosporidium oocysts and Cryptosporidium-infected cells in mouse model. Meanwhile, it could improve integrity of cell membranes and of the mucosal barrier, suggesting a potential in therapeutic applications against Cryptosporidium infection (Chen and Huang 2012).

Fig. 2.4 *Radix Sophorae Flavescentis* and *Sophora Flavescens Ait*. *Radix Sophorae Flavescentis* is the root of *Sophora flavescens Ait*. of family Leguminosae. It is extremely bitter in flavor and cold in nature. The cold property is capable of clearing heat. The bitter flavor could remove the dampness and purge fore downward. In addition, it could destroy intestinal parasites and induce diuresis

Kushe Decoction, consisting of *Radix Sophorae flavescentis* and *Fructus cnidii*, was successfully applied to treat human *Balantidium* infection on three cases and showed a clinical efficacy of 100 %, which was similar to metronidazole. The decoction could be used by oral and coloclysis administration (Su 1978).

2.6 *Fructus cnidii*

Fructus cnidii is the fruit of *Cnidium monnieri* (L.) Cusson. It is a classical heat-clearing and dampness-eliminating Chinese medicine with broad-spectrum activity against protozoa (*T. vaginalis*), helminthes (*Ascaris lumbricoides*), and some bacteria. It is widely used against *T. vaginalis* in China (Zhang et al. 1996) and usually combined with other Chinese medicines to treat Trichomonas intestinalis (Tang 1987; Qiu 2002). In vitro susceptibility testing demonstrates that *Fructus cnidii* has activity against trophozoite of *E. histolytica*, but the activity is lower than that of *Radix Pulsatillae* and *Artemisa annua* (Lan et al. 1996) (Fig. 2.5).

2.7 Other Traditional Chinese Medicines in Treatment for Intestinal Protozoan Infections

In addition to Chinese medicines discussed above, there are a few studies with other traditional Chinese herbs which also could treat intestinal protozoan infections. *Cortex Magnoliae Officinalis*, considered as a classical dampness-eliminating Chinese medicine and having antimicrobial effect, could treat intestinal amoebiasis alone successfully. Clinical trial had employed dosing two times daily (6 g/dose) for 4–9 days and shown effectiveness in 93.5 % in 46 adult patients (Sun 1960).

Fig. 2.5 *Fructus cnidii* and *Cnidium monnieri* (L.) Cuss. *Fructus cnidii* is the dried ripe fruit of *Cnidium monnieri* (L.) Cuss. of family Umbelliferae, with special aromatic smell, pungent, and cool taste. The best quality is *yellowish green* in color, pungent, and spicy smell under twisting and full-stacked. It could dry dampness, kill parasites, and relieve itching for external application, dispel cold, and dry dampness for internal application

Herba Calthae Membranaceae, an aquatic plant, is regarded as a delicious food and an antimicrobial Chinese medicine herb. Freshly chopped *Herba Calthae Membranaceae* is effective against *E. histolytica* (Wang and Liao 1962). It is given as 2–3 doses per day (20 g/dose) for 5 days, with obvious benefits regarding cost. *Radix et Rhizoma Thalictri*, having broad-spectrum antibacterial activity, has generally been considered as therapeutic Chinese medicine to treat dysentery. Depending on its shorter course and lower recurrence rate, it has been shown to be more potent against *E. histolytica* than emetine (Yunnan Kaiyuan Hospital 1959). *Folium Baeckeae* is a heat-clearing, detoxifying, and diuretic herb. The oil of *Folium Baeckeae*, distilled from the leaves of *Folium Baeckeae*, has been demonstrated 93.9 % effective in 115 patients infected with *T. hominis* (Beihai Town Hospital 1978) (Figs. 2.6 and 2.7).

In conclusion, Chinese medicine has been used for treating intestinal protozoan infections in human populations for a long time in China and other Asian countries. However, the anti-protozoa mechanism of most of Chinese medicines is not fully elucidated, which limits the widespread use in the world. At present, clinically resistant strains have appeared with longer repeat courses or higher doses of the original western medicine. It seems Chinese medicine may be the efficacious means of eradicating the intestinal protozoan infections to avoid potential parasite resistance.

Fig. 2.6 *Cortex Magnoliae Officinalis* and *Magnoliae Officinalis*. *Cortex Magnoliae Officinalis* is the dried bark of *Magnoliae Officinalis of family* Magnoliaceae, with pungent and bitter flavor. It is a deciduous tree growing to 20 m in height. The thick and *brown* bark is stripped from the stems, branches, and roots. It could eliminate dampness, relieve food stagnancy, clear away phlegm, and relieve asthma

Fig. 2.7 *Radix et Rhizoma Thalictri* and *Thalictrum foliolosum DC*. *Radix et Rhizoma Thalictri* is the dried root of *Thalictrum foliolosum DC* and *T. cultratum Wall* of family Ranunculaceae. It looks like horse tail. It could clear heat, dry dampness, and remove toxicity

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Chapter 3
Treatment Methods of Traditional Chinese Medicine for Toxoplasmosis

Fangli Lü

Abstract *Toxoplasma gondii* is an apicomplexan parasite that can cause toxoplasmosis, a widespread infection, with clinical spectrum ranging from a completely asymptomatic infection to multiorgan involvement. Novel medicines effective against both active and latent forms of the parasite are greatly needed. To review current evidence for antitoxoplasmosis by Traditional Chinese Medicine (TCM), our literature focused on five kinds of Chinese herbs, including *Torilis japonica*, *Zingiber officinale*, *Sophora flavescens*, *Astragalus membranaceus*, and *Scutellaria baicalensis* GEORGI in our present study, which are used in TCM for the treatment of allergic inflammatory diseases, infectious diseases such as bacterial infection, virus infection, helminthiasis, etc., and the mentioned TCM whose antiapicomplexan activity have been previously reported. In conclusion, TCM possess promising activity in vivo and in vitro against *T. gondii*, and the components of TCM deserve further study.

Keywords *Toxoplasma gondii* • *Torilis japonica* • *Zingiber officinale* • *Sophora flavescens* • *Astragalus membranaceus* • *Scutellaria baicalensis* GEORGI

3.1 Introduction

The apicomplexan parasite *Toxoplasma gondii*, the causative agent of toxoplasmosis, is an important pathogen for both human and animals. Although the combination of sulfadiazine and pyrimethamine has been used as therapy for this disease, these drugs can have serious side effects, especially in pregnancy. So far,

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no treatment is available to eradicate chronic infection in infected individuals. Therefore, new drugs are needed for treatment of *T. gondii* infections. To date, there has been a growing interest in natural herb extracts used in traditional medicine, which have been under consideration as the therapeutic reagents in the treatment of infectious diseases. *Torilis japonica* fruit has been used in therapeutic antimicrobial treatments in China, Japan, and Korea since ancient times, the ethanol extract of *T. japonica* fruit showed excellent antimicrobial activity against *Bacillus subtilis* ATCC 6633 spores and vegetative cells (Cho et al. 2008). Ginger, *Zingiber officinale* Roscoe, is a common spice and also a widely used medicinal plant in ancient China. Fresh, but not dried, ginger is effective against human respiratory syncytial virus (HRSV)-induced plaque formation on airway epithelium by blocking viral attachment and internalization (Chang et al. 2013). Ginger has antischistosomal activities against *Schistosoma mansoni* harbored in C57 mice (Mostafa et al. 2011). The extract of *Sophora flavescens* is effective against *Eimeria tenella* (Youn and Noh 2001). *Astragalus membranaceus*, a well-known traditional Chinese medicinal plant indigenous to China, the roots of *A. membranaceus*, Huang-qi, are amongst the most popular health-promoting herbs in China, their use dated back more than 2,000 years, and was recorded in Shen Nong's Materia Medica written in the Han dynasty (Mckenna et al. 2002). Studies from both pharmacology and clinical practices have demonstrated that *A. membranaceus* possesses immunostimulant properties and exhibits immunomodulating and immunorestorative effects both in vitro and in vivo (Cho and Leung 2007), which has been widely used as a tonic to enhance the body's natural defense mechanisms and to treat various immune disorders (Lee et al. 2003). *Scutellaria baicalensis* GEORGI is also a popular traditional Chinese herb widely used for the treatment of various inflammatory diseases in Asia. The roots of *S. baicalensis*, Huang-qin, have been widely employed for many centuries in the traditional Chinese herbal medicine as popular antibacterial, antiviral, and anti-inflammatory agents (Lin and Shieh 1996). Historically, *S. baicalensis* has been used to treat respiratory tract infection, diarrhea, jaundice, and hepatitis. Recent investigations showed it had broad anti-inflammatory activities (Hsieh et al. 2007).

It has been reported that the fraction of *T. japonica* can inhibit *T. gondii* proliferation (Youn et al. 2004); the methanolic extracts of both *Z. officinale* and *S. flavescens* Aiton have anti-*T. gondii* RH strain activity in vitro (Choi et al. 2008). In addition, the water extracts of *A. membranaceus* and *S. baicalensis* GEORGI demonstrated good efficacy in reducing replication of *T. gondii* in mouse models (Yang et al. 2010), and there are also remarkable in vitro activities of the two aqueous extracts against the tachyzoites of *T. gondii* (Yang et al. 2012). Therefore, Traditional Chinese Medicine (TCM) could provide a potent alternative therapy for *T. gondii* infection.

Fig. 3.1 Leaf of *Torilis japonica*

3.2 *Torilis japonica*

T. japonica, the Japanese hedge parsley, is a plant species in the genus *Torilis*. A substance known as torilin can be extracted from the plant and has been shown to be a potent inhibitor of 5-alpha reductase, the enzyme that converts testosterone to DHT (Park et al. 2003).

The roots and fruits of *T. japonica* are used medicinally in some provinces in China. The distribution of *T. japonica* is throughout China, except Heilongjiang, Nei Mongol, and Xinjiang (widespread as a ruderal in Asia and Europe), mixed forests in valleys, grassy places, especially in disturbed areas; 100–3,800 m (Figs. 3.1, 3.2, and 3.3).

To characterize the chemical component associated with antiprotozoal activity, specific fractions were isolated by HPLC and used for in vitro testing. These fractions were evaluated in vitro against *T. gondii*. Fractions of the herb extracts were serially diluted to final concentrations of 2.850–0.356 ng/ml in medium and added to wells containing replicating *T. gondii*. ^{3}H-uracil incorporation was used to determine parasite replication. In cultures infected with *T. gondii*, a fraction of *T. japonica* (*TJ*2) inhibited *T. gondii* proliferation by 99.2, 94.4, 88.6, and 27.0 % in the range from 2.850 to 0.356 ng/ml (Youn et al. 2004).

Fig. 3.2 Flowers of *Torilis japonica*

Fig. 3.3 Fruit of *Torilis japonica*

3.3 *Zingiber officinale*

Ginger or ginger root is the rhizome of the plant *Z. officinale*, consumed as a delicacy, medicine, or spice. It lends its name to its genus and family (Zingiberaceae). Other notable members of this plant family are turmeric, cardamom, and galangal. Ginger cultivation began in South Asia (Vyas et al. 2013) and has since spread to East Africa and the Caribbean. Ginger tea is a beverage in many countries, made from ginger root. In China, the tea is made by boiling peeled and sliced ginger to which brown sugar is often added, and it may be consumed both hot and cold (Figs. 3.4, 3.5, and 3.6).

3.3.1 Chemistry

The characteristic odor and flavor of ginger is caused by a mixture of zingerone, shogaols, and gingerols, volatile oils that compose 1–3 % of the weight of fresh ginger. In laboratory animals, the gingerols increase the motility of the

Fig. 3.4 *Zingiber officinale* (ginger) plant

Fig. 3.5 *Zingiber officinale* (ginger) flower

gastrointestinal tract and have analgesic, sedative, antipyretic, and antibacterial properties (O'Hara et al. 1998). Gingerols from ginger can kill ovarian cancer cells (Rhode et al. 2007; Kim et al. 2008; Choudhury et al. 2010). [6]-Gingerol (1-[4′-hydroxy-3′-methoxyphenyl]-5-hydroxy-3-decanone) is the major pungent principle of ginger, with numerous pharmacological properties including antioxidant, anti-inflammation, and antitumor-promoting properties. The chemopreventive potentials of [6]-gingerol present a promising future alternative to expensive and toxic chemotherapeutic agents (Oyagbemi et al. 2010). Ginger contains up to 3 % of a fragrant essential oil whose main constituents are sesquiterpenoids, with (−)-zingiberene as the main component. Smaller amounts of other sesquiterpenoids

Fig. 3.6 Fresh ginger rhizome or ginger root of *Zingiber officinale*

(β-sesquiphellandrene, bisabolene, and farnesene) and a small monoterpenoid fraction (β-phelladrene, cineol, and citral) have also been identified. The pungent taste of ginger is due to nonvolatile phenylpropanoid-derived compounds, particularly gingerols and shogaols, which form from gingerols when ginger is dried or cooked. Zingerone is also produced from gingerols during this process (Manjunatha et al. 2013).

3.3.2 Folk Medicine

The traditional medical form of ginger historically was called *Jamaica ginger*; it was classified as a stimulant and carminative and used frequently for dyspepsia and colic (Ghayur and Gilani 2005; Hu et al. 2011). It is also an integral part of many traditional medicines and has been extensively used in Chinese, Ayurvedic, Tibb-Unani, Sri Lankan, Arabic, and African traditional medicines, since antiquity, for many unrelated human ailments including common colds, fever, sore throats, vomiting, motion sickness, gastrointestinal complications, indigestion, constipation, arthritis, rheumatism, sprains, muscular aches, pains, cramps, hypertension, dementia, fever, infectious diseases, and helminthiasis (Ghayur and Gilani 2005). Significant anti-*T. gondii* RH strain activity was observed with *Z. officinale* methanolic extracts (EC50 = 0.18 mg/ml), which displayed a highly selective toxicity (selectivity = 10.1) (Choi et al. 2008).

3.4 *Sophora flavescens*

S. flavescens, a TCM, is a species of plant in the genus *Sophora* a genus of the Fabaceae family, which contains about 52 species, 19 varieties, and 7 forms that are widely distributed in Asia, Oceanica, and the Pacific islands. About 15 species in this genus have a long history of use in TCMs (Krishna et al. 2012). Ku shen (the root) (Zhu 1998) or kushenin (a pterocarpan, an isoflavonoid compound) is a typical traditional Chinese medicine that is found in this plant. It is commonly used for the treatment of viral hepatitis, enteritis, cancer, viral myocarditis, gastrointestinal hemorrhage (Yamazaki 2000), and skin diseases (such as colpitis, psoriasis, and eczema). Its roots contain quinolizidine alkaloids, including matrine and its oxide, matrine oxide (Yoshikawa et al. 1985) that interfere TNF-alpha and IL-6, suggesting that oxymatrine may inhibit the expression of the above proinflammatory cytokines (Zheng et al. 2005). Matrine also inhibited expression of Substance P and NK-1R in a human model of skin inflammation (Liu et al. 2007), as well as acting as an agonist at mu and kappa opioid receptors (Xiao et al. 1999; Higashiyama et al. 2005) (Figs. 3.7 and 3.8).

3.4.1 Chemistry

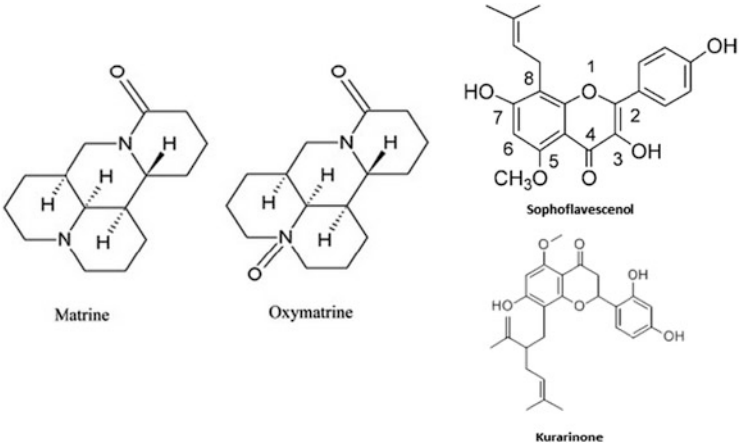
More than 20 alkaloids are found in *S. flavescens*. Oxymatrine, sophoridine, oxysophocarpine, and sophocarpine are the major alkaloids present (Liu et al. 2011). The prenylflavonoid 8-Prenylkaempferol can be found in *S. flavescens* (Chiou et al. 2011).

3.4.2 Composition

Specific components are: Matrine at 0.21–1.12 mg/g (seen as the main compound) and Oxymatrine at 1.94–8.77 mg/g, as well as the structurally related Sophoridine (0.13–0.62 mg/g) (Li and Wang 2004) and Oxysophorodine (Zhang et al. 2008), and there are also Kurarinol, as well as neokurarinol and norkurarinol (Kuroyanagi et al. 1999), etc.

Fig. 3.7 Plants of *Sophora flavescens* with flowers

Fig. 3.8 Ku shen (the root) of *Sophora flavescens*



3.4.3 Pharmacology

Matrine, one of the main components extracted from *S. flavescens*, possesses strong antitumor activities in vitro and in vivo. Inhibition of cell proliferation and induction of apoptosis are the likely mechanisms responsible for matrine's antitumor activities (Ma et al. 2008). It promotes hair growth, possibly due to 5-alpha-reductase inhibitory activity (Roh et al. 2002). Sophorae is anti-inflammatory and an antioxidant (Dong et al. 2010).

7,9,2′,4′-Tetrahydroxy-8-isopentenyl-5-methoxychalcone, a prenylated chalcone, can be effective against atopic dermatitis (Choi et al. 2010). *S. flavescens* may have the potential for treatment of chronic inflammatory disorders such as rheumatoid arthritis (Jin et al. 2010), and neuroprotective in focal cerebral ischemia (Park et al. 2009). A mix of *S. flavescens* and licorice (Glycyrrhiza glabra) gave better liver protective and antihepatocarcinogenic effects than licorice or *Sophora* alone. In addition, glycyrrhiza + sophora had a protective effect on immunosuppression, a strong nonspecific anti-inflammatory effect and reduced the incidence of sodium and water retention, protecting against pseudohypercorticosteroidism (Wan et al. 2009). Various compounds from the plant have exhibited significant antibacterial activities against the Gram-positive bacteria *Staphylococcus aureus*, *B. subtilis*, *S. epidermidis*, and *Propionibacterium acnes*. They also exhibited antiandrogen activities (Kuroyanagi et al. 1999). Sophoridine possesses antiviral activities against Coxsackievirus B3 (Zhang et al. 2006). Trifolirhizin, a pterocarpan flavonoid, was isolated from the roots of *S. flavescens* and showed potential anti-inflammatory and anticancer activities (Zhou et al. 2009). Sophora could possibly be used as a treatment for mast cell-derived allergic inflammatory diseases (Hong et al. 2009).

3.4.4 Antimicrobial Activity

Traditionally, plant extracts, such as roots of *S. flavescens*, have been used for microbial infections, which strongly reflecting that natural products are the major source of important antimicrobial agents. Prenylated flavonoids are chemical entities, which have an isoprenyl, a geranyl, a 1,1-dimethylallyl, and/or lavandulyl moiety as part of flavonoid backbone structure, which was isolated from *Sophora* species. Sophora flavanone G isolated from *S. exigua* showed strong antimicrobial activity against methicillin-resistant *S. aureus* with 3.13–6.25 μg/ml of MIC (Sato et al. 1995); Kurarinone, sophoraflavanone G, and kuraridin also showed strong antimicrobial activity against *S. aureus* and *Streptococus mutans* (Yamaki et al. 1990). Kuraridin, sophoraflavanone D, and sophoraisoflavanone A has the antimicrobial activity against fungi (*C. albicans* and *S. cerevisiae*), Gram-negative bacteria (*E. coli* and *S. typhimurium*), and Gram-positive bacteria (*S. epidermis* and *S. aureus*) (Sohn et al. 2004).

3.4.5 Antiviral Activity

Traditional Chinese medicinal herbs in the form of hot water extracts orally have been used as remedies against infectious viral diseases in China. Anagyrine, oxymatrine, and sophoranol isolated from *S. flavescens* have potent antiviral activity against respiratory syncytial virus (RSV) with IC50 values of 10.4 μg/ml and SI (CC50/IC50) values of 24.0, 12.0, and 24.0, respectively (Ma et al. 2002a, b). But it showed less significant activity against herpes simplex virus type 1 and type 2. Quinolizidine alkaloids from *S. alopecuroides* have very weak activity against HSV 1, coxsackie B2, measles, polio, semliki forest virus, and vesicular stomatitis virus (Zheng et al. 1997).

3.4.6 Antiprotozoal Activity

Inhibition of parasite replication by treatment using HPLC fractions of ethanol extracts of *S. flavescens* and *T. japonica* were compared to untreated controls. The alcoholic herb extracts of *S. flavescens* and *T. japonica* demonstrated good in vitro antiprotozoal activity against *T. gondii* and *Neospora caninum*. Most fractions prepared similar with the exception of TJ1 and SF5, which exhibited lower inhibitory rates almost concentrations as compared to the others against *T. gondii* (Table 3.1). All three HPLC fractions of *S. flavescens* and an HPLC fraction of *T. japonica* had some antiprotozoal activity against all dilution of *T. gondii* and *N. caninum* (Table 3.2) (Youn et al. 2003).

Table 3.1 Anti-*T. gondii* activity of HPLC fractions of *T. jiaponica* and *S. flavescens* extracts

	Reduction rate (%) of parasites (ng/ml of herb extracts)			
Group	2.850	1.425	0.713	0.356
Par + ve	40,432 ± 11,853			
Par –ve	1,112 ± 311			
TJ1	87.8	85.2	89.0	43.5
TJ2	99.2	94.4	88.6	27.2
SF1	99.6	95.2	88.8	60.6
SF2	96.9	88.7	89.1	48.1
SF3	88.5	89.9	92.3	68.2
SF4	94.9	95.4	83.6	52.9
SF5	82.1	88.0	78.2	62.7
Sulfadiazine	97.0	97.0	84.1	50.4

Par +ve: parasites and 80 % EtOH; Par −ve: host cells and solvent; TJ: *T. japonica*; SF: *S. flavescens*; ED cells: 50,000 cells/well; tachyzoiles of *T. gondii*: 100,000 cells/well (from Youn et al. 2003)

Table 3.2 Anti-*T. gondii* activity of a HPLC fractions of *T. japonica* (TJ2) and *S. flavescens* (SF1–SF3) extracts

HPLC fractions	Number of parasites	Par +ve	Reduction rate (%) of parasites (ng/ml of herb extract) 2.850	1.425	0.713	0.356
TJ2	100,000	70,297	95.4	79.5	33.6	18.3
	10,000	136,796	98.3	85.6	63.1	36.7
	1,000	28,321	95.8	90.7	54.8	39.7
SF1	100,000	72,887	87.2	49.4	21.0	20.2
	10,000	146,630	92.3	73.5	29.7	14.9
	1,000	42,835	96.2	84.3	75.6	36.7
SF2	100,000	71,055	87.3	55.8	35.5	15.7
	10,000	109,600	94.0	68.8	54.5	30.8
	1,000	28,442	91.6	76.8	31.1	16.3
SF3	100,000	69,779	91.4	37.3	14.1	3.7
	10,000	288,373	94.2	67.5	26.6	13.8
	1,000	100,556	95.4	81.5	55.3	−9.3

Par +ve: parasites and 80 % EtOH (from Youn et al. 2003)

To characterize the chemical component associated with antiprotozoal activity, specific fractions were isolated by HPLC and used for in vitro testing. These fractions were evaluated in vitro against *T. gondii*. Fractions of the herb extracts were serially diluted to final concentrations of 2.850–0.356 ng/ml in medium and added to wells containing replicating *T. gondii*. To determine the ability of each fraction to inhibit parasite proliferation, ^{3}H-uracil incorporation was used to determine parasite replication. In cultures infected with *T. gondii*, four fractions of *S. flavescens* (SF1–SF4) inhibited *T. gondii* proliferation by 99.6–60.6, 96.9–48.1, 92.3–68.2, and 95.4–52.9 % in the range from 2.850 to 0.356 ng/ml (Youn et al. 2004). *S. flavescens* Aiton methanolic extracts showed high anti-*T. gondii*

activity (EC50 = 0.20 mg/ml) and a high selective toxicity (4.6). This indicates that *Z. officinale* and *S. flavescens* Aiton extracts may be sources of new anti-*T. gondii* compounds (Choi et al. 2008).

3.5 *Astragalus membranaceus*

A. membranaceus (Latin): membranous milk-vetch root (English), huang qi (Chinese), ogi (Japanese), and hwanggi (Korean) is one of the important "Qi tonifying" adaptogenic herbs from the Chinese materia medica. The Chinese species *A. membranaceus* and the related *A. membranaceus var mongholicus* (synonym: *A. mongholicus*) are defined in the *Pharmacopoeia of the People's Republic of China* as Radix Astragali. It has been prescribed for centuries for general weakness, chronic illnesses, and to increase overall vitality. The genus *Astragalus* is a very large group of more than 2,000 species distributed worldwide. So far much of the pharmacological research on *Astragalus* is focused on its immune-stimulating polysaccharides and other active ingredients useful in treating immunodeficiency conditions. *Astragalus* has demonstrated a wide range of potential therapeutic applications in immunodeficiency syndromes, as an adjunct cancer therapy, and for its adaptogenic effect on the heart and kidneys. *Astragalus* root has been used to promote immune function and as a tonic to build stamina. Ancient Chinese texts record the use of *Astragalus* for tonifying the spleen, blood, and qi (No authors listed 2003) (Fig. 3.9).

3.5.1 Traditional Indications

A. membranaceus, a well-known traditional Chinese medicinal plant indigenous to China, the roots of *A. membranaceus*, Huang-qi, are amongst the most popular health-promoting herbs in China. Their use dates back more than 2,000 years and was recorded in Shen Nong's Materia Medica written during the Han dynasty (Mckenna et al. 2002). It is a widely used herbal product in China, other Asian countries, and some Western countries as an immune stimulant to be taken on first clinical signs of infection, and a tonic to enhance the body's natural defense mechanisms (Clement-Kruzel et al. 2008). In TCM, *Astragalus* is classified as an herb that tonifies the qi and is indicated for symptoms of spleen qi deficiency such as diarrhea, fatigue, and lack of appetite (Bensky and Gamble 1993). It also raises the yang qi of the spleen and stomach, thus addressing prolapses of organs such as the uterus, stomach, or anus. In this capacity, it can also address uterine bleeding. *Astragalus* tonifies the lung qi and is used in cases of frequent colds, spontaneous sweating, and shortness of breath (Bensky and Gamble 1993). Other traditional indications include wasting disorders, night sweats (Hong 1986), chronic ulcerations and sores (Bensky and Gamble 1993), numbness and paralysis of the

Fig. 3.9 Plants of *Astragalus membranaceus* with flowers and their sliced roots

limbs, and edema (from deficiency) (Bensky and Gamble 1993). Its properties are sweet and slightly warm. *Astragalus* is typically prescribed as a dried root, powdered, or in a decoction. Classically, it is prescribed in combination with other Chinese medicinal herbs, depending on the desired therapeutic effect and the specific TCM diagnosis. Studies from both pharmacology and clinical practices have demonstrated that *A. membranaceus* possesses immunostimulant properties and exhibits immunomodulating and immunorestorative effects both in vitro and in vivo (Cho and Leung 2007), which has been widely used as a tonic to enhance the body's natural defense mechanisms and to treat various immune disorders (Lee et al. 2003).

3.5.2 Active Constituents

The root of *Astragalus* species is known to be rich in polysaccharides, saponins, flavonoids, amino acids, and trace elements (Ma et al. 2002a, b). The main constituents of *A. membranaceus* include polysaccharides, saponins, flavonoids, amino acids, and trace elements (Ma et al. 2002a, b).

3.5.3 Polysaccharides

The polysaccharides found in *Astragalus* have received a great deal of attention, especially the polysaccharide fraction F3. They have been shown to play a role in immunomodulatory actions. Polysaccharides A, B, and C have been identified as glucans, and polysaccharide D as a heteropolysaccharide (McKenna et al. 2002).

3.5.4 Triterpenoid Saponins (Astragalosides)

Eleven major isoflavonoids and three major astragalosides in the xylem and bark of cultivated *Radix Astragali* (RA) from different cultivated regions of China were determined by HPLC. Constituents in either xylem or bark were divided into five groups according to their chemical structures Group 1, contained calycosin and related constituents; Group 2, contained ononin and related constituents; Group 3, contained (6aR,11aR)-3-hydroxy-9,10-dimethoxypterocarpan and related constituents; Group 4, contained (3R)-7,2′-dihydroxy-3′,4′-dimethoxyisoflavan and related constituents; and Group 5, contained astragalosides, compounds AG I, AG II, and AG IV (Song et al. 2008).

3.5.5 Flavonoids

Using HPLC–electrospray ionization mass spectrometry to analyze the flavonoids in the roots of *A. membranaceus* (*Am*) and *Astragalus mongholicus*, eight flavonoids were identified, including calycosin-7-O-beta-D-glucoside, calycosin-7-O-beta-D-glucoside-6′-O-malonate (2), ononin, (6aR,11aR)-3-hydroxy-9,10-dimethoxypterocarpan-3-O-beta-D-glucoside, calycosin, (3R)-7,2′-dihydroxy-3′,4′-dimethoxyisoflavan-7-O-beta-D-glucoside, formononetin-7-O-beta-D-glucoside-6′-O-malonate, and formononetin (Lin et al. 2000).

Accumulating evidence has indicated the importance of polysaccharide fractions of *A. membranaceus* in the modulation of immune functions both in human and experimental animals (Tan and Vanitha 2004; Cho and Leung 2007). Studies demonstrated that *A. membranaceus* exhibits immunomodulating and immunorestorative effect both in vitro and in vivo (Cho and Leung 2007) and have shown preliminary promise against the experimental coccidial infection when used in conjunction with vaccine (Guo et al. 2005). *A. membranaceus* has been used for more than 2,000 years in China to boost the body's general vitality and strengthening resistance to exogenous pathogens. The root of *Astragalus* species is known to be rich in polysaccharides, saponins, flavonoids, amino acids, and trace elements, which have been regarded as a potent tonic for increasing energy levels and stimulating the immune system (Wang et al. 2002; Block and Mead 2003) and has been shown to increase human lymphocyte proliferation, cytotoxic T-cell responses, and IgG production (Ma et al. 2002a, b). It has potential anti-inflammatory (Ryu et al. 2008) and anticancer effects (Auyeung et al. 2009). *Astragalus* polysaccharides and astragalosides showed strong promoting effects on the phagocytosis of *Mycobacterium tuberculosis* and on the secretion of interleukin (IL)-1 beta, IL-6, and tumor necrosis factor-alpha by activated macrophages from mice (Xu et al. 2007). The water extracts of *A. membranaceus* (*Am*E) demonstrated good efficacy in reducing replication of *T. gondii* in mouse models (Yang et al. 2010) and remarkable in vitro activities against the tachyzoites of

T. gondii (Yang et al. 2012). In addition, the protective immunity of ultraviolet (UV)-attenuated *T. gondii* can be markedly enhanced by aqueous extracts obtained from *Am*E coadministration, which suggests that the water extracts of *A. membranaceus* may have the potential to be used as effective vaccine adjuvant (Yang et al. 2010).

3.6 *Scutellaria baicalensis*

S. baicalensis GEORGI is another popular traditional Chinese herb, one of the 50 fundamental herbs used in TCM, widely used for the treatment of various inflammatory diseases in Asia. Historically, *S. baicalensis* has been used to treat high fever, respiratory tract infection, diarrhea, jaundice, and hepatitis. Several studies have reported that major compounds, such as baicalin and baicalein isolated from this medicinal herb showed antioxidative, anti-inflammatory effects (Huang et al. 2006; Zhang et al. 2011) (Fig. 3.10).

3.6.1 Chemistry

Several chemical compounds have been isolated from the root; among them, baicalein, baicalin, wogonin, and β-sitosterol are the major ones.

The roots of the traditional Chinese herbal medicine *S. baicalensis* GEORGI (*Sb*), Huang-qin, have been widely employed for many centuries as popular antibacterial, antiviral, and anti-inflammatory agents (Lin and Shieh 1996). The major constituents of *S. baicalensis* are flavonoids: baicalein, baicalin, wogonin, wogonoside, and skullcapflavone, which have been associated with various properties such as antioxidant, anti-inflammatory, antithrombotic, antibacterial, antiviral (Broncel 2007; Hsieh et al. 2007; Błach-Olszewska et al. 2008), and anticancer (Li-Weber 2009). The crude drug ingredients of *S. baicalensis* showed powerful antitrypanosomal (Yabu et al. 1998). In addition, the extracts of *S. baicalensis* (*Sb*) can improve the antimicrobial activity of some antibiotics (Yang et al. 2005). The water extracts of *S. baicalensis* GEORGI (*Sb*E) demonstrated good efficacy in reducing replication of *T. gondii* in mouse models (Yang et al. 2010) and remarkable in vitro activities against the tachyzoites of *T. gondii* (Yang et al. 2012). In addition, the protective immunity of ultraviolet (UV)-attenuated *T. gondii* can be markedly enhanced by aqueous extracts obtained from *Sb*E coadministration, which suggests that the water extracts of *S. baicalensis* GEORGI may have the potential to be used as effective vaccine adjuvant (Yang et al. 2010).

Fig. 3.10 Plants of *Scutellaria baicalensis* GEORGI with flowers and their sliced roots

3.7 Conclusions

TCM has been widely used to treat various diseases for thousands of years. Research efforts have focused on looking for natural products as alternative medicines with high effect, low cost, and good safety for the treatment of toxoplasmosis. This chapter summarizes the results of experimental studies on the treatment of *T. gondii* infection by TCM both in vivo and in vitro. Some water or methanolic extracts of TCMs, including *T. japonica*, *Z. officinale*, *S. flavescens*, *A. membranaceus*, and *S. baicalensis* GEORGI, seem to be effective on controlling *T. gondii* proliferation both in vivo and in vitro. However, all of these preliminary results remain to be further investigated. Therefore, an understanding of Chinese herbal medicines on toxoplasmosis is needed and the effective components of TCM need to be further studied.

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Chapter 4
Leishmania Infection in China

Jian-Ping Chen and Xiao-Xiao Chen

Abstract Visceral leishmaniasis was one of the most serious diseases in China before the 1960s in the last century. New VL has been successfully controlled in China, although some new cases were still found from certain regions in this country. The combination of mass chemotherapy of patients, proper treatment of the infected dogs (elimination and limited the number of the dogs), and sandfly control, particularly from the living environments is considered the key successful strategy for the control of leishmaniasis in China.

Keywords Visceral leishmaniasis • Infection • *Leishmania donovani* • Vector control • Dog treatment • China

4.1 Introduction

Leishmaniasis is a geographically widespread disease, which is caused by the genus *Leishmania* spp. (Herwaldt 1999; Desjeux 2004). In 1904, a German sailor who joined the Eight-Power Allied Forces in 1900 was reported to have died of visceral leishmaniasis (VL), as an autopsy revealed *Leishmania donovani* in his spleen, liver, and bone marrow. Around 1951, VL was one of the most important parasitic diseases, 530,000 cases in >650 counties of 16 provinces in the area north of the Yangtze River in China (Li et al. 2011; Guan 2009). After nationwide control measures (mass registration of patients for treatment, killing of infected dogs, and use of insecticides against sandflies), VL has been under controlled and essentially

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eliminated in the northeastern endemic regions since 1958; however, transmission of VL has not been interrupted in western China. Since the 1980s, the endemicity has been expanded with a corresponding increase in the number of reported incidences, resulting apparent in the recent outbreak of the disease that is due to rapid migration of large population in some regions of China (Guan et al. 2000). Although, VL seems to have reemerged with an increasing number of cases reported in the endemic regions, this disease is still well under control in China.

4.2 Epidemiology

New VL is mainly prevalent in six provinces in northwest China including Xinjiang, Gansu, Sichuan, Shaanxi, Shanxi, and Inner Mongolia, but particularly in Xinjiang, Gansu, and Sichuan. During the period of 1985–1988, 1,069 cases were reported in these six provinces, nearly over twice the number of cases ($n = 443$) identified between 1981 and 1984. Moreover, in the 1990s, about 2,629 cases were confirmed in 43 counties from these six provinces. A total of 2,450 VL cases were notified between 2005 and 2010, with a mean of 408 cases per year. Sixty-one counties were identified as endemic area with 2,224 (90–78 %) autochthonous cases, and the other 118 counties as nonendemic areas with 226 (9.22 %) imported cases. Among these cases, 97.71 % (2,394/2,450) were concentrated in Xinjiang (49.71 %, 1,218/2,450), Gansu (33.67 %, 825/2,450), and Sichuan (14.33 %, 351/2,450).

4.2.1 Geographical Distribution

Xinjiang. VL is widely distributed in the areas close to the Tarim Basin in the southern part, the Turpan Basin in the eastern part, and the Dzungarian Basin in the northern part of Xinjiang. Data from published studies indicated that in the last two decades, more than 50 % of the VL cases found in China were documented from Xinjiang (Zhang et al. 2005; Zheng et al. 2010). For instance, from 1996 to 2007, a total of 994 cases were found in Kashi City, 737 (74.14 %) of them were reported from Boshikeranmu Township (Guan et al. 2000). From 2005 to 2010, 1,208 cases were reported in Xinjiang with the anthroponotic type (AVL) and the desert subtype of zoonotic visceral leishmaniasis (DST-ZVL) accounting for 43.46 % (525/1,208) and 54.88 % (663/1,208), respectively, and 80 % (973/1,208) cases were reported from the Kashi area (Osman and Hou 2011). The VL outbreak occurred in Jiashi county, resulting in a 20-fold increase of the incidence rate of VL compared to the average annual incidence rate. In Kizilsu Autonomous Prefecture, no case was reported from 1983 to 1994, but 117 cases were recorded during the period of 1996–2007 with 92.73 % (102/110) from Artux City. With the growing economy and migration of the population, cases of leishmaniasis were reported from 34 cities

or counties in Xinjiang in 2010, and about 200 cases had been documented annually in this province in the past few years (Wang et al. 2012; Chai et al. 1997; Osman et al. 2007).

Sichuan and Gansu. VL is mainly found in mountainous areas of north Sichuan and south Gansu. In these two provinces, mountainous subtype zoonotic visceral leishmaniasis was very common. Prevalence of 59.43 % (63/106) and 26.9 % (7/26) in dogs was found, respectively, in the endemic regions of Sichuan in 2011 and Gansu in 1991 (Wang et al. 2011). From 2004 to 2008, 178 human VL cases were reported in the endemic regions of Sichuan in which 99.44 % (177/178) was reported in Jiuzhaigou, Heishui, and Maoxian Counties (Zhang et al. 2008a, b, c). More recently, from 2000 to 2010, 489 cases were reported in Sichuan comprising nearly 50 cases every year and thus indicates a significant reemergence trend in this province. In Gansu province, 1,751 cases were reported in 18 counties from 1985 to 1994 giving the greatest number of VL patients in China at that time (Wang et al. 2011, 2012). From 1996 to 2000, 266 cases were documented in the Longnan area, of them 81.95 % (218/266) cases were found in the Wudu and Wenxian Counties. This prevalence had declined markedly by 65 % compared to that during the period of 1987–1991. From January 2001 to June 2009, 933 cases were reported in Gansu province showing a decrease in number of cases since 1985–1994, but there is still the need for control and prevention of the disease in Gansu province (Wei et al. 1993; Zheng et al. 2010).

Shanxi, Shaanxi, and Inner Mongolia. In recent decades, only sporadic cases were documented in Shaanxi, Shanxi, and Inner Mongolia, with only 43, 18, and 64 cases being reported in each province, respectively, in the 1980s and 18, 15, and 24 cases in the 1990s. Currently, cases have only been found in Yan'an and Yulin in Shaanxi, but the current epidemiological situation is rarely studied. In Shanxi, only a few cases have been reported in Yangquan city and Wuxiang County of Changzhi City since 2001. While in Inner Mongolia, cases were mainly found in Ejina of Alax, but these were relatively rare with one to three cases each year (Guan 2009; Guan et al. 2000; Xiong 1992).

4.3 Types, Vectors, Hosts, and Transmissions of Visceral Leishmaniasis

The parasite is transmitted to man and other vertebrate hosts by the bite of blood-sucking female sandfly. In China, VL have been grouped into anthroponotic visceral leishmaniasis (AVL) caused by *L. donovani* and zoonotic visceral leishmaniasis (ZVL) with an animal host as the principle source of infection, and the later can be further grouped to mountainous subtype zoonotic visceral leishmaniasis (MST-ZVL) and desert subtype zoonotic visceral leishmaniasis (DST-ZVL) according to different geographical distributions and epidemiological

characteristics (Wang et al. 2011). They differ from each other either in vectors, hosts, or pathogens. So far as we known, only four phlebotomine sandfly species including *P. chinensis*, *P. wui*, *P. longiductus*, and *P. alexandri* were identified as natural transmission vectors of VL in China. VL patients are the sole infection sources in AVL endemic regions and in MST-ZVL endemics, dogs are considered the infection reservoirs. However, until present, in DST-ZVL endemic regions the infection sources are remained unclear even *Lepus yarkandensis* was questioned as wild animal reservoir hosts of *L. infantum*. Wang et al. reported that case numbers of AVL, MST-ZVL, and DST-ZVL respectively account for 22.75 % (506/2,224), 45.41 % (1,010/2,224), and 31.83 % (708/2,224) of the total in endemic regions during 2005–2010 (Wei et al. 1993; Zuo et al. 2007).

4.4 Population Distribution

The epidemiology of different types of VL in endemic regions of China is complex. Most of the VL cases are infants, preschoolers, and students. Male are greater than female in the proportion of total VL cases, especially in nonendemic regions (Xiong and Jin 2003; Wang and Gan 2011). Possibility of sandfly–human contacts based on the environment for living or working in and the ordinary lifestyle differences. VL cases are reported throughout the year and incidence peak is always observed which is in relation to sandfly season, but the overall distribution and trend in different months can be total distinct among different VL types (Wang et al. 2012).

4.5 Species of *Leishmania*

Although many studies have been done, the phylogenetic relationships among Chinese *Leishmania* isolates remain controversial (Yang et al. 2010; Cao et al. 2011). *L. donovani* and *L. infantum* are the main pathogens causing human leishmaniasis in China. *L. donovani* is the pathogen causing AVL while *L. infantum* is the pathogen causing both MST-ZVL and DST-ZVL. Moreover, CL (cutaneous leishmaniasis) which is prevalent in Karamay in Xinjiang is caused by *L. infantum* transmitted by *P. wui* (Wang et al. 2012). In addition, Guan et al. reported *Leishmania turanica* (*L. turanica*) isolated from the auricular tissues of naturally infected great gerbils (*Rhombomys opimus*) in Xinjiang for the first time in 1996. They also found that *L. turanica* is pathogenic to both monkeys and humans with *Phlebotomus mongolensis* and *Phlebotomus andrejevi* being the major vectors (Guan et al. 1995). Interestingly, *Leishmania gerbilli* was also isolated from auricular tissue of great gerbils, but this species was recognized as nonpathogenic to humans (Guan et al. 1994).

4.6 Clinical Aspects

VL also known as *kala azar*, is the most severe form of the disease, which has a mortality rate of almost 100 % when untreated. It is characterized by irregular bouts of fever, substantial weight loss, swelling of the spleen and liver, and anemia. The incubation period of VL is generally about 3 months; it may vary from 3 weeks to 18 months (Herwaldt 1999).

Fever is the first symptom to appear. Typically, it is nocturnal or remittent with a twice-daily temperature spikes. Sweating with chills but seldom rigor, accompanies the temperature spikes, less commonly, fever is continuous. Diarrhea and cough are frequently present.

Spleen is grossly enlarged by the third month, frequently occupying the entire left side of the abdomen. It is soft and nontender. Liver is enlarged but less conspicuous. It is soft with a smooth surface and a sharp edge. Lymphadenopathy is seen only in some cases of African kala-azar.

Normocytic and normochromic anemia is always present in kala-azar. Leucopenia, which white blood cell count as low as 1,000/mm^3, is a consistent feature. Hypergammaglobulinemia, peripheral edema, epistaxis, gingival bleeding, petechiae, and ecchymoses are the late manifestations. Immune complex glomerulonephritis and interstitial nephritis have also been described (Herwaldt 1999; Desjeux 2004).

4.7 Diagnosis

Early and accurate diagnosis followed by adequate treatment is a key step for control of VL. Diagnosis of VL is complex because its clinical features are shared by a host of commonly occurring diseases such as typhoid, tuberculosis, anemia, hepatitis, malaria and so on. The misdiagnosis rate was reported to be up to 30.2 % (26/86) in the initial diagnosis (Kang et al. 2009). Therefore, diagnosis of VL need a highly sensitive, specific, and be easily applied methods.

4.7.1 Etiological Diagnosis

Etiological method is the gold standard and the most reliable way for VL diagnosis because of its specificity. Specimens are usually acquired by aspiration from bone marrow, spleen, liver, or lymph node and examined microscopically. Among these assays, bone marrow aspiration is the most commonly used method for VL diagnosis with a sensitivity of 58.33–75 %. However, patients can suffer from pain and missed diagnosis is common if the parasite density is too low to be detected. Spleen

aspiration shows the highest sensitivity (90.6–99.3 %). Liver aspiration is safer but shows relatively lower sensitivities (76.9–95 %) (Hu et al. 1991).

4.7.2 Immunological Diagnosis

Dipstick (rK39). The dipstick test, a recombinant K39 antigen-based immunochromatographic strip test used to detect antileishmanial antibodies, has been widely used in China. Zuo et al. (2007) reported that during 1996 to September 2004, 1,204 VL patients of differing VL types in Xinjiang were tested using the rK39 dipstick and 1,169 (97.09 %) of them were positive, which indicated that the rK39 dipstick was highly sensitive, and quite suitable for field application in remote areas. However, some reports indicated that only 67.91 % (91/134) showed positive when 134 patients were tested using the rK39 dipstick and 67.47 % (56/83) were confirmed by the etiological method. The rK39 dipstick has also been used to diagnose canine leishmaniasis. Wang et al. (2011) reported that the positive rate detected by dipstick, ELISA, and PCR was 15.87 % (10/63), 61.9 % (39/63), and 87.3 % (55/63), respectively, suggesting it was less sensitive for asymptomatic infection in dogs.

ELISA. ELISA has been widely used to detect antibodies for VL diagnosis in China. Lei et al. (2010) tested 67 sera samples from VL patients using a double antigen sandwich ELISA (DAgS–ELISA) and the rK39 dipstick was also used to detect the specific antibody. The results indicated that a sensitivity of 68.7 % (46/67) was found by DAgS–ELISA and 67.2 % (45/67) was detected by rK39, respectively. Interestingly, ELISA-based methods used to diagnose infected dogs were showed a lower sensitivity. Wang et al. (2006) reported the evaluation of the potential of PCR, ELISA, and the rK39 dipstick for diagnosis on asymptomatic dogs and they found that the detection sensitivity of ELISA was only 22.22 % (16/72) with PCR showing a higher sensitivity up to 77.21 % (61/79).

McAB-AST. The monoclonal antibody–antigen spot test (McAB-AST) has been used for diagnosis of VL in China and is based on detection of specific circulating antigen with high sensitivity (90.57–100 %) and specificity (99.8–100 %). In 1994, Hu et al. (1994) reported that 100 % (15/15) sera of VL patients were positive and 94.12 % (16/17) of urine samples from VL patients were also positive indicating that urine could be a convenient potential sample for VL diagnosis. McAB-AST method was also used to diagnose infected dogs and gave high coincidence rate with bone marrow aspiration. According to Xu et al. (1993), positivity was found in 28 of 113 (24.8 %) dog sera and 30 of 113 dogs were confirmed positive by the etiological method. The coincidence rate was 93.6 %, indicating McAB-AST detection is a good alternative method for canine diagnosis.

Molecular Diagnosis. PCR-based assays targeting minicircle kinetoplast DNA (kDNA) for VL diagnosis showed various sensitivities with different primers. For

diagnosis, the 116 bp, 120 bp, and 297 bp fragments in the conserved region of the *Leishmania* minicircle kDNA are most often amplified. Hu et al. (2000) used PCR to amplify a specific kDNA fragment (297 bp) from *L. donovani* for VL diagnosis with a total positivity rate of 95.4 % (21/22). In addition, they found that the template derived from bone marrow, blood, and serum used in the study showed sensitivity of 90.91 % (20/22), 68.75 % (11/16), and 29.4 % (5/17), respectively. Wang et al. (2007) evaluated the status of asymptomatic VL infection in a human population in Wenxian County of Gansu using PCR, ELISA, and the rK39 dipstick. Results indicated that in the 269 blood samples, the positivity rate for PCR, ELISA, and the rK39 dipstick was 30.9 % (83), 24.2 % (65), and 0 % (0), respectively.

4.8 Control (Chemotherapy and Prevention)

4.8.1 Patient Treatment

In China, sodium stibogluconate (SSG) is typically used to treat VL patients using intravenous injection and has proved quite effective. Kang et al. reported that 86 cases were treated with SSG with a dosage at 120–150 mg/kg and 200–240 mg/kg of body weight for adults and children administered daily by intravenous injection for 6 days at the first course of treatment; 95.35 % (82/86) cases were cured in the first course (Kang et al. 2009). However, recently the efficacy of the conventional medication has been reduced due to drug resistance in these parasites. Amphotericin B is one of the most active antileishmanial for treatment of the drug-resistant strains, although it is costly and requires long-term administration (Yuan 2010).

4.8.2 Dog Control

Dogs are important reservoir hosts and sources of infection for VL in MST-ZVL areas. However, in those areas asymptomatic infected dogs are very common. Wang et al. (2006) reported that 100 of 106 (94.34 %) dogs examined showed no clinical signs. When large-scale dog elimination was carried out in Wenchuan, Lixian, and Maoxian County of Sichuan province during 1990–1993, case number apparently declined and only few cases have been reported since 1994 (Zhang et al. 2008a, b, c). These infected dogs are considered the main source of human VL in the endemic regions. This probably is one of the key reasons why VL reemerges after domestic dog elimination has been discontinued.

4.8.3 Sandfly Control

Deltamethrin-containing or cyfluthrin-containing pesticides have been widely used in China to control domestic or peridomestic sandflies and significant results were obtained. Zhang et al. (2008a, b, c) reported that 25 g/L high-performance cyfluthrin-containing emulsion was sprayed on the roof structure of indoor human dwellings and animal shelters in two villages in Xinjiang during 2005–2007 and no case were reported during 2006–2007 despite 49 cases being reported in the two villages previously 2003–2005. Dogs wearing pesticide-containing collars and bathed with pesticide were also recommended according to some studies (Xiong et al. 1995). In ZVL regions, insecticide-impregnated bed nets, window screens, and mosquito repellents were recommended as means to reduce human–vector contact (Jia et al. 1990).

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Chapter 5
Malaria in China

Ying Wang

Abstract As a global mosquito-borne disease, malaria is also one of the most important parasitic diseases in China. There is a long history of Chinese people battling with the malaria parasites. China is implementing a National Malaria Elimination Program. Now, a delightful progress has been made to control malaria after great efforts were involved. However, the prevalence of malaria is still serious in some places in China. And, Chinese government has to face many challenges to further control the infectious disease. Besides, clinical aspects, diagnosis methods, and control strategies were also discussed in this chapter.

Keywords Malaria • China • Epidemiology • Diagnosis • Control

5.1 Introduction

Malaria is a mosquito-borne infectious disease of humans and other animals. Up to date, five *Plasmodium* species were found being able to infect human, including *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, *and Plasmodium knowlesi*. It begins with a bite from an infected female *Anopheles* mosquito, which introduces the parasites (sporozoites) through saliva into the circulatory system. In the blood, the parasites travel to the liver to mature and reproduce. Malaria causes symptoms that typically include fever and headache, which in severe cases can progress to coma or death. The disease is widespread in tropical and subtropical regions such as Sub-Saharan Africa, Asia, and the Americas.

During the past decade, malaria incidence and mortality rates have been cut in all regions of the world, according to the report. In 2010, there were an estimated

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216 million cases of malaria in 106 endemic countries and territories in the world. An estimated 81 % of these cases and 91 % of deaths occurred in the WHO African Region. Globally, 86 % of the victims were children under 5 years of age. There were an estimated 655,000 malaria deaths in 2010, which is 36,000 lower than the year before. While the significant progress has been made to control the notorious disease during the past years, the mortality figures are still disconcertingly high (WHO 2011a–d).

Malaria is still one of the most important parasitic diseases in China (Pan et al. 2012).Chinese people have and are still being suffered seriously from malaria. There is a long history of Chinese people battling with the malaria parasites. Human malaria likely originated in Africa and has coevolved along with its hosts, mosquitoes, and nonhuman primates. The first evidence of malaria parasites was found in mosquitoes preserved in amber from the Paleogene period that are approximately 30 million years old (Poinar 2005). According to legend, the Chinese emperor Huang Di (Yellow Emperor, 2697–2590 BCE) ordered the compilation of a canon of internal medicine. The Chinese Huangdi Neijing (The Inner Canon of the Yellow Emperor) apparently refers to repeated paroxysmal fevers associated with enlarged spleens and a tendency to epidemic occurrence—the earliest written report of malaria (Cox 2002). For thousands of years, traditional herbal remedies have been used to treat malaria (Willcox and Bodeker 2004). Qinghao (*Artemisia annua*) was first recommended for acute intermittent fever episodes by Ge Hong as an effective medication in the fourth century Chinese manuscript *Zhou Hou Bei Ji Fang*, usually translated as "Emergency Prescriptions kept in one's Sleeve" (Wright et al. 2010). His recommendation was to soak fresh plants of the artemisia herb in cold water, wring it out, and ingest the expressed bitter juice in its raw state (Hsu 2006). Systematic screening of traditional Chinese medical herbs was carried out by a number of Chinese research teams consisting of hundreds scientists in the 1960s and 1970s (Tu 2011). Qinghaosu, later named artemisinin, was cold extracted in a neutral milieu (pH 7.0) from the dried leaves of *Artemisia annua* inspired by Ge Hong's recommendation (Wright et al. 2010).

5.2 Epidemiology

Malaria is widely distributed all over the world, with 1 billion people of the world exposed to malaria infection. In some places, the mortality is still high. So, malaria is a global problem. Malaria is also one of the major parasitic diseases in China. There were three main endemic areas in China on the history. South China is the highest endemic area, including Yunnan province and Hainan province. *Plasmodium falciparum* was prevalent in this area. And the main mosquito vectors were *Anopheles minimus* and *Anopheles dirus*. Central China and Yangtze Valley was another endemic area. *Plasmodium vivax* was predominant in this area. In central areas of China, the transmission of malaria is entirely due to *P. vivax* now. *Anopheles sinensis* is the main mosquito vector. Huang-Huai Region was also an

endemic area in history of China. Outbreaks of malaria occurred in 1860 and 1970. *Anopheles sinensis* was also the main mosquito vector.

In China, the reported trends in confirmed cases were predominantly downwards and the numbers of cases more than halved between 2000 and 2010 (WHO 2011a–d). China is implementing a National Malaria Elimination Program. According to the plan and aim of Ministry of Public Health of China, no local transmission will be found except the border areas in Yunnan Province before 2015. And, no local transmission will be found all over the country till 2017. Malaria elimination certification will be issued by WHO in 2020.

In China, 4,479 malaria cases were reported through the annual reporting system from 782 counties of 27 Provinces/Municipalities/Autonomous Regions (P/M/A) in 2011. Comparing with 7,855 cases in 2010, the total human malaria cases were reduced by 43 %. However, the number of malaria deaths increased to 33 from 19 in 2010. Although the annual incidence of the whole country was reduced to 0.0334/10,000, the incidence in some places is still very high. Among the 782 counties with reported cases, 2 counties had an incidence of more than 10/10,000 including Motuo (16.4660/10,000) in Tibet and Ruili (12.2352/10,000) in Yunnan, 10 counties in Yunnan and 1 county in Guizhou had an incidence between 1/10,000 and 10/10,000, and that of the others was below 1/10,000 (Xia et al. 2012).

The malaria areas and transmission got further confined in 2011. Out of the 4,479 malaria cases, a proportion of 29.3 % was reported as the indigenous cases who mainly distributed in the provinces of Anhui (40 %), Yunnan (25.8 %), Henan (12.6 %), Guizhou (10.4 %), and Hubei (6.1 %), a proportion of 66.4 % was reported as the abroad-imported cases who mainly distributed in Yunnan (36.5 %), Jiangsu (12 %), Henan (6.2 %), Sichuan (5.8 %), and Hunan (4.8 %). The remaining 4.3 % were domestically mobile cases (Xia et al. 2012).

In 2011, there were 81.7 % confirmed cases and 18.3 % clinically diagnosed cases. Among the confirmed cases, 56.7 % were *Plasmodium vivax* cases reported from 25 provinces, 40.2 % were *P. falciparum* cases reported from 22 provinces, 1.1 % were mixed infections of *P. vivax* and *P. falciparum* reported from 10 provinces, and the remaining 1.9 % were *P. malariae* or *P. ovale* cases reported from 14 provinces (Xia et al. 2012).

Among the provinces with malaria prevalence, Yunnan was still the major malaria area, which ranked No. 1 in China in terms of the case number, 1,522 cases. Among the cases, 301 were *P. falciparum* malaria accounting for 20.3 % of the national *Plasmodium falciparum* malaria figure. And, the 32 indigenous falciparum malaria cases were found only in Yunnan Province. Anhui province reported 644 malaria cases, though with a decrease of 65.5 % in comparison to that of 2010, ranked No. 2, accounting for 14.4 % of the country's malaria cases with an incidence of 0.1000/10,000. Differently, Hainan as previous key malaria province reported only nine malaria cases indicating 88.5 % decrease of the last year with an incidence of 0.0104/10,000, and ranked down to No. 25. In central China, another major malaria region, the case number decreased considerably. In Jiangsu, 374 cases were reported and decreased by 3.1 % with an incidence of 0.0508/10,000. In Henan, the number of reported cases was 358 and decreased by 59.9 %

with an incidence of 0.0387/10,000. In Hubei, 167 malaria cases were reported and decreased by 61.1 % with an incidence of 0.0277/10,000. Cases reported from other provinces occupied 31.4 % of the total. Respectively from Guizhou, Sichuan, Guangxi, Guangdong, Zhejiang, Hunan, and Shandong provinces, 100–200 malaria cases were reported; and the number of cases was less than 100 in the provinces of Fujian, Chongqing, Shanghai, Hebei, Beijing, Tianjin, Xinjiang, Ningxia, Jiangxi, Liaoning, Shaanxi, Shanxi, Gansu, and Tibet (Xia et al. 2012).

As all kinds of efforts are involved in control the notorious disease, a delightful progress has been made to decrease the prevalence of malaria. However, there are still many challenges for Chinese government to further control malaria. First, the grant for malaria control is insufficient as the Global Fund Malaria Project was stopped in this year. So, Chinese government has to deal with the new situation and increase the budget. Second, the transmission is still high in some places including the provinces of Anhui, Yunnan, Henan, Guizhou, Hubei, and Tibet. Third, imported malaria is threatening the health of Chinese people these years (Wang 2013; Liu et al. 2013). In 2011, imported malaria cases were dominant and widely distributed in the country including the four *Plasmodium* species, this could bring about high risks of reintroduction of malaria transmission in areas where malaria was effectively under control, particularly could cause more malaria deaths in the circumstances of increased imported *P. falciparum* malaria cases; therefore, provinces with more mobile population and imported cases need to pay higher attention. Although the prevalence of malaria was significantly decreased in China, the situation of the neighboring countries, including Myanmar, India, etc., is still worrying. Fourth, a number of clinically diagnosed but unconfirmed cases still exist in malaria elimination stage, which need to be addressed through strengthening local laboratory diagnosis capabilities (Xia et al. 2012). Fifth, the resistance of malaria parasites against antimalarial and mosquito vectors against insecticides is increasingly serious. Finally, the new malaria parasite species, *Plasmodium knowlesi*, is threatening Chinese people in some areas (Zheng et al. 2006; Zhu et al. 2006).

5.3 Clinical Aspects

The pathologic effects of malarial infection are the results of infected and uninfected red blood cells, the liberation of the metabolites of the parasite and the immunologic response of the host to the antigenic material and the formation of malarial pigments. An attack occurs because of the sudden liberation of merozoites, malarial pigment, and RBC debris into the blood stream.

In a typical case, paroxysms attacks of fever appear at regular intervals. Each paroxysm shows a succession of three stages: the cold stage lasting for 30 min to 1 h, the hot stage lasting for 1–4 h, and the sweating stage lasting for 1–2 h. In the atypical case, paroxysms of fever do not occur and the patient may have diarrhea, headache, abdominal pain, etc. In falciparum malaria, schizogony takes place in the

capillaries of the internal organs, the infected red cells tend to adhere to one another, and small vessels may become plugged. This may produce symptoms, which vary depending upon the organ involved. Examples are cerebral malaria, massive hemoglobinuria (blackwater fever) in which the urine becomes dark in color, because of acute hemolysis of RBC, acute respiratory distress syndrome, severe gastrointestinal symptoms, shock and renal failure, which may cause death. Cerebral malaria or severe anemia is quite common manifestation of severe malaria. Both parasite- and host-related factors contribute to the pathogenicity of the clinical manifestations of severe malaria. Cytoadherence of infected red blood cells to the vascular endothelium of different organs and rosetting are unique features of malaria parasites, which are likely to contribute to the vascular damage and the consequent excessive inflammatory/immune response of the host (Autino et al. 2012).

5.4 Diagnosis

5.4.1 Etiological Diagnosis

Laboratory diagnosis is confirmed by the demonstration of malarial parasites in the blood film under microscopic examination. In the thin film, it is easy to master because the red cell's morphology is maintained so that the malarial parasites are easy to recognize. But, several fields should be checked to find a parasite. On the contrary, it is easy to find parasites in only a few fields under the microscope in a thick film. But it is now easy to recognize the malarial parasites species as the red cells are completely lysed during the process of staining. So, the characteristic of malarial parasites are not easy to recognize, sometimes they may appear like flying birds, or eyes of birds.

The microscopic examination of blood smears is the most common malaria diagnosis method, and it has traditionally been considered as the gold standard test for malaria diagnosis (Moody 2002). Microscopy is an accurate tool, but the examination of blood films requires a well-trained staff, is time consuming, and is limited in sensitivity (Panel et al. 2010). Now, a variety of diagnostic methods for malaria are available such as antigen detection, fluorescence-based assays, and PCR.

5.4.2 Immunological Diagnosis

The use of antigen-based malaria rapid diagnostic tests (RDTs) was pioneered in the 1980s (Ling et al. 1986). Giemsa microscopy and RDTs represent the two diagnostics most likely to have the largest impact on malaria control today. Rapid

diagnostic tests for malaria do not require any special equipment and offer the potential to extend accurate malaria diagnosis to areas when microscopy services are not available (Bisoffi et al. 2009). Rapid diagnostic tests (RDTs) have become an essential tool in the contemporary malaria control and management programs in the world. There has been further progress in rolling out diagnostic testing, which is crucially important to separate malaria from other febrile illnesses. The number of rapid diagnostic tests delivered by manufacturers climbed from 45 million in 2008 to 88 million in 2010, and the testing rate in the public sector in the WHO African Region rose from 26 % in 2005 to 45 % in 2010 (WHO 2011a–d).

To evaluate the performance of two commonly used RDTs for malaria diagnosis in the China–Myanmar border area, a total 606 febrile patients in the China–Myanmar border were recruited and diagnosed for malaria infections by microscopy, two RDTs tests (Pf/Pan device and Pv/Pf device) and nested PCR. Compared to PCR, both microscopy and RDTs had lower sensitivities. RDTs had similar performance to microscopy for *P. falciparum* diagnosis but performed worse for *P. vivax* diagnosis. So, RDT products should be selected with higher sensitivity (and good specificity) for both *P. falciparum* and *P. vivax* diagnosis (Yan et al. 2013).

5.4.3 Molecular Diagnosis

PCR has been used to detect malaria parasites for decades. Nested PCR is useful for monitoring, identification, and diagnosis of malaria (Zhou et al. 2011). In nested PCR for malaria detection and *Plasmodium* species identification, universal and species-specific primers were designed according to the sequences of small subunit ribosomal RNA (SSU rRNA) gene of *Plasmodium* spp. (Shi et al. 2011). However, PCR is limitedly used in China, especially in field detection of malaria, because it requires special machine and reagents, well-trained staff and is expensive. PCR was usually used to verify the clinically diagnosed malaria cases and compare the sensitivity and specificity of microscopy and immunodiagnosis methods (Yin et al. 2013). Sometimes, PCR was used to identify the unusual *Plasmodium* species for example *Plasmodium knowlesi* (Zhu et al. 2006).

Loop-mediated isothermal amplification (LAMP) method is a simple, rapid, specific, and cost-effective nucleic acid amplification method (Notomi et al. 2000). It amplifies a nucleic acid using strand displacement DNA synthesis performed with Bst DNA polymerase at a constant temperature (ranging from 60 °C to 65 °C). LAMP provides high amplification efficiency. Turbidity caused by magnesium pyrophosphate, a by-product of the amplification reaction, is produced in proportion to the amount of amplified products. The presence of turbidity indicates the presence of amplicon. The white turbidity can be observed by the naked eye or measured with a turbidimeter.

Now, LAMP has been successfully developed for species-specific detection of human malaria parasites (Tao et al. 2011; Poon et al. 2006; Han et al. 2007). And, the sensitivity and specificity of LAMP for the analysis of blood samples of *P. vivax* patients, healthy subjects, and consecutive feverish patients in central China were compared with those of microscopy and nested PCR (Lu et al. 2012). The results showed that the sensitivity and specificity of LAMP and nested PCR were consistently high. So, LAMP is useful for diagnosis in field diagnoses instead of nested PCR. It is feasible to diagnose vivax malaria parasite in endemic areas of central China. However, in most malaria-endemic areas, which are often resource limited, current LAMP methods are not feasible for diagnosis due to difficulties in accurately interpreting results with problems of sensitive visualization of amplified products, and the risk of contamination resulting from the high quantity of amplified DNA produced. So, a visualized LAMP method was established by the addition of a microcrystalline wax-dye capsule containing the highly sensitive DNA fluorescence dye SYBR Green I to a normal LAMP reaction prior to the initiation of the reaction (Tao et al. 2011). This novel, cheap, and quick visualized LAMP method is feasible for malaria diagnosis in resource limited field settings in China.

As countries approach elimination, malaria diagnosis needs to change from diagnosing patients to actively detecting infections in all carriers including asymptomatic and low-parasite-load patients. However, few of the current diagnostic methods have both the throughput and the sensitivity required. A sandwich RNA hybridization assay was adopted to detect genus *Plasmodium* 18S rRNA directly from whole-blood samples from *Plasmodium falciparum* and *Plasmodium vivax* patients without RNA isolation. The assay's simple work flow, high throughput, and sensitivity make it suitable for active malaria screening (Cheng et al. 2013).

5.5 Control

5.5.1 Treatment of Patients and Elimination of Contagium

The treatment of falciparum malaria differs from *P. vivax* and *P. ovale* malaria and *P. malariae* malaria. Dangerous complications can occur in falciparum malaria and treatment must include both antiparasitic and supportive measures. In China, the standard treatment of *P. vivax* malaria is Chloroquine/primaquine treatment for 8 days. Differently, *P. falciparum* malaria is treated by artemisinin-based combination treatments (ACTs).

The biocrystallization inhibitor chloroquine was developed in Germany in the 1930s and was officially named Chloroquine in March 1946 (Krafts et al. 2012; Loeb et al. 1946). Chloroquine is an inhibitor of hemozoin production through biocrystallization and is one of the best antimicrobials ever developed. Quinine and chloroquine affect malarial parasites only at stages in their life cycle when the

parasites are forming hematin pigment (hemozoin) as a by-product of hemoglobin degradation. The drug target of chloroquine is host derived, which markedly delayed the emergence of resistance and it took *P. falciparum* 19 years to build resistance to chloroquine (Wellems and Plowe 2001). The first chloroquine-resistant strains of *Plasmodium falciparum* were detected around the Cambodia–Thailand border and in Colombia in the 1950s (Payne 1987). These resistant strains spread rapidly, resulting in a large increase in mortality from this disease, particularly in Africa during the 1990s (Snow et al. 2001). *Plasmodium vivax* is an important cause of malaria in many parts of Asia and South America. Although *P. vivax* is mostly still susceptible to chloroquine now, and resistance to the standard treatment (chloroquine) is now high in some places. According to the comparison of ACTs with alternative antimalarial regimens for treating acute uncomplicated *P. vivax* malaria, ACTs appear at least equivalent to chloroquine at effectively treating the blood stage *P. vivax* infection. Even where chloroquine remains effective this finding may allow for simplified protocols treating all forms of malaria with ACTs. Dihydroartemisinin–piperaquine may provide a longer period of posttreatment prophylaxis than artemether–lumefantrine or artesunate plus amodiaquine, which is likely to be a function of the long elimination half-life of piperaquine. This effect may be clinically important in high transmission settings whether primaquine is also given or not (Sinclair et al. 2011).

Artemisinin is a sesquiterpene lactone containing a peroxide group, which is believed to be essential for its antimalarial activity. Its derivatives, artesunate and artemether, have been used in clinics since 1987 for the treatment of drug-resistant and drug-sensitive malaria, in especially, cerebral malaria. These drugs are characterized by fast action, high efficacy, and good tolerance. They kill the asexual forms of *P. berghei* and *P. cynomolgi* and have transmission-blocking activity (Chotivanich et al. 2006). In 1985, Zhou Yiqing and his team combined artemether and lumefantrine into a single tablet, which was registered as a new medicine in China in 1992, and later it became known as "Coartem" (Weiyuan 2009). ACTs are now widely used to treat uncomplicated *P. falciparum* malaria, but access to ACTs is still limited in most malaria-endemic countries and only a minority of the patients who need artemisinin-based combination treatments actually receive them (Nosten and White 2007). Improved agricultural practices, selection of high-yielding hybrids, microbial production, and the development of synthetic peroxides will lower prices (White 2008; Hale et al. 2007). Worldwide, the volume of antimalarial medication delivered to the public sector has increased. In 2010, 181 million courses of ACTs were procured, up from 158 million in 2009, and just 11 million in 2005. ACTs are recommended as the first-line treatment for malaria caused by the most deadly malaria parasite, *Plasmodium falciparum* (WHO 2011a–d).

Drug resistance poses a growing problem in the treatment of malaria in the twenty first century, since resistance is now common against all classes of antimalarial drugs, with the exception of the artemisinins (White 2004). This situation has resulted in the treatment of resistant strains becoming increasingly dependent on this class of drugs. *Plasmodium falciparum* resistance to artemisinins, which was

confirmed on the Cambodia–Thailand border in 2009, has now also been identified at additional sites in Thailand, Myanmar, and Vietnam (Wongsrichanalai and Meshnick 2008; Vijaykadga et al. 2006). It raises the possibility that strains of malaria may have evolved that are untreatable with currently available drugs. Exposure of the parasite population to artemisinin monotherapies in subtherapeutic doses for over 30 years and the availability of substandard artemisinins have probably been the main driving force in the selection of the resistant phenotype in the region (Dondorp et al. 2010). WHO has recommended that all countries ban the marketing of oral artemisinin-based monotherapies, which have been one of the major factors fostering the emergence and spread of resistance. Despite continued international pressure, 25 countries still allow the marketing of oral artemisinin-based monotherapies and 28 pharmaceutical companies continue to market these products (down from 39 in 2010).

5.5.2 Control of Mosquito Vectors

Mosquito control is an efficient way to block the transmission of malaria disease. Reconstruction of environment is helpful to eradicate the breeding places of mosquitoes. Spray insecticides were commonly used to control mosquitoes during the past decades. Even now, it is still a main way to kill mosquitoes in China. However, the use of chemical insecticides is restricted because of the increasing resistance and environment pollution. So, people began to work on the new methods such as using biopesticides. Actually, mosquito nets, screen, or mosquito repellents are widely used now to protect the person from mosquito bites.

The World Health Organization (WHO) recommends Indoor Residual Spraying (IRS), the use of insecticide-treated mosquito nets (ITNs), and prompt treatment of confirmed cases with artemisinin-based combination therapies (ACTs) as the three primary means of malaria control. The use of ITNs to prevent malaria has been recommended as one of the most cost-effective means of combating the disease. In 2000, only 1.7 million (1.8 %) African children living in stable malaria-endemic conditions were protected by an ITN. That number increased to 20.3 million (18.5 %) African children using ITNs by 2007, leaving 89.6 million children unprotected (Noor et al. 2009). An increased percentage of African households (31 %) are estimated to own at least one ITN in 2008 (WHO 2011a–d). Most nets are impregnated with pyrethroids, a class of insecticides with particularly low toxicity. Long-lasting insecticidal nets and IRS have been the least expensive and most effective weapons in the fight against malaria by mosquito vectors control (N'Guessan et al. 2010). According to the new report, the number of bed nets delivered to malaria-endemic countries in Sub-Saharan Africa increased from 88.5 million in 2009 to 145 million in 2010. An estimated 50 % of households in Sub-Saharan Africa now have at least one bed net, and 96 % of persons with access to a net use it (WHO 2011a–d). The situation in China is different. Most Chinese do not like to use bed nets right now, as malaria is under control in most places in

China. Mosquito-repellent incense, repellent, and the physical methods like light trap are the preferred choices to avoid mosquito bites.

Pyrethrum is an economically important source of natural insecticide. Pyrethrins attack the nervous systems of all insects. A few minutes after application the insect cannot move or fly away and female mosquitoes are inhibited from biting (Duchon et al. 2009). Current malaria control efforts are heavily reliant on a single class of insecticides, the pyrethroids, which are the most commonly used compounds for indoor residual spraying, and the only insecticide class recommended—and currently used—on long-lasting insecticidal nets. In response to this emerging threat, WHO is currently working with a broad group of stakeholders to develop a Global Plan for Insecticide Resistance Management in malaria vectors.

Unfortunately, the problem of mosquito resistance to insecticides appears to be growing, although to date has not been linked to widespread failure of malaria vector control efforts. According to the World malaria report 2011, which includes data on insecticide resistance for the first time—45 countries around the world have identified resistance to at least one of the four classes of insecticides used for malaria vector control; 27 of these are in Sub-Saharan Africa. Resistance has been reported from all WHO Regions except the WHO European Region. India and malaria-endemic countries in Sub-Saharan Africa are of greatest concern due to widespread reports of resistance—in some areas to all classes of insecticides—combined with a high malaria burden.

5.5.3 Malaria Vaccines

A malaria vaccine would be of inestimable benefit to human beings. But until now an experimental malaria vaccine has been established only by some scientists in the laboratory and is not available for general use. It is not easy to establish malaria vaccine. The first successful continuous malaria culture was established in 1976 by William Trager and James B. Jensen, which facilitated research into the molecular biology of the parasite and the development of new drugs substantially. By using increasing volumes of culture medium, one can grow *P. falciparum* to higher parasitemia (above 10 %) (Trager and Jensen 1976; Schuster 2002). It provided the feasibility of developing malaria vaccines by continuous culture of human malaria parasites. Now, more and more scientists including some Chinese teams are working on developing an efficient malaria vaccine. Pan's lab is the most famous one. They have been focused on malaria vaccine research for more than 10 years (Li et al. 2010; Peng et al. 2010; Xue et al. 2010; Cao et al. 2009; Qian and Pan 2002; Pan et al. 2004; Zhang and Pan 2005; Zhang et al. 2007).

5.6 Research

The application of genomics to malaria research is now of central importance. With the sequencing of the three genomes of the malaria parasite *P. falciparum*, one of its vector *Anopheles gambiae*, and the human genome, the genetics of all three organisms in the malaria lifecycle can now be studied (Aultman et al. 2002). This breakthrough is expected to produce advances in the understanding of the interactions between the parasite and its human host as well as allowing the further study of the relationship between malaria parasites and mosquito vectors. It is likely that these will eventually lead to new therapeutic approaches (Winzeler 2008; Sahu et al. 2008). Another new application of genetic technology is the ability to produce genetically modified mosquitoes that are unable to transmit malaria, allowing biological control of malaria transmission (Ito et al. 2002). Now, research of malaria was focused on vaccine and immunology of human against malaria parasites, resistance of malaria parasites against antimalarial, resistance of mosquito vectors against insecticides, and the relationship between mosquito and Plasmodium including the innate immunity of anopheles mosquitoes.

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Chapter 6
Traditional Chinese Medicines Against Malaria

Wenyue Xu

Abstract Malaria, caused by the infection with the protozoa of the genus *Plasmodium*, is a major global public health problem. As an effective malaria vaccine is unavailable, prevention and controlling of malaria are mainly depended on the antimalarial drugs. Traditional medicine has contributed an important role to the combat against malaria in history, especially the discovery of the frontline antimalarial drug quinine from *Cinchona* and artemisinin from *Artemisia annua* L. However, *Plasmodium* strains resistant to first-line antimalarial drugs, such as chloroquine and sulfadoxine–pyrimethamine, have appeared and spread all over the world since 1960. Although artemisinin-based combination therapies were strongly recommended by WHO to prevent the development of *Plasmodium* strains resistant to artemisinin, the emergence of artemisinin-resistant malaria on the western border of Thailand was recently reported. So, the development of new antimalarial drugs is urgently needed. Traditional medicine is affordable and widely used in rural areas in Africa and elsewhere in developing countries and recommended as an alternative solution to malaria control. Furthermore, traditional medicine also provided the sources for discovery of new antimalarial drugs. Although hundreds of active products and their analogous, including alkaloid and nonalakloid, from the higher plants have been demonstrated to be potential efficacy against malaria parasites in vitro, in vivo assay, and clinical research of those candidates, active products and their analogous should be undertaken in the future. Beside the blood stage, their inhibitory role on the either liver or mosquito stage of malaria parasites should also be investigated, which is highly desirable in the context of the eradication of malaria all over the world.

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Keywords Malaria • Traditional medicine • China • Artemisinin • Quinine • Drug-resistance • Alkaloid • Nonalkaloid • Blood stage • Liver stage • Mosquito stage • In vitro • In vivo • Clinical research

6.1 Introduction

Malaria is caused by an infection with protozoa of the genus *Plasmodium*. It begins with the bite of an infected female *Anopheles*. After inoculation, sporozoites are then injected into the skin and rapidly invade the liver and followed with the development of liver stage for about 1 week. Merozoites released from the hepatocytes then invade the red blood cells and initiate the development of blood-stage, which is responsible for all clinical symptoms of malaria patients. If the malaria patients are bitten by the mosquito, the ingested gametocytes will further develop into sporozoite in the mosquito, which is known as the mosquito stage. It is reported that about 30–50 of *Anopheles* could transmit the malaria, and *Anopheles gambiae* is one of the major vectors in Africa. The *Plasmodium* pathogenic to humans includes *P. falciparum* (*Plasmodium falciparum*), *P. vivax* (*Plasmodium vivax*), *P. ovale* (*Plasmodium ovale*), *P. malariae* (*Plasmodium malariae*), and *P. knowlesi* (*Plasmodium knowlesi*). Of those, *P. falciparum* is the most virulent species causing the most severe cases of malaria, and malaria-associated complications and deaths. The infection of either *P. vivax* or *P. ovale* could lead to the relapse caused by their hypnozoites in the liver, which is a great barrier of the elimination of malaria worldwide.

Malaria is the most devastating disease worldwide in history. With the discovery of quinine and its analogous at the beginning of the nineteenth century, malaria was once controlled. However, as the parasites developed resistance to chloroquine in 1953, sulfadoxine–pyrimethamine in 1957, mefloquine in 1982, and the vectors became resistant to insecticides, the prevalence of malaria increased in the 1980s and 1990s. During the 1990s, child deaths caused by malaria increased by up to twofold in some parts of Sub-Saharan Africa. The disease also reemerged in several countries in Central Asia, Eastern Europe, and Southeast Asia. In 2006, there were 3.3 billion people at risk of contracting malaria, with 300–500 million new clinical cases, and nearly a million of deaths annually. With the launch of a new campaign of malaria eradication in 2007, the global adoption of highly effective artemisinin-based combination therapies (ACTs) as first-line treatments and the distribution of long-lasting insecticide-treated bednets have reduced the prevalence of malaria in many endemic regions. In 2010, there were about 225 million new cases occur annually, resulting in 781,000 deaths each year.

In China, there were about 30 million clinical cases of malaria annually, and malaria was prevalent almost in 75 % counties before 1949. With the widely use of ACTs and the economic development, Chinese malaria case has been reduced to only about 15,000 in 2009, and mainly distributed in Anhui, Yunnan, Henan, Guizhou, and Hubei provinces. Most cases were caused by the infection of

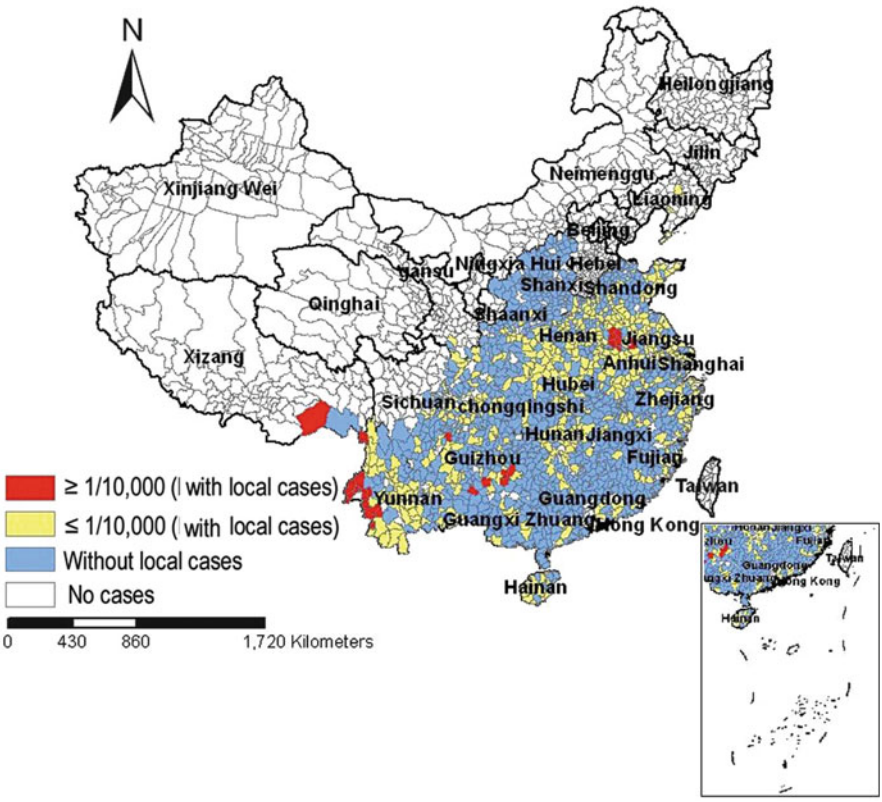

Fig. 6.1 Distribution of malaria in China in 2010. *Red* indicates the counties where local malaria cases were found in recent 3 years and the malaria rate ≥1/10,000; *yellow* indicates the counties where local malaria cases were found in recent 3 years, but the malaria rate ≤1/10,000; *blue* indicates the counties where local malaria cases were not found in recent 3 years; *white* indicates the counties without malaria cases in recent 3 years (Thanks to Jun Cao and Shaosen Zhou for providing the map)

P. vivax; only 7.1 % of cases were caused by the infection of *P. falciparum*, which was mainly found in Yunnan and Hainan provinces. In addition, the only 130 was caused by the infection of local *P. falciparum*, and most *P. falciparum* were introduced from abroad (Zhou et al. 2011) (Fig. 6.1). Nowadays, in China, the network of malaria control program has been established under the leadership of Department of Malaria, National Institute of Parasitic Disease, and Chinese Center for Disease Control and Prevention (CDC) (the address is No. 207 Rui Jin Er Road, Shanghai, China). Under this program, the local CDC continuously monitors the malaria and provides antimalarial drugs free to all the malaria patients, and hope the malaria in China could be eliminated by 2020.

As, *Plasmodium* strains resistant to artemisinin derivatives and to drug combination therapies have been reported recently (Phyo et al. 2012), and efforts to develop new antimalarial drugs continue being urgently needed now. Traditional

medicines are often trusted, affordable, and accessible in rural areas in Africa and elsewhere in developing countries (Graz et al. 2011) and have been playing a dominant role in the discovery of the major antimalarial drugs (quinine and artemisinin) (Kayser et al. 2003), so it is regarded as an alternative solution to malaria control, and sources for discovery of new antimalarial drugs (Graz et al. 2011).

6.2 The History of Chinese Traditional Medicine Against Malaria

Babylonian clay tablets from about 2600 BC were the earliest written records of antimalarial treatments. In Europe, the bark of the *Cinchona* tree was used for the treatment of malaria about three centuries ago. The treatment of malaria by the bark of the *Cinchona* tree was then introduced into South America after colonized by the Spanish and Portuguese. The active ingredient of the bark of the *Cinchona* tree against malaria was identified as quinine at the beginning of the nineteenth century. Quinine was then provided chemists with a template to successfully synthesize aminoquinoline-based antiplasmodial analogs, such as chloroquine, amodiaquine, primaquine, and mefloquine, and has saved more lives than any other drug known in history (Kayser et al. 2003).

China has a long history of using traditional herbal Qinghao to treat intermittent fevers. The first recording was dating from 168 BC, while the recipe against intermittent fevers in their acute phase was first written in *The Handbook of Prescriptions for Emergency Treatment* by Ge Hong during Jin Dynasty. In 1596, Li Shizen introduced Ge Hong's recipe in *Compendium of Materia Medica* (Klayman 1985).

The discovery of the active ingredient of Qinghao against malaria parasite was in 1972 (Hien and White 1993; Klayman 1985). In the 1960s, Chinese government decided to aid the North Vietnamese in their war with the USA, but chloroquine-resistant *P. falciparum* malaria was great threat to the soldiers. Under the instructions of Chairmen Mao and Premier Zhou, a nationwide program called project 523 to search for new antimalarial drugs was initiated on May 23, 1967 (Miller and Su 2011).

Under the leadership of the project 523 office, a malaria research group, led by Professor Youyou Tu from the Institute of Chinese Meteria Medica, Chinese Academy of Chinese Medical Sciences (CACAMS), mainly worked on the extraction and isolation of constituents with possible antimalarial activities from Chinese herbal materials. Youyou Tu and her colleagues investigated more than 2,000 recipes of Chinese traditional herbs and identified 640 hits that had possible antimalarial activities. Although more than 380 extracts obtained from ~200 Chinese herbs were evaluated against a mouse model of malaria, only the extract from an *Artemisia annua* L. showed to inhibit against parasite growth by 68 %. However,

Fig. 6.2 A Handbook of Prescriptions for Emergencies by Ge Hong (284–346 CE). (**a**) Ming dynasty version (1574 CE) of the handbook. (**b**) "A handful of Qinghao immersed with 2 L of water, wring out the juice and drink it all" is printed in the fifth line from the right

subsequent studies could only achieve 12–40 % inhibition. To explain the contradictory results, they carried out an intensive review of *The Handbook of Prescriptions for Emergency Treatment* by GeHong (Fig. 6.2). After reading the ancient Chinese medical description, "take one bunch of Qinghao, soak in 2 L of water, wring out the juice and drink it in its entirety", professor Tu reasoned that the heating involved in the conventional extract step might have destroyed the active components, and that extraction at low temperature might be necessary to preserve the antimalarial activity. Indeed, a much better extract was obtained after switching from ethanol to ether extraction at low temperature (Tu 2011).

However, the extract was still toxic. Professor Tu then removed an acidic portion from the extract, and the remaining neutral extract was found to be reduced toxicity and improved antimalarial activity. In October 1971, they found the neutral extract exhibited 100 % inhibition on the growth of both rodent malaria parasite *Plasmodium berghei*, and simian malaria parasite *Plasmodium cynomolgi*. The follow-up clinical trials with patients infected with both *Plasmodium vivax* and *Plasmodium falciparum* in Hainan province also produced encouraging results. This finding represented the breakthrough in the discovery of artemisinin. Professor Tu presented her findings at a 523 meeting held in Nanjing on March 8, 1972.

In 1972, Tu's team identified a colorless, crystalline substance with a molecular weight of 282 Da, a molecular formula of $C_{15}H_{22}O_5$, as the active component of the extract. With the assistance of a team at the Institute of Biophysics, Chinese Academy of Sciences, the structure of artemisinin was determined and showed that artemisinin is a sesquiterpene lactone with a required antimalarial

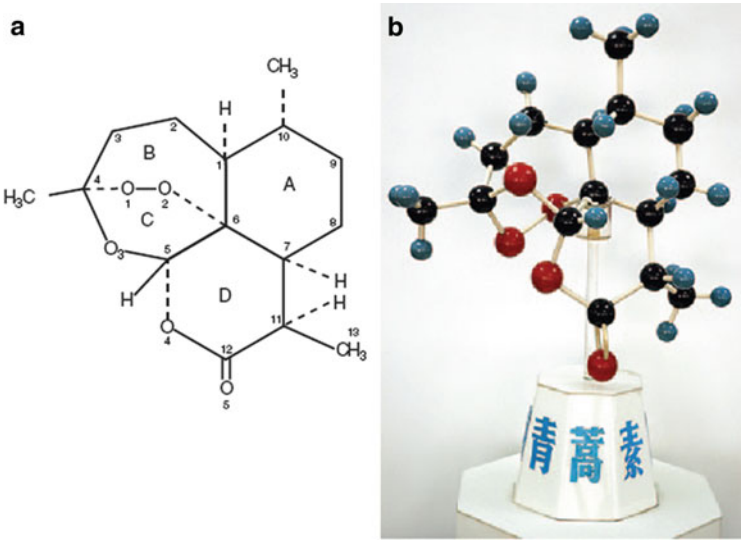

Fig. 6.3 Artemisinin. (**a**) Molecular structure of artemisinin. (**b**) A three-dimensional model of artemisinin. Carbon atoms are represented by *black balls*, hydrogen atoms are *blue*, and oxygen atoms are *red*. The Chinese characters underneath the model read Qinghaosu

endoperoxide (Fig. 6.3). To our exciting, professor Tu won the Lasker-DeBakey Clinical Research Award in 2011 for her discovery of artemisinin (Miller and Su 2011).

Two clinical studies headed by Professor Gouqiao Li compared artemisinin and mefloquine. These studies showed that Artemisinin works quickly within hours compared to mefloquine's slow parasite clearance, and suggested that artemisinin requires another drug in combination to prevent recurrence and development of resistance, due to its short half-life. Later, Nick White, who was working in Thailand as a professor at Oxford, began the study of artemisinin derivatives. He confirmed its rapid activity and the need for a partner drug to clear the parasitemia and became the primary proponent for the use of artemisinin derivatives in combination therapy, which is now the standard treatment worldwide. In 2010, he was honored by the Canadian Gairdner Award for this important work (Miller and Su 2011).

6.3 Established Drugs of Traditional Chinese Medicines Against Malaria

Quinine is a 4-methanolquinoline alkaloid isolated from the bark of *Cinchona* species (Rubiaceae) (Fig. 6.4) in 1820 by Pelletier and Caventou. It is considered as the prototype for the development of synthetic anti-malarial drugs belonging to

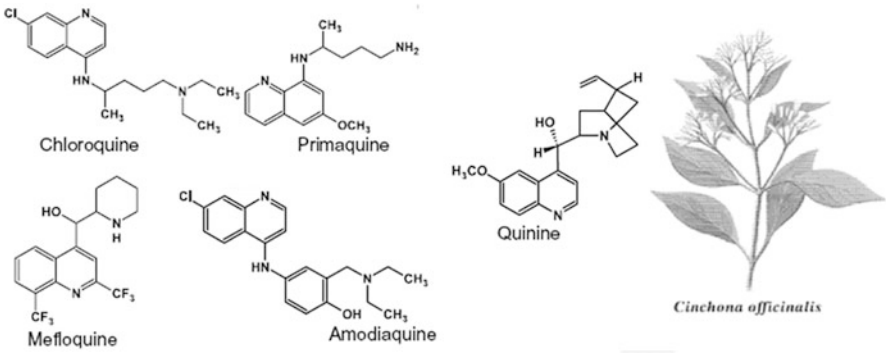

Fig. 6.4 Qinghao and the structure of Artemisinin and its derivates. (*Left*) Molecular structures of Quinine and its 4- and 8-aminoquinolines, such as Chloroquine, Primaquine, Mefloquine, and Amodiaquine. (*Right*) *Cinchona officinalis*

the classes of 4- and 8-aminoquinolines, such as chloroquine and primaquine (Batista et al. 2009). Before the advent of artemisinin (ART), chloroquine was the only effective and affordable drug used for the treatment of malaria. However, the appearance of drug-resistant *P. falciparum* strains since 1958, in particular to chloroquine, has made the treatment of malaria increasingly problematic in virtually all malarious regions of the world (Winter et al. 2006).

Quinine is a blood schizonticidal and weak gametocide against *P. vivax* and *P. ovale*. It is still very effective and widely used in the treatment of acute cases of severe *P. falciparum*, and especially useful in areas where there is known to be a high level of resistance to Chloroquine, Mefloquine, and sulfa drug combinations with pyrimethamine. Quinine is also used in pose-exposure treatment of individuals returning from an area where malaria is endemic. The mechanism of action of quinine and quinoline compounds in general has not been fully resolved, but widely held hypothesis involves the inhibition of parasite heme detoxification. As an alkaloid, it is accumulated in the food vacuoles of *Plasmodium* species, especially *P. falciparum*. It acts by inhibiting the hemozoin biocrystallization, thus facilitating an aggregation of cytotoxic heme. Similarly, the 4-aminoquinoline chloroquine also controls the conversion of toxic heme to hemozoin by inhibiting the biocrystallization of hemozoin, thus poisoning the parasite through excess levels of toxicity. Other potential mechanism through which it may act include interfering with the biosynthesis of parasitic nucleic acids, the formation of a chloroquine–heme or chloroquine–DNA complex (Willcox and Bodeker 2004).

The treatment regimen of Quinine is complex and is determined largely by the parasite's level of resistance and the reason for drug therapy (i.e., acute treatment or prophylaxis). The World Health Organization recommendation for Quinine is 8 mg/kg three times daily for 3 days (in areas where the level of adherence is questionable) and for 7 days (where parasites are sensitive to Quinine). In areas where there is an increased level of resistance to Quinine, 8 mg/kg three times daily for 7 days is

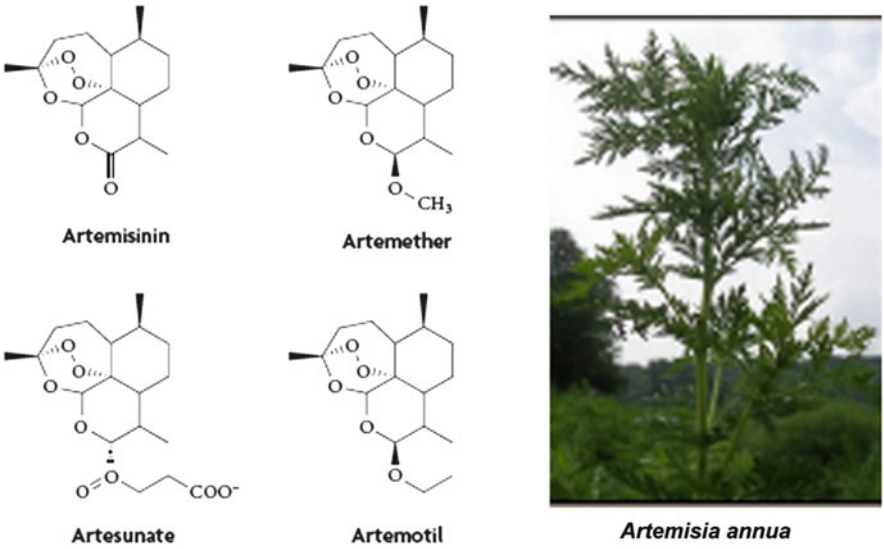

Fig. 6.5 The molecular structures of *Artemisinin* and its derivates and *Artemisia annua*. (*Left*) Molecular structures of Artemisinin, and its derivates Artemether, Artesunate, and Artemotil. (*Right*) *Artemisia annua* (Qinghao in Chinese)

recommended, combined with Doxycycline, Tetracycline, or Clindamycin. In China, the 4-aminoquinolines Chloroquine is combined with the 8-aminoquinolines Primaquine for the treatment of *P. vivax* and *P. oval*.

Artemisinin (*ART*) is also known as qinghaosu (or artemisinin; *su* means "basic element" in Chinese) in Chinese. It is an endoperoxide sesquiterpene lactone isolated in 1972 from the leaves of *Artemisia annua* (Fig. 6.5). *Artemisia annua* had been documented in 340 AD as a treatment for fevers in a medical book called "Zhou Hou Bei Ji Fang" by Ge Hong (Klayman 1985). Clinical trials in China in a large number of patients showed that artemisinin was highly effective in clearing parasitemia and reducing symptoms in patients with malaria, including some with chloroquine-resistant malaria and/or cerebral malaria (Jiang et al. 1982; Li et al. 1984).

However, the half-life of artemisinin is short, and recrudescence frequently occurs following treatment. In order to reduce the problem of recrudescence as well as improve its formulation properties, several derivates, such as dihydroartemisinin, artemether, arteether, and artesunate, have been made. In the body, sodium artesunate is very rapidly hydrolyzed to dihydroartemisinin, which is more active than artemisinin against *plasmodia*. In contrast, the metabolism of both artemether and arteether is slower than that of artemisinin, and there is less of problem with recrudescence (Cui and Su 2009).

In common with chloroquine, the mode of action of artemisinin depends on heme, but the mechanism is different and depends on the presence of the

endoperoxide group. Studies have shown that the reaction of the endoperoxide group with the iron in heme results in the production of highly reactive free radicals that are believed to kill the parasite by alkylating parasite molecules such as protein and DNA. Several "target" parasite molecules have been proposed including the parasite sarcoplasmic–endoplasmic reticulum ATPase, PfATP6 (Cui and Su 2009).

Artemisinin has a very different mode of action than conventional antimalarials, this makes it very useful in the treatment of resistant infections; however, in order to prevent the development of resistance to this drug, it is only recommended in combination with another nonartemisinin base therapy. For example, Artesunate–Amodiaquine combination potentially provides the alternative regime where levels of Chloroquine resistance are high. Dosage is recommended as 4 mg/kg of artesunate and 10 mg/kg of Amodiaquine per day for 3 days. Artesunate–Mefloquine combination has been used as an efficacious first-line treatment regimen in areas of Thailand for many years. The standard dose required is 4 mg/kg/day of artesunate plus 25 mg/kg of Mefloquine as a split dose of 15 mg/kg on day 2 and10 mg/kg on day 3. Artesunate–Sulfadoxine/Pyrimethamine is a well-tolerated combination, but the overall level of efficacy still depends on the level of resistance to Sulfadoxine and Pyrimethamine thus limiting its usage. It is recommended in doses of 4 mg/kg of artesunate per day for 3 days and a single dose of 25 mg/kg of sulfadoxine–pyrimethamine. In China, Artesunate–Amodiaquine, Dihydroartemisinin–Piperaquine, or Artesunate–Piperaquine combination is used for the treatment of *P. falciparum*, and Primaquine is added to either above combination and used for the treatment of *P. vivax*.

Lapachol (Kayser et al. 2003), a prenylated naphthoquinone from *Tabebuia* sp. (Fig. 6.6), provided the new pharmacophore that lead ultimately to the development of atovaquone, a synthetic 2-alkyl-3-hydroxy-1,4-naphthoquinone. Atovaquone is a highly lipophilic drug and an analog of ubiquinone (coenzyme Q), the lipid soluble mobile carrier that receives electrons from Complex I (NADH dehydrogenase or NADH-Q oxidoreductase) and Complex II (succinate dehydrogenase or succinate-Q oxidoreductase) in the electron transport chain and conveys them to Complex III (cytochrome bc1 or coenzyme Q-cytochrome coxidoreductase).

In *Plasmodium*, the site of action of atovaquone appears to be the cytochrome bc1 complex (Kessl et al. 2003). In essence, the drug acts by the irreversible and selective inhibition of mitochondrial electron transport and parallel processes such as ATP synthesis. In addition, obstruction of the electron transport chain ultimately inhibits de novo pyrimidine biosynthesis since dihydroorotate dehydrogenase, a key enzyme in pyrimidine biosynthesis, is unable to transfer electrons to ubiquinone.

Atovaquone is developed by The Wellcome Foundation Ltd. for treatment of multidrug-resistant falciparum malaria. Clinical study showed that using atovaquone alone resulted in approximately 67 % cure rate, but with a marked decrease in susceptibility to the recrudescent parasites. Although the combination of atovaquone and proguanil (Malarone) was effective in eliminating erythrocytic forms of *P. vivax*, but parasitemia recurred in most patients (Looareesuwan

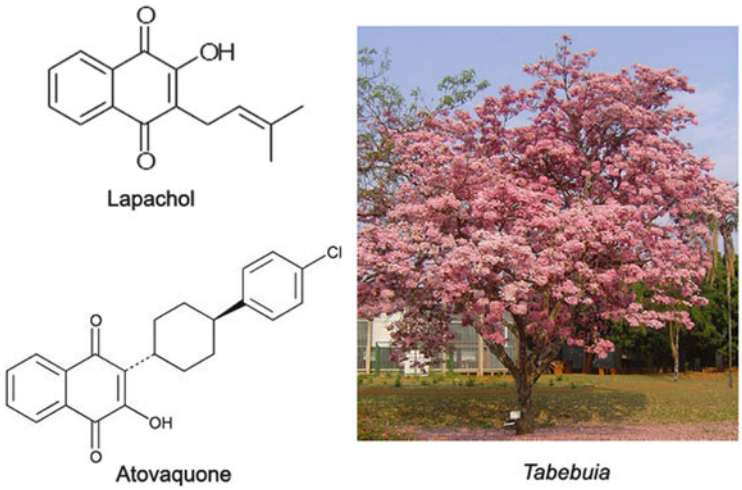

Fig. 6.6 The structure of Lapachol and Atovaquone and *Tabebuia*. (*Left*) Molecular structures of Lapachol and Atovaquone. (*Right*) *Tabebuia* in field

et al. 1996). Therefore, Atovaquone is not preferred to treatment of malaria in China or others countries.

6.4 Development of New Drugs of Traditional Chinese Medicines Against Malaria

The discovery of first-line antimalarial drugs of quinine and artesunate has saved millions of malaria patients' lives. However, the *Plasmodium falciparum* strains resistant to either chloroquine or sulfadoxine–pyrimethamine have appeared and spread since 1960, and strains resistant to artesunate combined treatment were also recently reported in the border of Thailand (Phyo et al. 2012). Malaria-endemic regions are now faced with an unprecedented situation in which only affordable treatment options are rapidly losing therapeutic efficacy. This is a pressing need for the discovery of new pharmacophores and novel molecules targets in the light of the drug-resistant crisis. Tradition medicine is not only considered as complementary or alternative solution in malaria control programs but also as template for the discovery of new leader antimalarial drugs.

In rural areas in Africa and elsewhere in developing countries, traditional medicines are often trusted, affordable, and accessible, as they are made from locally available plants or other elements. A large proportion of the population continues to rely on traditional medicine practitioners and local medicinal plants for primary health care, as a choice or when there is no access to other medicine. It is

estimated that traditional plants are used as a first-line treatment for malaria by 25 % of people in malaria-endemic countries (and up to 75 % in some areas), and over 1,277 plant species from 60 families are used in this way (Willcox and Bodeker 2004); therefore, traditional medicine was suggested as complementary or alternative solution in malaria control programs (Graz et al. 2011). A significant development was the setting up in 1999 of Research Initiative on Traditional Antimalarial Methods (RITAM) by Dr. G. Bodeker of the Global Initiative for Traditional System of Health (GIFTS of Health). RITAM is an international network of researchers and others interested in the use and evaluation of local herbal medicines used for the prevention and/or treatment of malaria and also local methods of vector control (http://giftsofhealth.org/ritam/).

Although many natural plants have a great potential efficacy for malaria treatment, their effects are usually unstable due to the diverse preparation approaches. Great efforts have been made to extract the active constituents from the antimalarial plants, which could lead to the production of newer drugs.

There are two major classes of natural products, alkaloids and nonalkaloids, which exhibit antimalarial activity. Over 100 alkaloids from higher plants have been reported to exhibit high activity against malaria parasites, and some of these were more potent than chloroquine. According to the structure, antimalarial alkaloids could be grouped into bisbenzylisoquinoline, naphthoquinones, indoloquinoline, furoquinoline, tetrahydroquinoline, indole alkaloids, benzofenantridine alkaloids, and acridone alkaloids (Oliveira et al. 2009). Nonalkaloidal natural products with antimalarial activity belong to the classes of terpenes, limonoids, flavonoids, chromones, xanthones, and anthraquinones (Batista et al. 2009). Alternatively, the antimalarial natural products could be divided into another two groups: one is the highly active compound, but with complex structure, so that no possibility of practical synthesis can be foreseen, and another one is with moderate to low activity but relative simple structures, and their synthesis and/or of their analogous could be undertaken. Plant species producing compounds of the first group are potential candidates for the development of phytomedicines, whereas the second group would represent templates to synthetic drugs.

However, the antiplasmodial activity of most natural products from many plant species only have been evaluated in vitro assay, few of them have been evaluated in vivo for their antimalarial activity and cytotoxicity (Batista et al. 2009; Bero et al. 2009; Oliveira et al. 2009), and fewer was moved to clinical research. In addition, many of antiplasmodial natural products were in low concentrations in plant species and usually as part of complex mixtures making their isolation and purification highly expensive. This hampers the development of phytomedicines, as the active compounds should be used as chemical–biological markers to guarantee the product quality.

Furthermore, the antimalarial activity of most natural products was evaluated against the blood stage, their effects on both liver and mosquito stages and mosquito should be stressed in the future, which could not only prevent the onset and relapse of malaria but also block the malaria transmission.

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Chapter 7
Liver Diseases (Abscesses, Tissue Cysts and Tumours) Caused by Parasites

Achim Harder and Heinz Mehlhorn

Abstract A variety of parasites—protozoans, cestodes, trematodes, nematodes or pentastomida—reside in the human liver or invade this organ and are responsible for inflammation resulting in hepatitis. Among the protozoans *Entamoeba histolytica* is of high importance. In the cyst fluid of the liver abscess, not only purulent components are visible but also some amoeba so-called magna forms. Among cestodes *Echinococcus granulosus* and *E. multilocularis* preferentially reside in the liver leading to often large cysts. Those of *E. multilocularis* grow by propagation of tiny omnipotent cells similar to typical cancer cells. Within the trematodes *Schistosoma* spp., *Clonorchis sinensis*, *Opisthorchis viverrini* and the juvenile stages of *Fasciola hepatica* have to be considered, since they may initiate true liver carcinoma. Among the large group of human nematodes, only *Capillaria hepatica*, migrating nematode larvae (such as larva 2) and the adults of *Ascaris lumbricoides*, larvae of *Strongyloides stercoralis*, hookworms, *Toxocara canis* and microfilariae of different filariae play a role in inducing liver inflammation, but never lead to cysts, abscesses or even carcinomas.

Keywords Liver diseases • Hepatitis • Abscesses • Tissue cysts • Tumour • Cancer • *Entamoeba histolytica* • Amoebom • Echinococcosis • *Echinococcus* species • Trematodes • *Schistosoma* species • *Opisthorchis* species • *Clonorchis sinensis* • *Fasciola hepatica* • Bilharziosis • Schistosomiasis • *Capillaria hepatica*

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7.1 Abscesses Caused by Protozoa

7.1.1 Entamoebiasis: Entamoeba histolytica

Taxonomy	
Subregnum	Protozoa
Phylum	Amoebozoa
Class	Lobosea
Order	Amoebida
Family	Entamoebidae

The life cycle of *Entamoeba histolytica* comprises two morphologically distinct developmental stages: the vegetative stages (minuta and magna forms), also known as trophozoites, and the cyst stage (Figs. 7.1, 7.2 and 7.3) (Mehlhorn 2008; Tannich and Burchard 2010; WHO 1997).

Trophozoites are uninucleate cells with a diameter of 20–40 μm containing a typical nucleus (spherical and central karyosome); they show pronounced gliding movements by formation of a single apical pseudopodium; cysts are spherical, quadrinucleate and have diameters from 10 to 20 μm. *Entamoeba histolytica* is usually transmitted by the faecal–oral route by ingestion of infectious cysts (contaminated food), but transmission through relevant homosexual practices is also possible. Multiplication of *Entamoeba histolytica* takes place only in the intestine. After ingestion of the typically quadrinucleate cysts, which unlike the trophozoites can remain infectious outside the body for months and are not destroyed by the acid contents of the stomach, the uninucleate trophozoites capable of multiplication develop in the small intestine. In the lower colon renewed encystment with two subsequent nuclear divisions takes place. An infected person can pass up to 500 million cysts per day along with the faeces. The incubation period of amoebiasis is very variable. The time between infection and the occurrence of clinical symptoms can vary between a few days and weeks. There are also cases, where no symptoms occur. The average incubation period for an amoebic liver abscess is up to 5 months.

Natural infections with *Entamoeba histolytica* are limited to man and a few Old World monkey species. People in tropical and subtropical countries in whom *Entamoeba histolytica* is endemic are usually associated with low socioeconomic status and poor hygienic conditions; travellers to such countries and male homosexuals are endangered. Worldwide half a billion of people are infected with *E. histolytica*, ten millions of these suffer from invasive amoebiasis and about 100,000 people are hit by complications, especially from liver abscesses.

The prevalence of *Entamoeba histolytica* depends primarily on the number of cyst passers and the local hygienic conditions. Under the hygienic conditions, which are standard in Western Europe, the infection usually does not or poorly spread.

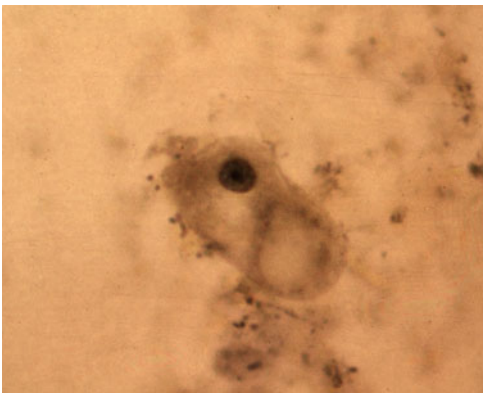

Fig. 7.1 Light micrograph (LM) of a minuta form of *Entamoeba histolytica*

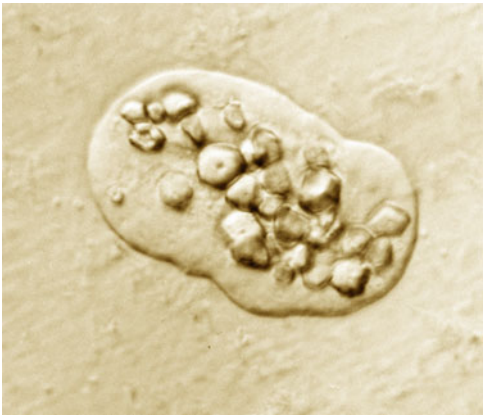

Fig. 7.2 Light micrograph of a magna form of *Entamoeba histolytica*

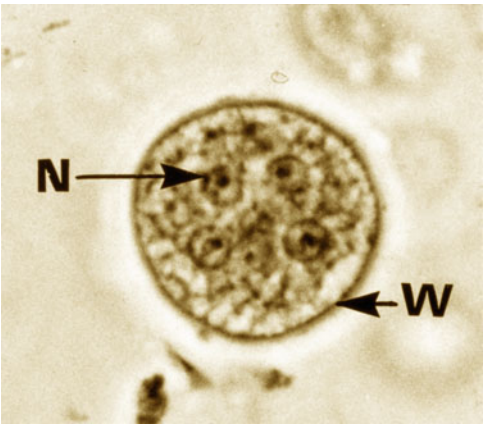

Fig. 7.3 LM of a cyst of *Entamoeba histolytica* (N = nucleus, W = wall)

The pathogenicity of *Entamoeba histolytica* is based primarily on the ability to destroy host tissue and cells. Surface receptors, cysteine proteinases and pore-forming peptides, called amoebopores, of the amoebae play a decisive role in this process. The virulence of individual amoeba isolates can vary depending on the level of expression of these molecules, which however, are essential to penetrate the intestinal wall and thus become a magna form. Antigen variability has not yet been shown for *Entamoeba histolytica*.

7.1.1.1 Intestinal Entamoebiasis

Intestinal amoebiasis is linked with enteritis or colitis of varying severity. Typically, there are ulcerative mucosal lesions with diarrhoeal stools containing blood and mucus (Fig. 7.4).

7.1.1.2 Extraintestinal Entamoebiasis

Magna forms of the amoebae can spread to other organs by haematogenous dissemination and lead to abscess formation. In more than 90 % of these cases, there are extraintestinal manifestations in the liver (Fig. 7.5). In rare cases, there can be complications such as peritonitis after perforation of an amoebic liver abscess or migration from the liver into the lung or pericardium. Infections with *Entamoeba histolytica* regularly induce a specific B- and T-cell response. Even in the case of asymptomatic intestinal infections, serum antibodies to *Entamoeba histolytica* can be detected in more than 80 % of cases.

7.1.1.3 Differential Diagnosis and Diagnosis

In the case of enteritis or colitis, other intestinal infectious pathogens should always be ruled out. In the case of marked mucosal lesions, an ulcerative colitis should be considered. An amoebic liver abscess should be differentiated from a bacterial abscess. Further differential diagnoses are echinococcosis or a necrotic tumour.

The diagnostic workup of invasive amoebiasis depends on the site of manifestation of the illness. (a) In the case of intestinal amoebiasis, rectoscopic or colonoscopic detection of appropriate mucosal lesions and direct detection of the pathogen are the primary measures. The latter is performed by examination of the stools (Figs. 7.1, 7.2 and 7.3) or by histological detection of amoebae in biopsy material. (b) In extraintestinal amoebiasis, imaging procedures such as ultrasound and computed tomography are used to detect appropriate organ manifestations and structural defects. At the same time, serological detection of specific antibodies to *Entamoeba histolytica* is an important, often pivotal diagnostic tool.

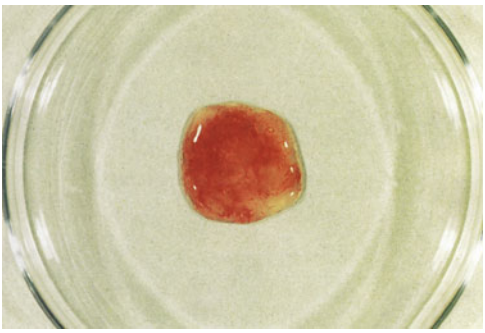

Fig. 7.4 Slimy, bloody diarrhoea (dysenteriae) as result of an infection with *Entamoeba histolytica*

Fig. 7.5 Macrophoto of a liver with abscesses due to *E. histolytica*

For differentiation from other apathogenic intestinal amoebae (e.g. *Entamoeba dispar*), particularly in the case of asymptomatic infections, *Entamoeba histolytica* should also be characterised immunologically or genetically.

Immunological characterisation using monoclonal antibodies to specific epitopes of *Entamoeba histolytica* is also available as well as the genetic characterisation on the basis of specific DNA sequences, e.g. within the rRNA gene.

The genome of *Entamoeba histolytica* is currently being sequenced. It probably comprises 20 megabases, which are distributed over 14 chromosomes.

7.1.1.4 Treatment

Infections with *Entamoeba histolytica* must always be treated (Chacín-Bonilla 2012). Asymptomatic intestinal *Entamoeba histolytica* infections should be treated with diloxanide furoate or paromomycin for 10 days. All *Entamoeba histolytica* infections with intestinal or extraintestinal clinical manifestations should be treated first with metronidazole (30 mg/kg body weight/day divided into three daily doses for 10 days) to kill amoebae, which have already penetrated the tissues, followed by treatment with diloxanide furoate or paromomycin to eliminate the remaining

intraluminal forms. Drug resistance to metronidazole has been suspected in individual cases but has not yet been scientifically confirmed.

7.2 Hepatitis Caused by Cestodes

7.2.1 *Hydatidosis, Echinococcosis, Echinococciasis:* Echinococcus granulosus

Taxonomy	
Subregnum	Animalia
Phylum	Platyhelminthes
Class	Cestoda
Order	Cyclophyllidea
Family	Taeniidae

7.2.1.1 Life Cycle and Transmission

The 2–5-mm-long adult tapeworm with its 2–5-mm-long strobila lives in the intestine of the dog and some other carnivores, is provided with 37–42-µm-long large and 29–34-µm-long small hooks at the rostellum, 3–4 (–6) proglottids and a uterus with lobed lateral sacculations (Figs. 7.6 and 7.7). The cystic larva (metacestode) takes the form of a fluid-filled, generally unilocular cyst (hydatid) that grows by expansion (Figs. 7.8, 7.9 and 7.10), it may contain daughter cysts in its interior and in extreme cases it attains a diameter of up to 30 cm. Protoscolices (Fig. 7.10) bud from blood capsules of the inner germinal layer and may break free. In the fluid, these together with calcareous corpuscles form what is known as hydatid sand (Mehlhorn 2008; Kern et al. 2000a, b; WHO 1996, 1997).

Transmission to humans (and other intermediate hosts) takes place by means of oral ingestion of the eggs of *E. granulosus* originating from the faeces of dogs or other definitive hosts (foxes and cats).

The adult forms of *E. granulosus* parasitising the intestine of the definitive host contain 500–1,000 taeniid eggs (32–39 × 24–26 µm) in their gravid proglottids (Fig. 7.11). After the terminal proglottids with eggs are excreted with the faeces, they are orally ingested by an intermediate host. Here occurs hatching in the intestinal tract of the larva (oncosphere) present in the egg membrane, penetration of the wall of the intestine. Oncospheres spread via the bloodstream to the liver, where the majority settle. Other oncospheres are transported further to the lungs and continue to develop there. Approximately 10–20 % passes through the lung and then travel via the systemic circulation to a wide range of organs. The oncosphere develops into a vesicular structure that grows by means of expansion to form a cyst

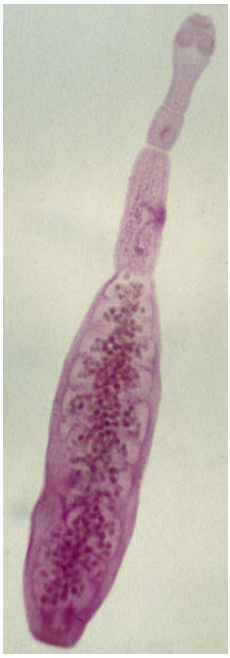

Fig. 7.6 Light micrograph of an adult *Echinococcus granulosus* worm

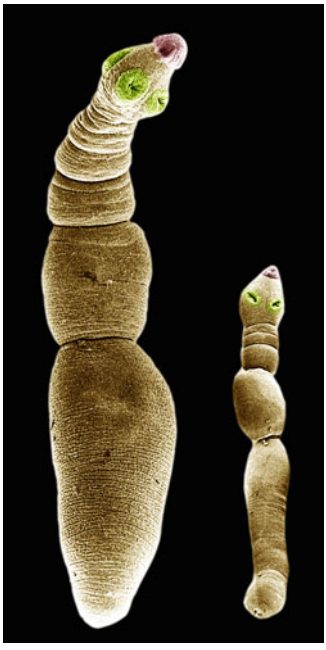

Fig. 7.7 Scanning electron micrograph of adults of *Echinococcus granulosus* and *E. multilocularis* (*right*) worms

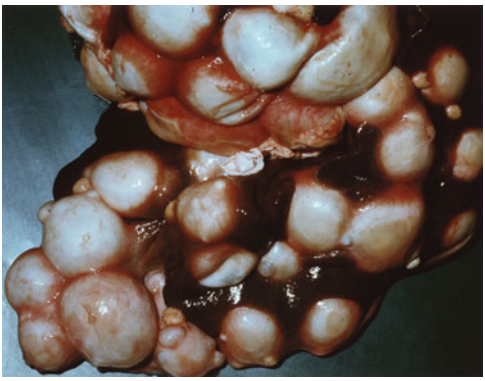

Fig. 7.8 Liver showing several cysts of *Echinococcus granulosus*

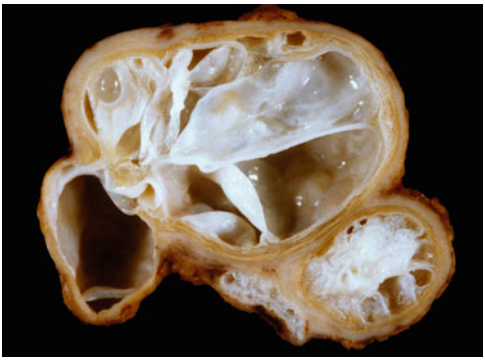

Fig. 7.9 Section through three differently advanced cysts of *Echinococcus granulosus*

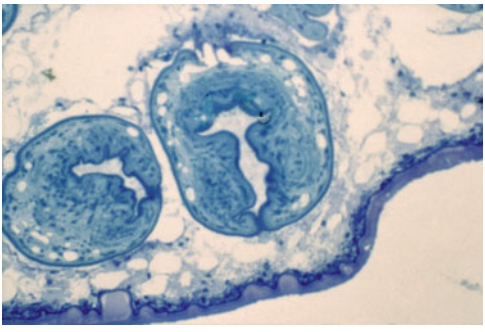

Fig. 7.10 Light micrographs of two protoscolices inside a hydatid

and inside contains protoscolices. The definitive host is infected by ingestion of such cysts. In the intestinal tract of the definitive host, each protoscolex matures into an adult tapeworm. The incubation period in humans to produce cysts is assumed to be over 5 years.

Echinococcosis is primarily a zoonosis with a broad spectrum of definitive and intermediate hosts. The most important definitive host is the domestic dog, although the wolf, coyote, dingo, hyena, jackal and some other carnivorous mammals may

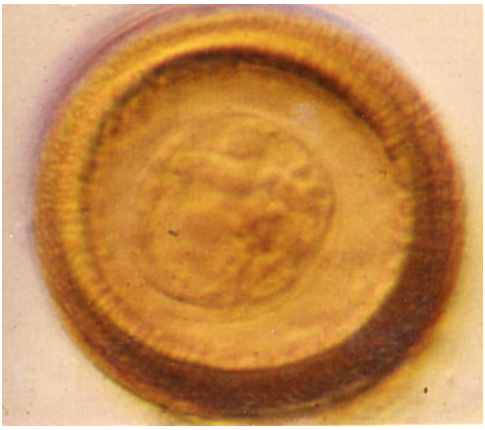

Fig. 7.11 Light micrograph of a typical *Echinococcus* egg with the included onchocerca larva

also harbour the adult form of *E. granulosus*. Mammals with an herbivorous or omnivorous diet serve as intermediate hosts. Those that play the most important role are domestic animals such as cattle, sheep, goats, pigs and horses. A number of wild animals (e.g. buffalos, bisons, antelopes, gazelles, elks and reindeer) may also host the metacestodes of this species. Humans constitute a dead-end intermediate host, as they generally do not allow transmission to the definitive final host.

Dog owners who feed their dogs scraps of raw meat are amongst those most at risk. In Germany, however, the risk is very low, as autochthonous infections are rare. Statutory meat inspection prevents meat containing cysts being sold as dog food.

As a zoonosis, the form of echinococcosis caused by *E. granulosus* is still distributed worldwide. It is particularly common, for example, in Mediterranean countries, broad regions of Central and South America and India. Dogs brought home from holiday destinations may therefore be infected. Cuddling of these animals may lead to human infections. *Echinococcus* eggs from the animals' coat or muzzle can also easily end up on carpets, chairs, etc., which may then again lead to infection of humans, too (Kern 2010; WHO-Arbeitsgruppe zur Echinokokkose 1997).

Cystic Echinococcosis

Infestation of humans with metacestodes of *E. granulosus* is known as cystic echinococcosis or *E. granulosus* infection. The slow growth of the parasite explains why the disease generally does not become manifest for several years post-infection. As a result of the expansive growth of the cyst, this form of echinococcosis presents as a space-occupying lesion predominantly in the liver or the lung (approx. 70–90 % of cases) (Figs. 7.8 and 7.9). With the gradual expansion, major vessels and structures may become compressed and occluded. The clinical presentation is extremely varied, depending on the number, size and site of the cysts. Cystic echinococcosis may give rise to febrile secondary infections, abscesses or

fistulisation. A major concern is cyst rupture, leading to secondary echinococcosis, as each of the released protoscolices may develop into a new cyst. The cysts usually grow slowly and are often detected by chance during routine investigations. There is a broad spectrum of symptoms, depending on the organ affected. Infestation of the liver, for example, causes a sensation of pressure in the upper abdomen and jaundice, whereas haemoptysis and expectoration of cyst contents with protoscolices may occur if the site of infestation is the lung. Clinical differentiation is often made as follows, depending on the organ involved.

Hepatic Cystic Echinococcosis

Well-demarcated cyst(s) usually appear with a characteristic honeycomb parenchymal pattern on an ultrasound scan (Fig. 7.9). On this basis, the cyst(s) is/are classified and staged in stages 1–6 using a classification proposed by a WHO working group (WHO 2001). If the cysts rupture into the abdominal cavity, this may lead to life-threatening peritonitis. Furthermore, seeding occurs in a process known as secondary echinococcosis. Compression of the efferent bile ducts leads to post-hepatic jaundice. If a cyst ruptures in the biliary tree, the cyst contents are discharged and, if small daughter cysts pass through the papilla, yellowish grape-like cysts will be detectable in the stools. Cholangitis often develops as a complication as a result of ascension of the pathogen.

Pulmonary Cystic Echinococcosis

Cyst(s) ranging in size from that of a tennis ball to that of a child's head is/are well demarcated and may be mistaken for circular foci of varying origin on plain X-rays. Clinical symptoms occur if a cyst latches onto the bronchial system. High fever, eosinophilia and pulmonary infiltrates are characteristic clinical manifestations. The clear cyst fluid with membrane fragments and hydatid sand may be expectorated via the bronchial tree. Rupture of the cysts and collapse of the endocysts results in the water-lily sign as a result of air permeating between ectocysts and endocysts. Bacterial secondary infections are a common complication.

Manifestation in Other Organs

Any organ is suitable as a nesting site for the development of the metacestode. The morphological correlate is the same for all the large parenchymatous organs, namely a well-demarcated mass with a host capsule. If the bones and muscles are involved, however, the presentation is polycystic. Differentiation from *E. multilocularis* in bone is therefore difficult.

7.2.1.2 Differential Diagnosis and Diagnosis

It is currently not known why the humoural immune response is so slow to respond to longstanding infection and symptom manifestation. High titre antibodies occur only in the event of cyst rupture and an allergic reaction. The cellular immune response in persistent infection is as yet poorly understood.

Different differential diagnoses need to be considered for the individual stages of the liver cysts visualisable with ultrasound. Benign liver cysts may be confused with stage 1 of hepatic cystic echinococcosis. Calcified haematomas, calcified abscesses and also liver metastases have to be considered as possible differential diagnoses for stages 5 and 6. With pulmonary cysts, there is characteristic bulging of the circular foci, in which homogeneously clear contents, possibly with daughter cysts, are detectable with diagnostic imaging techniques. A typical presenting feature in other parenchymatous organs is the well-demarcated host capsule. With infection of bone, the presentation is polycystic, making differential diagnosis difficult.

A blood count generally shows only moderate eosinophilia. Once a cyst has burst, excessive eosinophilia is detectable. Immunoglobulin E levels are generally raised. The following techniques are used for detection of cystic echinococcosis.

Ultrasound and computed tomography provide characteristic morphological images of the lesion. In the liver, the cysts usually show a bicycle-spoke structure. The WHO classification 2001 characterises the stage of the disease. The cysts of *E. granulosus* are well demarcated and are embedded in a host capsule known as the pericyst. The surrounding wall of the pericyst varies in thickness, depending on the organ involved. Calcifications are occasionally to be found. In the case of degenerated cysts, the tissue is compressed and the lesion may calcify completely.

A number of test methods have been developed for the detection of antibodies. The most commonly used are indirect immunofluorescence, indirect haemagglutination tests and ELISA. Serological tests generally allow differentiation of this disease from infestation with metacestodes of *E. multilocularis*. Negative results are nevertheless obtained in up to 50 % of cases. This applies in particular to pulmonary cystic echinococcosis.

Macroscopic features are the characteristic cysts and daughter cysts. Diagnostic fine needle biopsy is contraindicated. If the cyst is accidentally punctured, protoscolices and hooks are detectable in the clear cyst fluid. This confirms the diagnosis. PAS-positive laminated membranes are found in degenerated cysts.

A few DNA probes for *E. granulosus* have been described. They have not yet been fully validated on clinical specimens, however.

7.2.1.3 Treatment

In the past few years, there has been a considerable change in the strategies for the treatment of hepatic cystic echinococcosis. Whereas surgery was previously

considered the treatment of choice, this is nowadays reserved only for certain morphological cyst stages. When performing surgical procedures, it is recommended that the endocyst is opened up and the cyst wall is disinfected with 10–20 % NaCl solution. With more radical procedures, the cyst(s) is/are enucleated. The cyst(s) can then be resected together with the host capsule.

For easily accessible cysts of the liver and particular cyst stages, a technique known as Puncture–Aspiration–Instillation–Reaspiration (PAIR) can be used. This involves ultrasound-guided puncture of the cyst, removal and analysis of the cyst contents, injection of a scolicidal solution (70–90 % alcohol or 15–20 % NaCl solution) and reaspiration after a brief incubation period. This method has a promising success rate. The treatment should take place at specialist centres.

Drug therapy of cystic echinococcosis with the benzimidazole derivatives mebendazole or albendazole is a key cornerstone of the treatment plan (WHO 1996). Following curative surgery, treatment is recommended for 3 months. Drug therapy is essential before, during and after treatment with the PAIR method. Drug therapy alone has proved successful at various treatment centres, leading after a period of months or years to degeneration of the cyst and curing of the disease. Both anthelmintics are able to stop growth of the cysts' germinal layer and the protoscolices. A 3-month period of treatment with albendazole at a dosage of 10–15 mg/kg BW per day is recommended. Longer treatment periods may be necessary, depending on which organ is affected. The anthelmintic therapy is often commenced preoperatively. The logic behind this is that it will reduce the high pressure inside the cyst and so prevent the risk of seeding during surgery. This thinking is not backed up by studies, however, and the WHO recommendation that pre-treatment with anthelmintics should be confined to just a few days before surgery therefore still applies (WHO 1996). Treatment can be monitored on the basis of measured concentrations of the anthelmintics. The extent to which the dose of the drug correlates with effective control of growth of the parasite in vivo has not been proven, however. Resistance to anthelmintics has not been described to date.

7.2.2 *Alveolar Echinococcosis:* Echinococcus multilocularis

Synonym
Small fox tapeworm

Taxonomy

Subregnum	Animalia
Phylum	Platyhelminthes
Class	Cestoda
Order	Cyclophyllidea
Family	Taeniidae

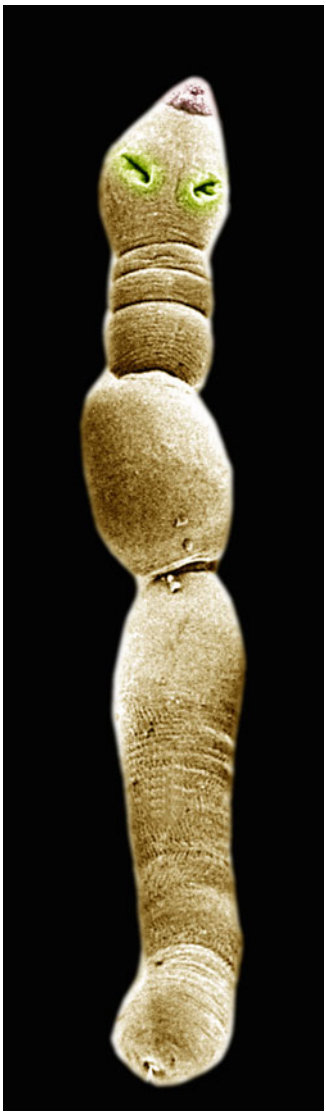

Fig. 7.12 Scanning electron micrograph of an adult worm of *Echinococcus multilocularis*

7.2.2.1 Life Cycle and Transmission

The 1.2–3.7-mm-long tapeworm is mainly found in the intestine of the red fox (*Vulpes vulpes*) and dogs amongst some other carnivores, with approximately 31-µm-long large and approximately 27-µm-long small hooks on the rostellum, 4–5 (2–6) proglottids and a sacciform uterus (Figs. 7.12 and 7.13). The larva (metacestode) of the alveolar type (Fig. 7.14) takes the form of a structure made up of multiple small vesicles that grows by infiltration by means of exogenous

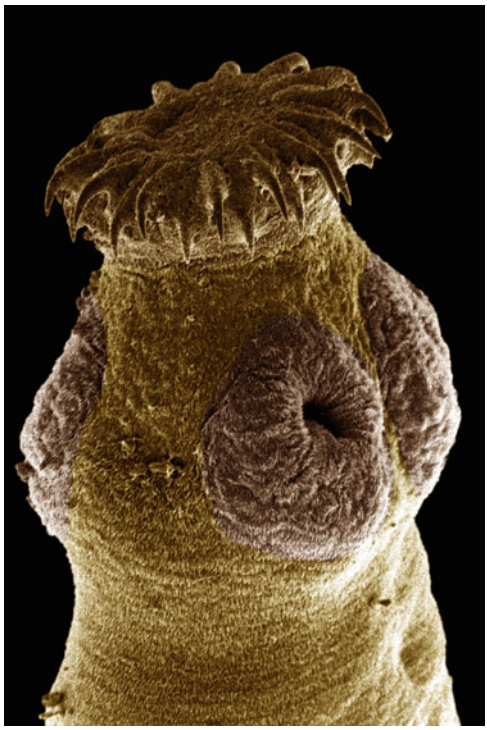

Fig. 7.13 Scanning electron micrograph of the scolex of an adult *Echinococcus multilocularis*

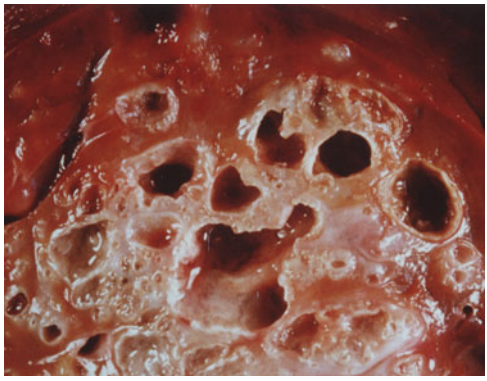

Fig. 7.14 Human liver with numerous hollows due to alveolar caverns of the *Echinococcus multilocularis* caverns

budding. Individual vesicles are mere millimetres in size, with a gelatinous matrix inside and protoscolices formed from the germinal layer (Kern et al. 2000c, d; WHO 1997, 2001).

Transmission to humans (amongst other intermediate hosts) takes place by oral ingestion of the eggs of *E. multilocularis* originating from the faeces of red foxes or other definitive hosts (mainly dogs). Contact to egg-contaminated hair of final hosts and consumption of wild berries (blueberries, strawberries, etc.) contaminated with

fox faeces or inhalation of helminth eggs swirled up during work in fields are thought to be responsible, too.

The adult form of *E. multilocularis* is a parasite in the small intestine of the definitive host. The number of eggs formed in the gravid proglottids depends on the host species and the age of the infection. In young worms, it is 200–500 per segment but later declines dramatically. Excretion of taeniid eggs (32–39 × 24–26 μm) within the proglottids with the faeces of the definitive final host. After oral ingestion by an intermediate host, the larva (oncosphere) present in the egg membrane in the intestinal tract hatch, penetrate the intestinal wall. Oncospheres spread via the bloodstream to the liver, lodge in the liver and develop into a multivesicular metacestode (permanently growing by means of exogenous budding) via omnipotent cells, which may also be easily transferred to other organs. Protoscolices are formed internally. Infection of the definitive host occurs as a result of consumption of the intermediate host, only in the definite host parasites mature into adult worms. The incubation period in humans is assumed to be 10–15 years.

The parasitosis caused by *E. multilocularis* is primarily a zoonosis, though with a distinctly narrower host spectrum than is the case with *E. granulosus* infection. The red fox (*Vulpes vulpes*) is the principal definitive host in Central Europe with infestation rates for *E. multilocularis* of way over 50 % in the main endemic areas. The Arctic fox (*Alopex lagopus*) plays a key role in Arctic regions. In addition, other canines (domestic dog, wolf, raccoon dog, etc.) also serve as definitive hosts. Humans constitute a dead-end intermediate host. The spectrum of natural intermediate hosts includes in particular species of the rodent family Cricetidae, with the field mouse (*Microtus arvalis*) as the most important species in Central Europe. Other *Microtus* species, not only bank voles, muskrats, lemmings but also house mice, brown rats and other rodents may harbour the metacestodes of *E. multilocularis*.

Anyone who works in the farming and forestry industries in endemic areas should be considered to be at increased risk. Some professional associations reckon that an occupational disease claim for compensation can be made for alveolar echinococcosis.

The zoonosis caused by *E. multilocularis* is limited in its distribution to particular regions of the northern hemisphere. In Europe, these areas are Southern and Eastern France, Germany and Austria. The disease is also prevalent in the Czech Republic, Slovakia, Poland and Bulgaria as well as Turkey, from where the endemic area extends eastwards as far as Siberia and the Northern islands of Japan. Its geographical distribution is likewise considered to include Alaska, Canada and the Central Northern states of the USA. As the prevalence in the definitive host populations increases, so the affected area expands, increasing the risk of infection for humans.

7.2.2.2 Alveolar Echinococcosis

The primary site of infection with *Echinococcus multilocularis* in humans in 98 % of cases is the liver (Fig. 7.14). The slow growth of the mass in the liver generally causes no symptoms. The disease is therefore often only diagnosed by chance. At advanced stages, systemic symptoms such as night sweats, weight loss and fatigue are experienced and are suggestive of a malignant disease. Additional symptoms may occur as a result of compression of major vessels in the liver. The pathogen's growth by infiltration causes different signs and symptoms according to which organs are affected.

Abdominal organs are receptive to growth of the alveolar mass (drop metastases). Lymph nodes are also colonised. Haematogenous dissemination of detached germinal layer cells (e.g. undifferentiated cells act like tumour cells) may lead to seeding in other organs. Such metastasis is favoured by immunosuppression. The spread of the parasitic mass at the time of diagnosis is currently described using an anatomical distribution pattern similar to the TNM system (PNM classification of the European Echinococcosis Working Group.

The chronic persistence of the parasitic lesion is as yet only little understood. There is TH2-weighted immunomodulation, as a result of which the parasitic infiltrate is presumably tolerated by the host organism for many years. Immunological control is assumed to play a role in the case of "abortive" lesions. In such cases, the effective cellular immune response is able to kill the parasite. The infection is cured, leaving a calcified lesion. It is not known how often this happens. Studies on immunogenetic predisposition are available and provide initial indications of possible resistance to the pathogen in the presence of particular HLA-DR characteristics. Rapid progression and metastasis have been described in the immunosuppressed.

A humoural immune response is generally detectable in cases of *E. multilocularis* infection. The cellular immune response is characterised by marked expression of IL-10 and partially explains the chronic persistence of the pathogen.

7.2.2.3 Differential Diagnosis and Diagnosis

The poor definition of the liver lesion with calcifications and central necrotic cavities is morphologically similar to the presentation of hepatocellular carcinoma. Early manifestations may give the impression of haemangiomas. Tiny intrahepatic calcifications are currently considered to be a manifestation of recovery from the parasitic disease, i.e. of the abortive form of alveolar echinococcosis.

Eosinophilia, which is rarely diagnostically informative, does not occur. Elevation of the immunoglobulin E level, on the other hand, is detectable in active alveolar echinococcosis. Imaging and serological techniques have to be used for diagnosis.

Macroscopic and histopathological evidence of the *E. multilocularis* metacestode is conclusive. A diagnostic biopsy is not recommended on account of the risk of dissemination of larval tissue.

The alveolar liver echinococcus is seen on ultrasound and CT scans as a grape-like tumour with scattered marginal calcifications. It is poorly differentiated from the rest of the liver tissue. It is not uncommon to see central necrotic cavities that give the impression of a cyst. The irregular texture, the poor differentiation and scattered calcified deposits are indicative. Diagnostic considerations must include a malignant lesion. Depending on which organs are affected, different imaging techniques may be informative.

Specific antibodies are detectable in over 90 % of cases, allowing differentiation from cystic echinococcosis. False-positive serological reactions do occur.

Various DNA probes are available. They have not yet been validated, however, for clinical diagnosis.

7.2.2.4 Therapy

Surgery

Since at the tips of the alveococcus small omnipotent cells protrude into the healthy tissues, surgery is not recommended (except the alveococcus cyst is restricted to a liver lobe).

Chemotherapy

For those inoperable lesions, chemotherapy on its own with albendazole or mebendazole leads to consolidation and regression by stopping the growth of the parasite. On the basis of current knowledge, treatment therefore has to be continued for life. The action of benzimidazoles against *E. multilocularis* infection is exclusively parasitostatic. The cellular target molecules for benzimidazoles are β-tubulins as essential components of the cytoskeleton. Variable sensitivity of the *Echinococcus* β-tubulin to benzimidazoles could explain drug resistance.

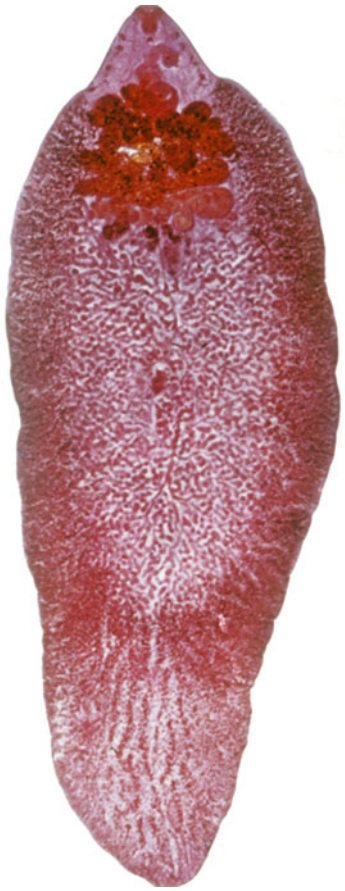

Fig. 7.15 Light micrograph of an adult *Fasciola hepatica* (*coloured*)

7.3 Hepatitis Caused by Trematodes

7.3.1 Fasciolosis, Fascioliasis: Fasciola hepatica

Taxonomy	
Subregnum	Animalia
Phylum	Platyhelminthes
Class	Trematoda
Subclass	Digenea
Order	Echinostomata
Family	Fasciolidae

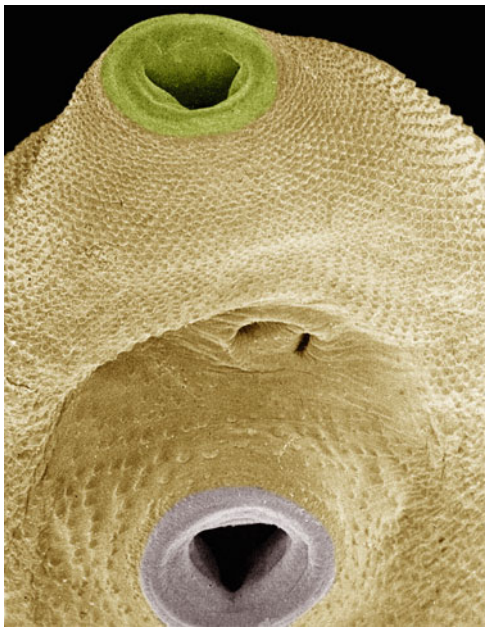

Fig. 7.16 Scanning electron micrograph of the anterior region of an adult *Fasciola hepatica* fluke showing the oral and the larger ventral sucker, the in-between situated two small genital openings and the typical scaly surface (by toothed tegumental scales)

7.3.1.1 Life Cycle and Transmission

Humans are infected predominantly with the large liver fluke (*Fasciola hepatica*), which grows to a size of 14 × 30 mm (Fig. 7.15). The giant liver fluke (*F. gigantica*) occurs only exceptionally and with a limited geographical distribution, growing to up to 70 mm in length in its natural host. The surface of these worms is fortified by numerous scales (Fig. 7.16).

Humans are infected as a result of oral ingestion of the metacercariae that are usually attached to plants. It is sufficient for such plants to be casually placed in the mouth.

The eggs of the adult flukes living in the bile ducts (Fig. 7.20) are excreted via the common bile duct with the faeces and need to reach water in order to develop further. This results in the development within 1–2 weeks inside the egg of a ciliated larva, the miracidium, which hatches from the egg membrane and actively seeks out the intermediate host (snails such as *Lymnaea*). Here, asexual reproduction involving a number of stages takes place, leading to the formation of cercariae. These leave the snail, swim around in the water, ultimately attach themselves to grasses and encyst to form the metacercaria, the infectious stage that can live for months, primarily under moist conditions (Mehlhorn 2008).

Once orally ingested, the young flukes hatch in the small intestine, bore their way through the intestinal wall and migrate through the abdominal cavity to the liver. After 6–8-week migration in the hepatic parenchyma, the flukes break

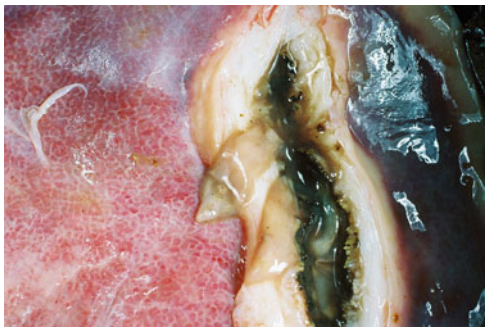

Fig. 7.17 Cut off bile duct with adult yellowish appearing *Fasciola* flukes

through into the bile ducts, their final resting place (Fig. 7.17). Sexual maturity is reached approximately 10 weeks after infection (prepatency period).

F. hepatica occurs worldwide and is particularly common in humid regions with a high rainfall that meet the biotope requirements of the amphibious intermediate host snails (*Lymnaea* spp., also known as mud snails). The main hosts are domestic and wild ruminants. Infections in other herbivores and omnivores are also not uncommon. Clinically and economically significant, often epidemic diseases may be triggered in domestic ruminants.

F. gigantica occurs in tropical zones of Asia and Africa, in Southeast Asia and the Pacific region, often alongside *F. hepatica*. Isolated areas of distribution are found in the Middle East and in the Southern republics of the former USSR. The host spectrum is the same as that for *F. hepatica*.

Infection of humans with *F. hepatica* occurs worldwide and usually sporadically. Group infestations have been reported in England, France and North Africa. Approximately 400 cases per year had been documented in France between 1970 and 1982, with higher rates in some regions. Infestation of humans with *F. gigantica* is rare, though cases are known from the Pacific and Asian region.

7.3.1.2 Disease and Symptoms

Fascioliasis is an acute or chronic disease of the liver following infestation of humans with liver flukes. The degree of infestation in humans is usually only slight. Infections are therefore often clinically inapparent, particularly in the prepatent period. In the early stage, approximately 2 weeks after infection, larval migration gives rise to perihepatitis with systemic symptoms such as fever, exhaustion and loss of appetite. Leucocytosis, eosinophilia and elevated IgE levels are generally observed.

Once the adult parasites have colonised the bile ducts, inflammation and later fibrosis and calcification of the latter occur. The anaemia that occurs at a later stage of the disease is caused by the sustained consumption of blood by the flukes. Besides intermittent fever, the symptoms experienced by patients are anorexia, weight loss, pruritus and pain usually localised under the right costal margin.

Occasional obstruction of the bile ducts by the migration of the worms leads to recurrent periods of jaundice. Ectopic sites of infection with the parasite are connective tissue and CNS.

7.3.1.3 Differential Diagnosis and Diagnosis

Conditions that must be ruled out are perihepatitis of other aetiology in the acute stage and cholestasis of other origin and gallstones at the chronic stage.

In the prepatent period lasting 10 weeks or more, serological methods (indirect immunofluorescence test, indirect haemagglutination and ELISA) can be used for aetiological clarification, although cross-reactions are likely, particularly with other trematodes (*Opisthorchis* and *Schistosoma* spp.). After the prepatent period, characteristic operculated eggs measuring 90 × 150 µm (*F. hepatica*) and 90 × 190 µm (*F. gigantica*) are detectable in the stools. The eggs are not regularly excreted. Repeated testing is therefore necessary and egg detection is not always possible. Egg detection on a single occasion is not sufficient, as liver fluke eggs consumed with contaminated beef liver (sausage) also pass through the intestine unchanged.

7.3.1.4 Treatment

Efficient treatment is achieved with triclabendazole (single dose of 10 mg/kg). Treatment with bithionol (2 × 20 mg/kg/day, every other day for 2 weeks) is also recommended. Furthermore, praziquantel for 3 days when the daily dose is given (divided) at three intervals.

Resistance in treatment of human fasciolosis is unknown at present. By contrast, there are severe problems in veterinary medicine in treating *Fasciola hepatica* infections with all available common fasciolocidal drugs.

7.3.2 Clonorchosis, Clonorchiasis: Clonorchis sinensis

Taxonomy	
Subregnum	Animalia
Phylum	Platyhelminthes
Class	Trematoda
Subclass	Digenea
Order	Opisthorchiata
Family	Opisthorchiidae

7.3.2.1 Life Cycle and Transmission

The lanceolate parasite, *Clonorchis sinensis*, which is a transparent pink colour while alive, grows to a size of 3–5 × 8–15 mm (Figs. 7.18 and 7.19). It has characteristic branched testes arranged in pairs on the posterior third of the body (Fürst et al. 2011; Mehlhorn 2008; Ehrhardt et al. 2010).

Transmission, Multiplication and Incubation Period

Humans are infected by consumption of raw or inadequately prepared freshwater fish, particularly carp, the second intermediate host in the development cycle.

If the small, operculated worm eggs (maximum 35 μm in length) find their way into water with the faeces of infected vertebrates and are ingested there by snails (*Bulinus* and *Parafossarulus* species), asexual reproduction then takes place in this first intermediate host in the course of development via a number of stages. Cercariae that are released from the snails actively penetrate small freshwater fish (predominantly cyprinids), encyst in muscle and form metacercariae. After being consumed in raw fish, the young flukes hatch in the duodenum, migrate via the common bile duct to the distal bile ducts, attach themselves to the epithelium, mature into adult flukes and start to lay eggs after 2–4 weeks (prepatency). The parasite can survive in the definitive host for 20–25 years (patency).

More than 1 billion of people live at risk of infection with one or more foodborne trematodiasis including *Clonorchis sinensis*, *Opisthorchis viverrini*, *Opisthorchis felineus*, *Fasciola* spp., *Fasciolopsis buski* and other intestinal flukes as well as the lung flukes *Paragonimus* spp. There are more than 50 millions of people infected with these trematode infections and about 8 millions diseased. Deaths are estimated to be about 7.000 annually.

C. sinensis occurs throughout Asia from Indochina to Japan. Besides humans, cats, dogs, pigs, various small predators (mustelids) and rats are also infested, these animals constituting a reservoir that is difficult to control.

Endemic areas are Korea, Vietnam, Taiwan, China and Hong Kong, where prevalences of over 50 % are common. In Japan, however, infestation of humans has today become a rarity.

7.3.2.2 Disease and Symptoms

Confined to Asia, clonorchiasis is a disease of the liver, primarily the biliary system, as a result of infection with the Chinese liver fluke. *C. sinensis* causes inflammatory and proliferative changes in the bile ducts, the severity of these changes being dependent on the number of flukes and the duration of the infestation. Infection is manifested initially in terms of exhaustion, loss of appetite and gastrointestinal

Fig. 7.18 Light micrograph of an adult *Clonorchis sinensis* (*coloured*)

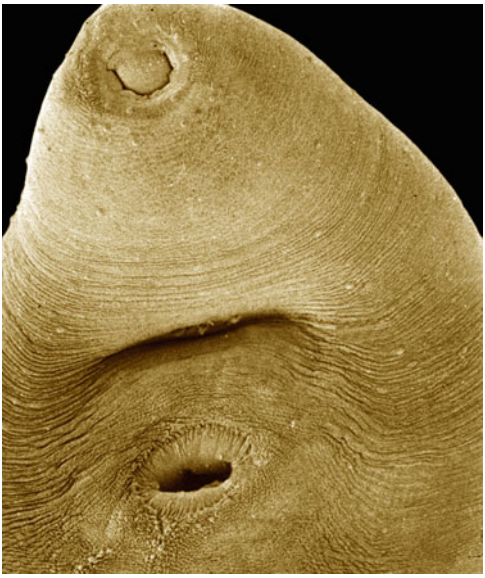

Fig. 7.19 Scanning electron micrograph of the ventral side of an adult *Clonorchis sinensis* fluke. Note the smooth, unscaly surface

symptoms such as diarrhoea and meteorism. Severe infections are followed by pain in the right epigastric region, hepatomegaly, fever, jaundice and occasionally urticaria. Massive infestation as a result of repeated infections (more than 1,000 flukes per patient may be seen) leads to severe disorders of general well-being with dizziness, tremor, seizures and weight loss. Cirrhosis of the liver, oedema and ascites occur with persistent infections.

Pancreatitis and gallstones are common. Bacterial superinfections (usually *E. coli*) and cholecystitis are common complications.

Infection predisposes to cholangiocarcinomas, which occur in cases of long-term infections.

7.3.2.3 Differential Diagnosis and Diagnosis

Cholangitis and cirrhosis of the liver of other origin need to be ruled out, depending on the stage and the severity of the infection. The clinical presentation resembles that seen in cases of infestation with other *Opisthorchis* species or *Dicrocoelium* spp.

The yellowish-coloured, operculated eggs, approximately 17 × 30 μm in size, are microscopically detectable in stools or duodenal fluid. Egg excretion may commence just 14 days after infection. Because they are so small, the eggs are easily missed in stool specimens. Concentration techniques should therefore be used.

Serological techniques and PCR techniques have also proved useful for diagnostic purposes (Müller et al. 2007). Eosinophilia is observed in the acute stage.

7.3.2.4 Treatment

The disease is treated with praziquantel (3 × 25 mg/kg/day for 2 days) or albendazole (400 mg/day for 7 days).

As with most fluke infections, the introduction of praziquantel constituted a significant advance in the treatment also of clonorchiasis. The eggs disappear from stools and duodenal fluid a few days after treatment.

7.3.3 *Opisthorchosis, Opisthorchiasis:* Opisthorchis felineus, O. viverrini

Other name(s) of the agents of disease
Liver fluke, cat liver fluke and oriental liver fluke

Taxonomy

Subregnum	Animalia
Phylum	Platyhelminthes
Class	Digenea
Order	Opisthorchiida
Family	Opisthorchiidae
Species	*Opisthorchis felineus*, *O. viverrini*

Fig. 7.20 Light micrograph of an unstained adult *Opisthorchis viverrini* worm, which is transparent so that the inner organs become visible

7.3.3.1 Life Cycle and Transmission

Dorsoventrally flattened hermaphrodite trematodes measure 7–25 mm in length and 2–5 mm in width. They have a blind-ending branched intestine and two suckers. Solid testes are located in the hind part of the body (Fig. 7.20).

Man is infected almost only by eating raw or uncooked carp flesh. In some endemic areas, fish from other families are also possible sources of infection.

The genus *Opisthorchis* belongs to the group of triheteroxenous helminths (having one definitive and two intermediate hosts). Developmental cycle starts with excretion of the eggs with the stools of the definitive host. Further development occurs only in fresh water by ingestion of miracidia containing eggs by the first intermediate host (snails: Bithyniidae and other families). Different larval stages develop in the snail (sporocyst, redia and cercaria). Cercariae are released and infect the second intermediate host (carp: Cyprinidae). Here, development of infectious metacercariae occurs. After ingestion of the metacercariae by the definitive host, the bile ducts are colonised with adult worms. Egg excretion begins about 3–4 weeks after infection (= prepatent period).

The main multiplication phase of the liver fluke takes place at the larvae stage in the first intermediate host (snail). In the definitive host (man and mammals) the number of adult worms corresponds to the number of infectious stages ingested

(one metacercaria develops to one adult worm). Multiplication in the definitive host consists only in the production of eggs, which must leave the body in order to undergo further development. An incubation period cannot usually be defined as the development of clinical symptoms depends on the number of—usually cumulatively—ingested metacercariae and the duration of the infection. In the case of simultaneous massive infection, the first symptoms can be expected 1–3 weeks after infection.

Man is the definitive (= final) host. In addition, there is a broad spectrum of reservoir hosts in piscivorous mammals (Canidae, Felidae, etc.). People who eat raw or partially cooked (carp) fish or parts of these fish are at high risk. The distribution of opisthorchosis is limited to Eurasia. It occurs wherever eating habits allow infection. The range of *O. felineus* extends from Spain eastwards to Siberia, whereas *O. sinensis* occurs only in East Asia and *O. viverrini* only in Southeast Asia (Thailand and Laos). Autochthonous *Opisthorchis* infection is now extremely rare in Western and Central Europe (Mehlhorn 2008; Ehrhardt et al. 2010; Fürst et al. 2011).

7.3.3.2 Diseases and Symptoms

Opisthorchosis: The disease is caused by bile duct parasites whose infectious stages (metacercariae) are released in the duodenum from where they migrate directly to the bile ducts (occasionally also to the pancreatic duct). Adult worms cause dilation of the bile ducts with hypertrophy of the epithelium and fibrous wall thickening. The severity of the disease is determined by the duration (untreated up to 10 years) and the intensity of infection (as many as 12,000 worms have been found at autopsy). The acute phase (only with simultaneous intake of numerous metacercariae) is associated with fever, epigastric and upper abdominal symptoms and diarrhoea. The chronic phase is characterised by fever, colicky abdominal pains, sometimes bile duct obstruction and hepatomegaly. In addition, there may be secondary bacterial infections, cholelithiasis, abscesses, cirrhosis, oedema, ascites and cholangiocarcinoma as complications. The immune response produced by liver flukes neither kills the parasites nor protects against reinfection.

7.3.3.3 Differential Diagnosis and Diagnosis

The differential diagnosis includes bile duct diseases of other origin with appropriate symptoms.

Microscopy. The only proof of the presence of opisthorchosis is detection of the operculated eggs measuring 28–35 μm in length and 11–19 μm in width in the stools or bile. Excretion of eggs begins about 3–4 weeks after infection (prepatent period). As the differentiation of eggs among *Opisthorchis* species and from those of numerous other species of small intestinal flukes is very difficult, the diagnosis should be made by specialised parasitological or tropical medicine laboratories.

Serology. No tests of high specificity and sensitivity are currently available. PCR was shown to help in diagnosis (Müller et al. 2007).

7.3.3.4 Treatment

The drug of choice is praziquantel (Biltricide®). Dosage: 40 mg/kg once or 25 mg/kg body weight three times on the same day.

7.3.4 Schistosomiasis, Schistosomosis, Bilharziasis: Schistosoma *species*

Other names of the agents of disease
S. intercalatum, S. japonicum, S. mansoni, S. mekongi, Bilharzia species, *Distomum bilharzias*e, blood fluke, intestinal blood fluke, bladder fluke, Japanese blood fluke and coupled or paired worms

Taxonomy

Subregnum	Animalia
Phylum	Platyhelminthes
Class	Trematoda
Subclass	Digenea
Order	Schistosomatida
Family	Schistosomatidae

7.3.4.1 Life Cycle and Transmission

Schistosomes are diecious, approximately 1–3-cm-long flukes (Figs. 7.21 and 7.22). The male carries the female in a ventral groove (*canalis gynaecophorus* or gynaecophoral canal). Two suckers are used for attachment to and movement within blood vessels (veins of the mesenteric or vesical plexus). The simple, blind-ending intestine is filled with ingested erythrocytes. The tegument consists of a syncytium with a double surface membrane. The eggs (mean size in length 65–170 µm according to species) each contain one larva (miracidium) and have a characteristic spine (Mehlhorn 2008; Richter and Ruppel 2010).

Transmission of schistosomes to humans is possible in freshwater containing the cercariae (fork-tailed larvae) excreted by the snails. The cercariae can actively penetrate the skin within a short space of time. Since the snail species that serve as intermediate hosts occur only in (sub)tropical climatic zones, transmission can only take place in waters in those regions. Construction of irrigation systems in Africa in

Fig. 7.21 Light micrograph of an unstained couple of *Schistosoma mansoni*. Both intestines appear *black* due to the ingested host blood

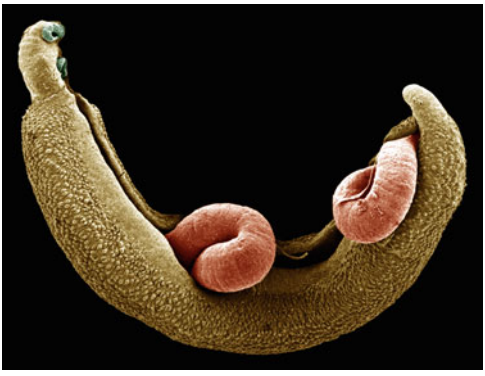

Fig. 7.22 Scanning electron micrograph of a couple of *Schistosoma mansoni*, where the male carries the female

particular has led to a dramatic increase in reproduction of the intermediate host snails, resulting in increased transmission.

Schistosomes are two-host helminths with different definitive and intermediate hosts: adult flukes reside in the definitive = final host, humans. Here, egg excretion with stools or urine occurs—depending on the species. Miracidia hatch in freshwater, penetrate an intermediate host (particular freshwater or amphibian species of snail), and larval development and multiplication occur inside the snail (sporocysts and cercariae). Thereafter, cercariae swarm into the water and actively penetrate percutaneously into humans, migrate within the definitive host, mature into adult worms and colonise the species-specific venous plexuses.

The blood flukes' principal multiplication phase takes place at the larval stage in the intermediate host snail. The number of worms harboured by the definitive host

(man) is the same as the number of penetrating cercariae (one cercaria develops to one male or female adult worm according to its sex chromosomes). Multiplication in the definitive host consists solely of the production of eggs. These must find their way into water in order to develop further.

An incubation period is definable only if a host is simultaneously infested with numerous cercariae. Penetration by the cercariae may lead shortly afterwards to cercarial dermatitis, followed a few weeks later by the febrile systemic disorder known as Katayama syndrome.

While *S. intercalatum* is almost exclusively a human parasite, *S. mansoni* also occurs in a number of rodent species, too. Also, the form of schistosomiasis caused by *S. japonicum* is a zoonosis with cattle, buffalo, pigs, rodents, etc. as definitive hosts. In the case of *S. mekongi*, dogs and pigs act as an animal reservoir.

Anyone living in endemic areas with occupational contact with water (e.g. rice-growing, laundry work) is exposed to the risk of *Schistosoma* infection. Similarly, anyone travelling to the tropics is at risk of contracting schistosomiasis when swimming or coming into contact in some other way with infected waters.

Schistosomiasis can only occur in regions in which the vector snails live and *Schistosoma* eggs pass into waters with faeces and/or urine. About 800 millions of people worldwide live at risk of infection with schistosomes in 76 countries in tropical and subtropical regions. It is estimated that way over 200 million people are infected worldwide: in Africa and the Middle East with *S. haematobium* (this species does not affect the liver but the urinary tract) or *S. mansoni*, in Latin America with *S. mansoni*, in China and the Philippines with *S. japonicum*, in Laos and Cambodia with *S. mekongi* and in tropical Africa with *S. intercalatum*. 120 millions of people are estimated to be sick from this infection; however, the death rate is somewhere between 15,000 and 280,000 cases.

7.3.4.2 Diseases and Symptoms

The signs and symptoms of schistosomiasis (=bilharziasis) vary according to the species and the duration of the disease, though all are directly or indirectly a consequence of man's immune response to the parasite's different stages (Richter and Ruppel 2010).

Acute Schistosomiasis

This disease is characterised by a toxaemic phase (including fever, diarrhoea, exhaustion and other non-specific symptoms; "Katayama syndrome") as a result of passage of the larvae of *S. japonicum* through the lung and their rapid growth (immune response to metabolic products) in the mesenteric veins. Formation of large granulomas (Fig. 7.23) is found around eggs that are deposited in the capillaries (particularly liver, intestine and bladder). Leucocytosis with marked

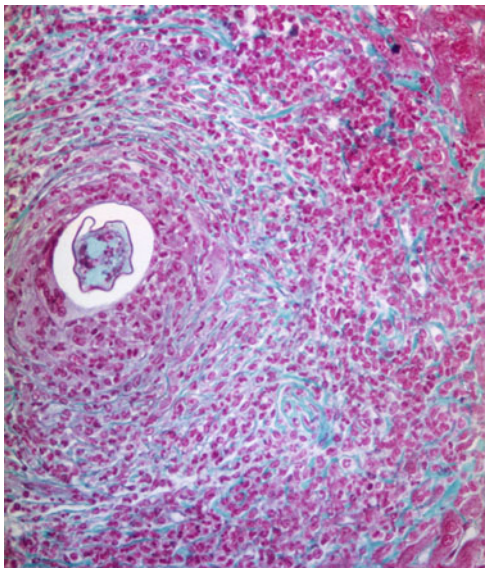

Fig. 7.23 Light micrograph of a section through a liver granuloma surrounding the centrally located egg of *S. mansoni*

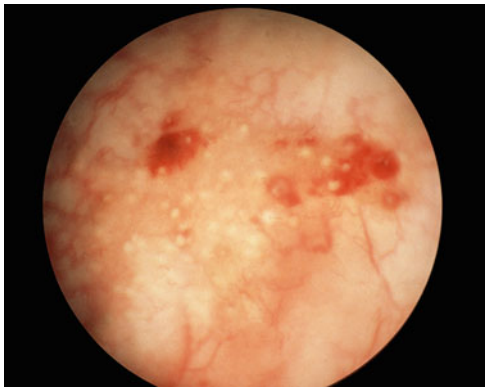

Fig. 7.24 Endoscopy photo of the inner surface of a human bladder showing many granulomas due to protruding eggs of *Schistosoma haematobium*

eosinophilia. In mild infections and also in tourists, early symptoms either do not occur or remain unrecognised.

Chronic Schistosomiasis

All symptoms are a direct or indirect consequence of the granulomatous reaction to the parasite eggs. Though the granulomas are smaller in the chronic stage than in the acute stage (immunomodulation), the quantity of eggs (i.e. of worm pairs) and the duration of the infection (many years or even lifelong without treatment) determine the course of the disease. In endemic areas, bilharziasis is relatively rarely fatal but often causes infirmity. Mild infections are not threatening.

Fig. 7.25 Light micrograph of an egg of *S. mansoni*

A distinction is made between urinary and intestinal schistosomiasis, depending on the site of the worms and the organs in which the eggs are deposited (Fig. 7.24).

Intestinal Schistosomiasis

Caused by the eggs of *S. mansoni*, *S. japonicum*, *S. mekongi* and *S. intercalatum* that are deposited primarily in the intestine and the liver (Figs. 7.25 and 7.26) or are excreted with the stools. The granulomatous inflammatory reaction around the eggs leads in the large intestine in particular to mucosal hyperaemia, ulceration and later also granulomatous proliferation and bleeding. Of even greater significance with the first two of the aforementioned species, however, is that the liver is damaged by granulomas that form around eggs that are carried via the bloodstream to the presinusoidal bifurcations of the portal vein. Chronic phlebitis, periportal fibrosis and finally "pipestem fibrosis" develop as a result of the granulomas in the liver. Even though the periportal fibrosis does not affect the hepatic parenchyma between areas of fibrosis and does not interfere with its functioning, it nevertheless leads to portal hypertension. Consequences of this may be as follows: formation of ascites (particularly with *S. japonicum*), oesophageal varices and hepatosplenomegaly.

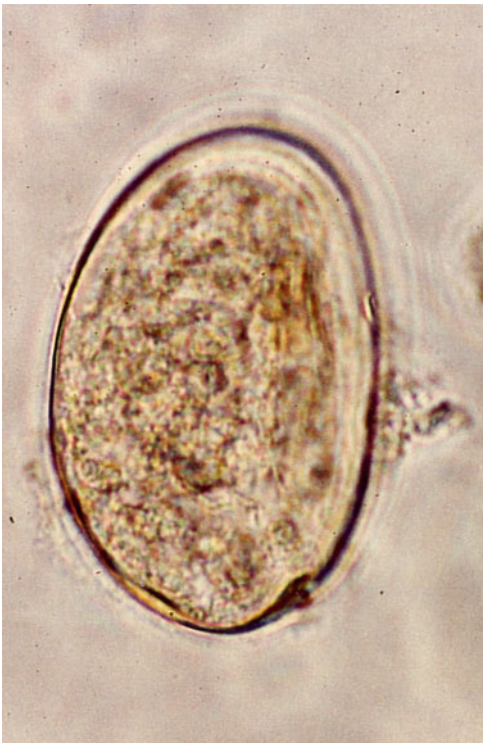

Fig. 7.26 Light micrograph of an egg of *S. japonicum*

Worm eggs may ultimately pass via portosystemic collaterals into the lung and lead to pulmonary hypertension and cor pulmonale. The signs and symptoms associated with intestinal schistosomiasis range, for example, from exhaustion and abdominal pain via diarrhoea with loss of blood and protein through to massive variceal bleeding as a cause of death. Chronic intestinal schistosomiasis persists for years and its severity depends on the intensity and duration of the infestation. Overall, it constitutes a disease that is triggered by the immune response in humans to the worm eggs.

Bladder Schistosomiasis

This disease can be recognised by bloody inclusions in the urine due to egg-derived haemorrhages in the bladder (Figs. 7.24 and 7.27) Many cases of bladder cancer have been reported.

7.3.4.3 Differential Diagnosis and Diagnosis

Differential diagnoses for both intestinal and urinary schistosomiasis are various diseases of other origin, e.g. Katayama syndrome needs to be distinguished from

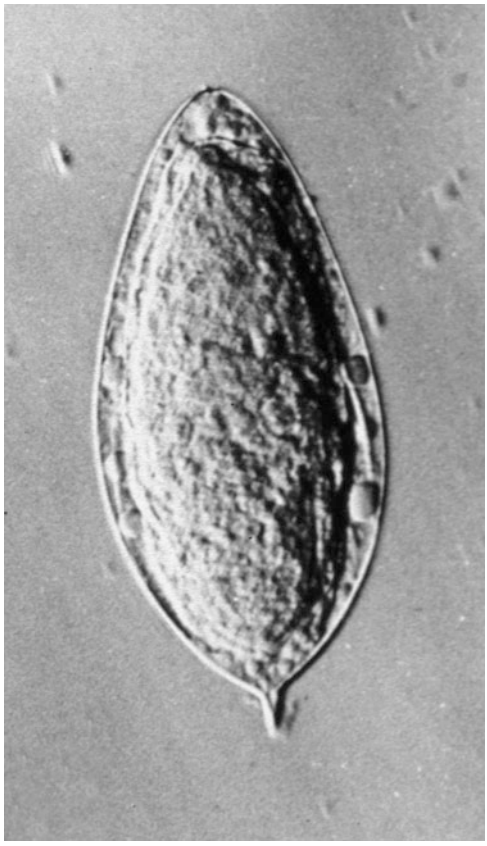

Fig. 7.27 Light micrograph of an egg of *S. haematobium*

paratyphoid fever and typhoid fever. Furthermore, a number of parasitic and other infections need to be excluded, both in the acute and the chronic phase of the different forms of schistosomiasis.

Direct detection in smears, sediment or after concentration takes place by means of microscopic examination of stools or urine for schistosome eggs, the shape of which is species specific. A 24-h urine specimen or several grams of stool may be necessary for this purpose. Stool may contain the eggs of *S. mansoni* (114–175 × 45–68 µm, with lateral spine), *S. intercalatum* (140–240 × 50–85 µm, with terminal spine), *S. japonicum* (70–100 × 50–65 µm, with a small sized spine) and *S. mekongi* (65–67 × 56–59 µm, with a small-sized spine) (Figs. 7.25, 7.26 and 7.27). Quantitative methods (10-ml urine filtrate, stool smear using the Kato-Katz method) are epidemiologically important. Where very few eggs are excreted, the miracidium hatching test has proved successful. This involves placing the miracidia present in the eggs in a flask to hatch. Because of their positive phototaxis, these then migrate into an illuminated, attached side arm of the flask, from which they can be removed.

Responses in the host take the form of formation of IgG, IgA, IgM and IgE, activation of various immunocompetent cell populations and circulation of immune complexes. The parasite protects itself by mimicry of host protein and by proteases that lead to inactivation of antibodies and complement factors. Immune processes at the schistosomula stage may, however, also protect against reinfection and superinfection.

Detection of antischistosomal antibodies in the serum by indirect immunofluorescence, indirect haemagglutination and/or ELISA. All tests are generally based on antigen preparations (worms or eggs) of *S. mansoni* and cross-react with other *Schistosoma* species. Evaluation of titre steps and possible cross-reactions with other parasitoses should be performed by institutes of parasitology or tropical medicine. Tests for circulating *Schistosoma* antigens in serum are likewise suitable for the detection of schistosomiasis.

7.3.4.4 Treatment

Praziquantel (Biltricide®) is the drug of choice against all *Schistosoma* species. The dosage is 2 × 30 mg/kg body weight (BW) (*S. japonicum*) or 3 × 20 mg/kg BW (all other species), administered orally in 1 day. Side effects, if they occur at all, are only minor (possible exceptions: massive infections and cerebral schistosomiasis). True resistance has not yet been shown.

7.4 Hepatitis Caused by Nematodes

7.4.1 Capillariasis: Capillaria hepatica

Other name of the agent of disease
No other specific name; disease: hepatic capillariasis

Taxonomy

Subregnum	Animalia
Phylum	Nematozoa
Class	Nematodes
Subclass	Adenophorea
Order	Trichocephalida
Family	Capillaridae

7.4.1.1 Life Cycle and Transmission

The pathogen is *Capillaria hepatica*, a nematode as slender as a human hair, measuring up to approximately 10 cm in length in the adult stage, with marked hepatotropism. The parasite is prevalent worldwide and its principal hosts are rodents, predominantly rats, in which prevalence rates may be as high as 80 % or more. Infection in humans is rare. Approximately 40 confirmed cases, in total, have been reported in North and South America, Africa, Asia and Central Europe, although it is likely that a number of cases remained unreported (Mehlhorn 2008; Burchard and Löscher 2010).

Humans are infected by oral ingestion of the parasite's embryonated (larva-containing) eggs.

C. hepatica parasitises the hepatic parenchyma within which it migrates. At the same time, the adult female worms lay characteristic eggs with two flattened polar plugs. The eggs, which are deposited in the bore holes and are later encapsulated in tubular granulomas, reach the outside world only by means of maceration on the death of the host or, if infected animals served as prey via the digestive tract of the predator (cannibalism also plays a role in the case of rats). Once in the open, an infective larval stage develops inside the egg and this is then ingested by humans with contaminated food. The larva hatches from the egg membrane in the intestine, migrates through the intestinal wall and passes via the bloodstream to the liver. Sexual maturity and hence commencement of egg laying are attained after 3–4 weeks (prepatent period).

7.4.1.2 Diseases and Symptoms

There are only rare cases of disease caused by single (few) liver-specific roundworms, while serious consequences occur in the event of massive infections.

The clinical presentation depends on the burden of infection. Light infection, occasionally reported as a post-mortem finding, would appear to be asymptomatic. In heavy infections, which in children may prove fatal, an acute course is to be expected at around the commencement of egg laying, these symptoms reflecting the destruction of large areas of liver parenchyma. In such cases, unexplained extrahepatic symptoms (e.g. lung disorders) have been described in the pathogenesis. Moderate infections are associated with uncharacteristic upper abdominal pain and hepatomegaly. Leucocytosis, eosinophilia and hypergammaglobulinaemia regularly occur.

7.4.1.3 Differential Diagnosis and Diagnosis

Focal or disseminated damage to the liver parenchyma may have diverse origin. Worm nodules with convoluted parasites and/or egg strings encapsulated in

granulomas are often to be found in the subcapsular region and are laparoscopically detectable. In patent infections, the diagnosis can be confirmed by biopsy, revealing eggs measuring 30 × 50 μm with two flattened polar plugs (one at each pole). There are no serological methods available.

7.4.1.4 Treatment

Mebendazole is effective according to findings in animal experiments; however, it is not very promising for humans since the liver damages are based on egg-blocked blood vessels and the fact that drugs have no effects on the eggs.

7.5 Conclusions

This chapter presented a series of different parasites, which may form abscesses, tissue cysts or which may induce cancer in the liver of humans or other hosts. While intestinal stages of these parasites are mostly susceptible to chemical medicines, the peculiar extraintestinal stages often withstand any chemotherapeutical measurement. Therefore, it seems worthwhile to look onto the remedies that might be obtained from plant-based extracts.

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Chapter 8
Praziquantel

Achim Harder

Dedicated to my former teacher Prof. Dr. H. Debuch.

Abstract Praziquantel is the most common antiparasitic drug against all types of trematodes and cestodes. Although discovered in Germany at Bayer Company, it came first on the market in China due to the high pressure of severe diseases such as schistosomiasis in Asia. This very efficient and safe compound is today the gold standard in the treatment of human and animal infections due to Platyhelminthes. Thus all plant-derived products—old and new ones—have to be compared with its high standards.

8.1 Introduction

Praziquantel, the well-known widely used antischistosomal and anticestodal drug in human and veterinary medicine, is now 40 years of age. The anthelmintic activity of praziquantel (PZQ) (Fig. 8.1) was discovered by a joint cooperation between Bayer AG and E. Merck in 1972 (Andrews et al. 1983; Harder 2002). Over 30 years ago, PZQ was introduced in the field for chemotherapy against human schistosomiasis (Cioli et al. 2012). Due to its high efficacy, excellent tolerability, and its simple way of application, this drug is useful for individual and mass treatment. From the beginning up to now, there are 4,028 publications dealing with this compound indicating its extraordinary importance.

PZQ has a broad-spectrum anthelmintic activity against parasitic trematodes and cestodes in vertebrates and humans. A major initial problem was the price of PZQ, but market competition started after the Korean company Shin Poong obtained a process patent and initiated a series of price cuts that brought the cost of a treatment

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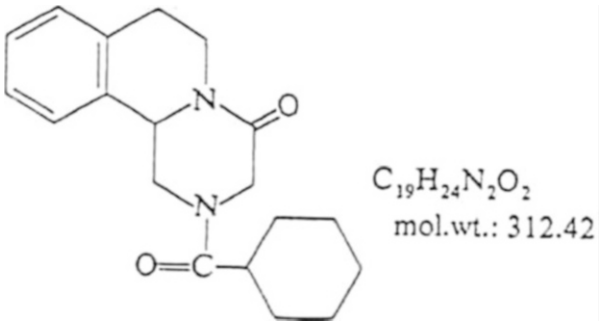

Fig. 8.1 Structural formula of praziquantel: 2 cyclohexylcarbonyl (1,2,3,6,7,11b) hexahydro-4H-pyrazino (2,1-a) isoquinolin-4-one

to the present level of US$0.2 for a child or US$0.3 for an adult. Both, the wide spectrum of activity and the reasonable price soon made PZQ the drug of choice for schistosomiasis, whereas all other existing medications, such as oxamniquine and metrifonate, were practically abandoned.

8.2 Anthelmintic Activities of Praziquantel

8.2.1 Trematodes

The therapeutic efficacy of PZQ is reviewed in detail by Andrews et al. (1983). The drug has a high therapeutic activity against schistosomes (*Schistosoma mansoni*, *S. haematobium*, and *S. japonicum*) in mice, mastomys, Syrian hamster, and monkeys after oral, subcutaneous, or intramuscular administration. It exerts high cure rates in man even 6 months after a single or a 1-day treatment. Therefore, PZQ is ideally suited for large-scale treatment directed in controlling schistosomiasis (Andrews et al. 1983; Cioli et al. 2012).

PZQ is active against all schistosome species infecting humans. This is an important feature, especially in those areas where more than a single *Schistosoma* species is present. This is typical in Africa where *Schistosoma mansoni* and *Schistosoma haematobium* are often coendemic. Today, PZQ is used in mass treatment programs, in which every year several million people, mostly school children, are treated irrespective of a previous diagnosis of infection. When administered as a single oral dose of 40–60 mg/kg bodyweight, it exerts cure rates ranging between 60 and 90 % (Cioli et al. 2012). Most importantly, even those individuals who are not completely cured have drastic reductions in the number of excreted schistosome eggs, an indication that the parasite load has been correspondingly decreased. Schistosomiasis is a chronic disease that typically evolves into serious pathology in patients with high parasite loads over many years, and a reduction of the worm load greatly reduces the likelihood of long-term sequelae (Cioli et al. 2012).

In addition to blood flukes, PZQ is highly efficacious against small liver flukes *Dicrocoelium dendriticum*, *Clonorchis sinensis*, *Opisthorchis viverrini*, *O. felineus*, *Metorchis conjunctus*, lung flukes *Paragonimus ohirai*, *P. philippensis*, *P. westermani*, *P. heterotremus*, *P. ecuadoriensis*, intestinal flukes *Nanophyetus salmincola*, *Heterophyes heterophyes*, *Metagonimus yokogawai*, *Echinostoma* species, *Gastrodiscoides hominis*, *Fasciolopsis buski*, and other flukes either in animal hosts or in man (Andrews et al. 1983). However, PZQ lacks efficacy against *Fasciola* species despite many efforts regarding drug dosing, formulation, etc. Therefore, PZQ cannot be used for normal treating infections with *Fasciola hepatica*, neither in animals nor in man.

8.2.2 Cestodes

PZQ shows high efficacy against adult and larval cestodes in many animal host models and also in man (Andrews et al. 1983). Thus, activity is found against *Hymenolepis* spp., *Diphyllobothrium* spp., *Mesocestoides corti*, *Taenia* spp., *Echinococcus* spp., *Dipylidium caninum*, *Spirometra erinacei*, *Joyeuxiella pasquali*, *Moniezia* spp., *Stilesia hepatica*, *Avitellina centripunctata*, and other adult cestode species listed in Andrews et al. (1983). Against larval cestodes PZQ shows variable efficacies. It exerts 100 % efficacy against *Cysticercus fasciolaris* in the mouse, *Multiceps multiceps* (*Coenurus*) in sheep, *Taenia pisiformis* in rabbits, *Cysticercus tenuicollis* in pigs, *Cysticercus ovis* in sheep, *Cysticercus bovis* in cattle, and *Cysticercus cellulosae* in pig muscles and brain (Andrews et al. 1983).

8.3 Praziquantel Used in Anthelmintic Products

Biltricide® is the PZQ product used against blood, liver, lung, and intestinal flukes in humans (Mehlhorn 2008). Droncit®, the drug for veterinary medicine, was introduced in 1975 for treatment of cestode infections in tablet formulation, as solution for injection for cats and dogs or as spot-on formulation for cats. Cestocur® is used for treatment of sheep against tapeworm infections in an oral liquid drench formulation.

PZQ is constituent in so-called allwormer combinations with two, three, or even four active antinematodal ingredients (pyrantel, fenbendazole, oxfendazole, oxibendazole, febantel, abamectin, milbemycin oxime, and emodepside) for curing dogs and cats. PZQ is also used in combinations with active nematocidal ingredients for use in cattle, sheep, and lambs (fenbendazole, abamectin, and moxidectin), and for horses (pyrantel, ivermectin, abamectin, and moxidectin) (Mehlhorn 2008) (Table 8.1).

Table 8.1 Praziquantel products alone or in combination with other antiparasiticides in human and veterinary medicine (× = effective)

		Antiparasitic activity				
PZQ alone or in combination with	Trade name and company	Trematodes	Cestodes	Intestinal nematodes	Heartworm prevention	Ectoparasites
Alone (human)	Biltricide (Bayer, others)	×				
Alone for dogs and cats	Droncit, Bayer		×			
Alone for sheep	Cestocur (Bayer)		×			
Milbemycin oxime for dogs and cats	Milbemax (Novartis)		×	×	×	
Milbemycin oxime/lufenuron for dogs and cats	Sentinel (Novartis)		×	×	×	Fleas
Abamectin/oxibendazole for dogs	Canimax (Virbac)		×	×	×	
Pyrantel/oxantel for dogs	Canex (Pfizer)		×	×		
Pyrantel/Febantel for dogs	Drontal Plus (Bayer)		×	×		
Fenbendazole/pyrantel for dogs	Fenpral (Riverside Vet Products)		×	×		
Pyrantel for dogs and cats	Drontal (Bayer)		×	× (not whipworm)		
Fenbendazole for dogs and cats	Caniquantel Plus (IDT), Feligel (CP Pharma)		×	×		
Oxibendazole for dogs and cats	Endogard (Virbac)		×	×		
Febantel for dogs and cats	Vercom Paste (Bayer)		×	×		
Emodepside for cats and dogs	Profender Spot-On, Profender tabs (Bayer)		×	×		
Macrocyclic lactones (ivermectin, abamectin, and moxidectin) for horses	Different companies		×	×		

8.3.1 Pharmacokinetics

PZQ is absorbed from the intestine after oral application between 80 and 100 %. Absorption is very rapid; maximal serum concentrations are reached within 1 h in all species. Invasion half-life varies between 0.1 and 0.3 h. PZQ is distributed throughout the body and reaches high plasma levels in tissues of all organs. Therefore, PZQ comes in close contact with larval and adult stages of cestodes and trematodes, which reside in very different locations of the host (Mehlhorn 2008). PZQ reaches the liver via the portal vein where it is metabolized extensively at a high rate by major biotransformation. 80 % of the drug is reversibly bound to protein in plasma of various laboratory animals. 75–82 % of an oral or intravenous dose is excreted with urine and feces. Elimination is nearly complete after 24 h. 60–80 % of PZQ are eliminated through the kidneys, while the remainder is eliminated from the body in the bile and excreted with the feces (Andrews et al. 1983).

8.3.2 Safety and Tolerability of Praziquantel

PZQ has a very favorable safety profile; mutagenicity tests were always negative and no systemic adverse effects, no reproductive toxicity, and no carcinogenicity were detected in animals after repeated administration of high doses. It is safe after the oral or parenteral application. There is a wide margin of safety for PZQ as shown in acute and chronic toxicity studies in mice, rats, rabbits, and dogs. Up to five times, the recommended dosage is tolerated without adverse effects in cats and dogs. Tenfold overdosing may cause transitory vomiting and sign of depression (Mehlhorn 2008). Clinical observations over many years have confirmed initial safety predictions. Unwanted side effects after treatment are not uncommon, but they are usually mild and short lived. Some of the more severe reactions are probably due to a massive release of parasite material from dying worms rather than a direct effect of the drug on the host. PZQ is active upon a single oral administration. This, together with its lack of serious side effects, makes drug distribution easy without direct medical supervision, as it is currently done in primary school settings (Cioli et al. 2012).

8.3.3 Problems with Praziquantel

Three negative aspects of PZQ need to be mentioned (Cioli et al. 2012). The first aspect is the fact that it is not active against juvenile schistosomes. When administered in the first few days after infection, PZQ is apparently effective, but activity rapidly drops until it reaches insignificant levels around week 4 and then

starts rising again to attain maximal efficacy around week 7. Such a biphasic curve of activity is common to other, but not all, antischistosomal drugs and its biological basis is so far unexplained (Cioli et al. 2012). The practical consequence of this phenomenon is that, in areas of very active transmission of infection, many people are likely to harbor immature worms at the time of treatment, with the result of correspondingly low cure rates. The dramatically low cure rates (28–36 %) achieved with PZQ in northern Senegal most likely rely on this phenomenon.

A second, but less serious problem is that all commercially available PZQ is a racemic mixture of two isomers, and only one exerts antiparasitic activity, while the other one contributes to side effects and is responsible for the bad smell and taste of the drug tablets that, being twice the necessary size, are often difficult to swallow for small children. Some promising progress towards the stereoselective synthesis of PZQ has been achieved recently (Cioli et al. 2012).

A third problem is that the mechanism of action of PZQ has not been elucidated, which hinders the rational development of improved PZQ derivatives (Cioli et al. 2012).

8.4 What Is Known About the Mechanism of Action of Praziquantel

8.4.1 Threshold Concentrations of Praziquantel

A prerequisite for an analysis of the mechanism of action of PZQ is the information on the concentration to which the parasites are exposed when the host organism is given a therapeutic dose. As schistosomes live in the vasculature, drug levels determined in samples of peripheral venous blood give a good approximation of the levels actually encountered by the parasite. One slight complication is that some schistosome species live in the portal vein, in which the concentration of PZQ is known to be elevated during the phase of enteric absorption of the drug. Favorable to the calculation of concentrations encountered is the lack of antischistosomal effect of the PZQ metabolites formed by the mammalian host. Thus, only the unchanged drug has to be considered. Furthermore, while schistosomes take up PZQ readily, they only bind it loosely and drug is readily lost in drug-free media. PZQ is evenly distributed through all organ systems of the parasite. In addition, schistosomes are unable to degrade PZQ (Andrews et al. 1983).

The simultaneous study of pharmacokinetics and therapeutic efficacy of PZQ in animal models allows the estimation of a threshold concentration to which the parasite must be exposed in order to be eliminated, as well as the time period involved. The threshold concentration is around 1 μM, and the period of exposure required is about 6 h. These conditions are met in patients receiving antischistosomal therapy. Only those effects of PZQ that are induced by concentrations of about 1 μM are likely to be relevant for the understanding of

the mechanism of action of PZQ against schistosomes and cestodes (Andrews et al. 1983).

8.4.2 The Schistosomal Tegument and Musculature

The tegument of schistosomes has developed some remarkable features. In adult worms, it is a cilialess, cytoplasmic but noncellular, metabolically active layer (Mehlhorn 2008). The primary functions of the tegument are protection of the parasite against the host's immune attack and maintenance of survival. The tegument is involved in the absorption of nutrients, in osmoregulation and excretion. In terms of protection, the parasite incorporates host antigens on its tegumental surface and so camouflages itself effectively. In terms of survival, the tegument serves as an important site of nutrient uptake and ion and water regulation. It further contains various enzymes involved, e.g., in ATP generation both by oxidative pathways and from reduced glutathione, and it maintains a membrane potential ranging between −35 and −41 mV. The ionic composition of the tegument is different from that of the serum surrounding the parasite and also from that of erythrocytes. All these phenomena suggest the existence of proton pumps operating in the tegument, which maintain ionic gradients and a pH value of the tegument of 7.8. The schistosomal tegument is much more than a mere envelope—it is an organ in its own right.

The surface of the tegument of some species is studded with pits and spines. The pits lead to branched and sometimes interconnected channels, which contain serum. The enormous increase in surface area of the parasite imparted by pits and channels is undoubtedly of value for an absorptive organ. Histologically, the tegument is a syncytium with a thickness of about 4 μm. Its cell nuclei are sunk below the circular muscle layer. The apical surface of the tegument is a specialized version of the normal phospholipid bilayer membrane, a so-called heptalaminate membrane, which is formed by the stacking of two trilaminate membranes on top of each other. This special adaptation is known to occur in other blood-dwelling flukes and may be related to the intravascular habitat. The tegument is not a static structure, but its components underlie rapid rates of turnover. The heptalaminate surface membrane, with a half-life of about 2–3 h, appears to be replaced continually by material provided through multilaminate vesicles produced in the cell bodies of the tegument (Mehlhorn 2008).

The inner cell membrane of the syncytial tegument is connected to finger-like protusions of parenchymal cells (Mehlhorn 2008), the cell body of which is situated below the circular and longitudinal muscle bundles. These connections pass the basal lamina. The primary cellular, ciliate, and nucleate epithelium get lost during schistosome development. An underlying syncytium with degenerating nuclei is formed de novo and comes into contact with the underlying parenchymal cells via their finger-like protusions. The parenchymal cells contain a large nucleus and all other typical cellular organelles, but there are apparently two types of cells with

respect to the storage of glycogen; one type is closely filled with glycogen granules and the other is not (Mehlhorn 2008).

Four different types of muscles occur and appear mainly as smooth muscles. Muscles of body wall of adult platyhelminthes consist of two layers of muscles just below the syncytial tegument and its basal lamina. The outer bundles of the circular musculature and the inner longitudinally running muscles are both embedded in an electron-lucent amorphous layer of connective tissue. The muscle cells contain thick, thin, and intermediate filaments. The thick ones are myosin filaments, the smaller-sized are actin filaments and run parallel to the thicker ones (Mehlhorn 2008).

8.4.3 Primary Effects of Praziquantel

Two striking phenomena are observable in trematodes and cestodes after exposure to PZQ. There is a rapid vacuolization of the syncytial tegument and simultaneously an instantaneous tetanic contraction of the musculature (Andrews et al. 1983). In vitro, both changes occur at concentrations equivalent to therapeutic serum levels at 1 μM within 30 s after contact with the drug. Both effects are also seen in vivo, when worms of *Schistosoma mansoni* and *S. japonicum* shift from the mesenteric veins to the liver within minutes after oral drug application (Andrews et al. 1983). Vacuolization of the tegument is observable as early as 15 min after oral treatment of mice infected with *S. mansoni*.

8.4.4 Morphological Effects

Within seconds after exposure to 0.5 μM PZQ, rapid structural damage of the surface of the tegument ensues. Schistosomes develop bleb-like structures, which can be seen from the outside and are protrusions of the surface membrane. The outer surface of the tegumental membrane appears unchanged and the surface coat remains intact (Becker et al. 1980). This tegumental blebbing is followed within minutes by increasing vacuolization, which develops from the basal region of the tegument, and by the formation of larger balloon-like surface exudates, which still contain the tegumental membrane (Fig. 8.2). Immunologically, the parasite surface begins to change. Previously, unexposed parasite antigens are exposed within 30 min of treating the mouse host (Xiao et al. 1981; Harnett and Kusel 1986). The process of vacuolization and ballooning of the tegument continues and eventually parts of the tegument are sloughed off. Within 4 h after treatment of infected mice, phagocytic cells are found associated with the parasite. Invasion of the parasite by phagocytes is in full progress after 17 h of treatment and results in the lysis of the parasite tissues within a few days (Table 8.2).

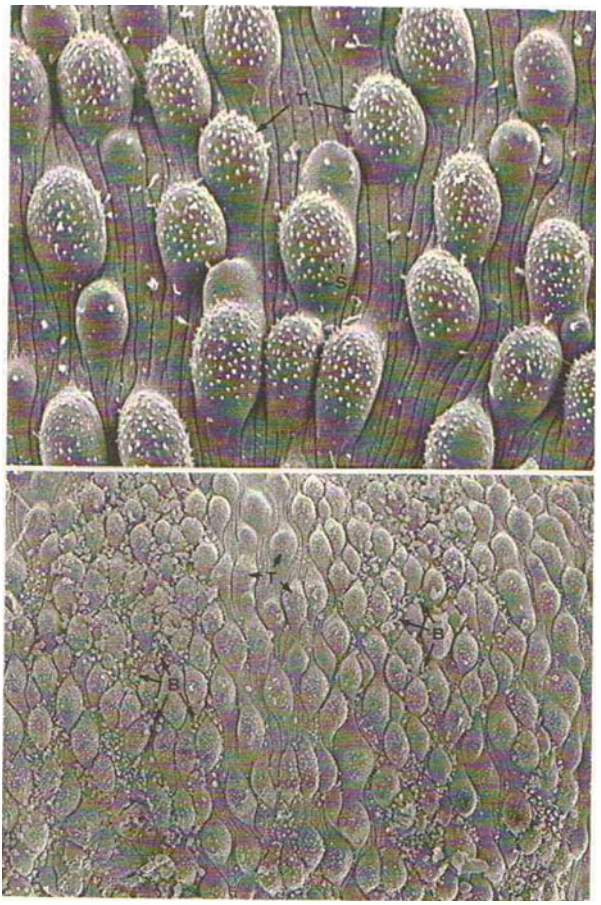

Fig. 8.2 Scanning electron micrograph of the dorsal tegument of *Schistosoma mansoni* provided with tubercles (T) and spines (S). *Above*: untreated control, ×950. *Below*: after 60 min exposure to 30 μM praziquantel. The dorsal tegumental surface shows numerous bleb-like lesions (B), ×300

How tegumental damage is brought about cannot be answered by mere morphological and ultrastructural studies. Similar tegumental alterations have also been observed in schistosomes exposed to many different conditions or compounds such as, hypo- or hypertonic media, in vitro culture, hyperimmune serum, complement, major basic protein, lectins, and several antischistosomal compounds. It thus appears that the morphologically visible damage is not directly related to the nature of the noxious agent encountered by the schistosome, but rather a general reaction to adverse conditions. One point, however, is noteworthy. PZQ induces surface blebbing within seconds, while many minutes or hours are required by the other agents. PZQ causes tegumental alterations qualitatively similar to those found in schistosomes in a large number of other digenean trematodes living in the lungs, livers, or intestines of their hosts and also in all of the several cestode species studied so far (Andrews et al. 1983). The extent of tegumental damage caused may vary between different parasite species.

Table 8.2 Primary and secondary effects or praziquantel induced in trematodes and cestodes as a function of time after drug exposure

Helminth species	Drug concentration	Observed effect	Time after drug exposure of observable effect
Primary effects			
Schistosomes	0.32–0.6 μM	Surface blebbing, vacuolization	Seconds
		Spastic paralysis	Seconds
		Increase in muscle tension	Seconds
S. mansoni	1 μM	Ca^{2+} influx	Seconds
Effects associated to primary membrane effects			
S. mansoni		Depolarization or alterations of ion fluxes (Na^+, K^+); uptake of glucose and adenosine, lactate excretion, glycogen breakdown, alterations of membrane associated enzymes: ATPase	Seconds until minutes
Secondary effects			
S. mansoni		Phagocytic cells associated with parasite	4 h
S. mansoni		Invasion of phagocytes	17 h
S. mansoni		Lysis of parasite's tissues	Few days

8.4.5 Muscular Contraction

Contraction of the schistosome musculature can be monitored more exactly than the development of surface damage. Half maximal contraction is obtained just 11 s after the addition of 1 μM PZQ (Pax et al. 1978). The PZQ-induced contraction depends on the presence of Ca^{2+} ions. Depletion of external Ca^{2+} or addition of an excess of Mg^{2+} abolishes drug-induced contraction (Redman et al. 1996). Most inhibitors of known neurotransmitters of *S. mansoni*, as well as other pharmacologically active agents, do not antagonize this contraction-inducing effect of PZQ. This lack of interaction of PZQ with neuroceptive sites together with the requirement for Ca^{2+} ions has led to the idea that PZQ directly or indirectly activates a Ca^{2+}-dependent contraction of the parasite's musculature (Andrews et al. 1983). It has been shown that 1 μM PZQ induces a rapid increase in the rate of uptake of external Ca^{2+} into schistosomes, which is already measurable after 1 min. However, it has not yet been shown that the concentration of Ca^{2+} ions actually increases within schistosome muscle cells when they contract under the influence of the drug.

PZQ does not act like an ionophore because it does not transfer Na^+ or Ca^{2+} ions from an aqueous medium into an organic phase, although some ionophores (X537 and A23187) were shown to induce contraction in schistosomes (Pax et al. 1978). Another possibility that might have explained the action of PZQ on Ca^{2+} flux was ruled out when it was found that the Ca^{2+} channel blocker D-600 did not prevent PZQ-induced muscular contraction in schistosomes (Fetterer et al. 1980). Furthermore, La^{3+} and Co^{2+} ions, known to interfere with Ca^{2+} binding and flux across biological membranes in experiments with other tissues, were also without effect on

PZQ-induced contraction (Pax et al. 1978). Fluoxetine (FXT), a specific inhibitor of serotonin uptake in mouse brain and in schistosomes, was the only pharmacological agent that interfered with both the influx of Ca^{2+} and the induction of musculature contraction. All these experiments suggest that PZQ affects Ca^{2+} flux across biological membranes by interaction with sites not classically associated with the regulation of Ca^{2+} transport (Andrews 1985).

8.4.6 Coupling Between Tegument and Muscle Cells

PZQ exerts its effects within the tegument and muscle cells of schistosomes (Redman et al. 1996). As mentioned above, the surface of the tegument is a highly complex double bilayer, in which lipids and proteins may be organized into domains characterized by different properties and compositions (Redman et al. 1996). Indeed, there are differences in the distribution and extent of PZQ-induced damage to the surface of different life cycle stages of schistosomes observable. Moreover, domains in the double-bilayered surface membranes show different responses to PZQ: in one domain fluidity was increased, while in the other it was decreased (Lima et al. 1994a, b). These differences in susceptibility to PZQ suggest that membrane composition may be an important factor in the drug's action (Redman et al. 1996).

The PZQ induced Ca^{2+} influx across the tegument followed by a muscular contraction almost 10 min after drug exposure may be explained by the draining of sequestered Ca^{2+} from pools within schistosomes and existence of PZQ-sensitive sites present in the worm's tegument (Redman et al. 1996). The presence of these PZQ-sensitive sites in the surface would explain why the tegument is so susceptible to PZQ-induced damage (Redman et al. 1996).

Moreover, the tegument is electrically coupled to muscle cells. Therefore, an increase in intrategumental Ca^{2+} might lead to increased Ca^{2+} in sarcoplasmic reticulum, which then could induce muscular contraction (Redman et al. 1996). The electrical coupling between tegument and muscle cells would explain why any tegumental changes will immediately produce changes in muscle cells. This means that PZQ may produce muscle contraction by interacting with tegument rather than affecting muscle cells directly (Redman et al. 1996). The anatomical basis between the tegument and muscle cells could be due to junctional complexes (Redman et al. 1996). However, PZQ-sensitive sites are also present in muscle cells. Redman et al. (1996) conclude that the relationship between PZQ and Ca^{2+} influx is mediated by Ca^{2+} permeable ion channels located in the membranes of the tegument and muscle cells. This view of the mechanism of action did not change until now, since Ca^{2+} channels in schistosomes are the only sites identified so far as the molecular target of PZQ resulting a toxic influx of the Ca^{2+} into the worm (Doenhoff et al. 2008). However, a big problem is that the evidence for this model remains indirect. Nevertheless, this remains the most attractive model

among the numerous hypotheses that have been advanced on PZQ mechanism of action until now (Cioli et al. 2012).

8.4.7 *Tegument-Mediated Effects of Praziquantel on Carbohydrate Metabolism of* Schistosoma mansoni

Although PZQ-induced alterations of carbohydrate metabolism belong to the so-called secondary effects of this drug, it was from a study of these secondary effects that the next step towards unraveling the mechanism of action of PZQ developed.

As pointed out above, tegumental vacuolization and contraction are very rapid effects and regarded as primary effects of PZQ. The finding that FXT interferes with the ability of PZQ to induce contractions led to an investigation of the effect of FXT and other cationic amphiphilic drugs, so-called membrane-active compounds and PZQ on the carbohydrate metabolism of *Schistosoma mansoni* (Harder et al. 1987a, b). The amphiphilic cationic drugs chlorpromazine, imipramine, amitriptyline, propranolol, and fluoxetine and the electrically neutral PZQ all stimulate glucose uptake and lactate excretion in a qualitatively similar way. Quantitatively, however, there are significant differences. On the one hand, the five amphiphilic drugs exert almost identical alterations, with a maximal stimulatory effect at concentrations from 10 to 100 μM and inhibitory effect at 1 mM. PZQ, on the other hand, is much more effective, being maximally stimulatory at 0.1 μM and inhibitory above 1 μM. Combination experiments during which schistosomes were first incubated with chlorpromazine or FXT at a concentration of 50 μM followed by the addition of 0.1 μM PZQ did not result in a reduced stimulation of glucose uptake and lactate excretion. Addition of higher concentrations of PZQ, however, abolished the chlorpromazine-induced stimulatory effects. This can be explained by a higher affinity of PZQ than that of chlorpromazine for the same binding site, the tegumental membranes.

The combined effects of serotonin and PZQ on the carbohydrate metabolism were also studied, because serotonin receptors had been implicated as possibly mediating the effects of PZQ (Harder et al. 1987a, b). The stimulatory effect of serotonin on glucose uptake and lactate release was not observed in parasites that had already been stimulated by the addition of 1 mM fluoxetine or 10 μM PZQ. At lower concentrations of both drugs, serotonin can exert its stimulatory effect. As one of the amphiphilic cationic drugs studied, FXT is a specific inhibitor of serotonin uptake. It has been found that PZQ inhibits serotonin uptake of *Schistosoma mansoni* as effectively as FXT. Both drugs inhibit the Na^+-dependent and the Na^+-independent serotonin uptake by approximately 40 % at a concentration of 10 μM. At the same concentration, PZQ also inhibits glucose uptake and lactate excretion, while FXT is not inhibitory but enhances glucose uptake as well as lactate excretion. This indicates that PZQ is not a specific inhibitor of serotonin

uptake (Harder et al. 1987a, b). Its inhibitory effect is presumably caused by perturbation of the tegumental environment of receptor proteins. A similar explanation has been suggested for the inhibitory effect of PZQ on two other tegumental enzyme systems: the ATPase of *S. mansoni* (Nechay et al. 1980) and different phosphatases of the cestode *Cotugnia digonophora* (Pampori et al. 1985).

Amphiphilic cationic drugs, such as chlorpromazine, are known to affect different membrane-associated processes. They stabilize membranes, as seen by the decrease of relative hemolysis and replacement of Ca^{2+} ions from membranes, and they release serotonin from rat mast cells indicating a change in the permeability to uncharged solutes through membranes. A certain range of concentrations (30–100 μM chlorpromazine) is known as the range of prelysis, and it is just in this range of concentrations that chlorpromazine induces maximal glucose uptake, lactate excretion, and also maximal motility of *S. mansoni*. As PZQ and the amphiphilic cationic drugs have qualitatively similar effects on carbohydrate metabolism, it is not unreasonable to assume that PZQ is also a membrane affecting drug.

8.4.8 Praziquantel–Phospholipid Interactions

8.4.8.1 In Vitro Experiments

First investigations into the interactions between PZQ and phospholipids were conducted with liposomes by electron microscopy. The following observations were made: liposomes of dipalmitoylphosphatidylcholine (DPPC) in presence of imipramine, fluoxetine, or PZQ (molar ratio lipid/drug 2:1) do not have contact with each other. In the presence of Ca^{2+} (molar ratio lipid/Ca^{2+} 2:1), liposomes appear to be close together, but addition of imipramine, FXT, or PZQ hinder a close contact of liposomes. The situation is quite different when liposomes of the acidic dipalmitoylphosphatidylglycerol (DPPG) are examined. While these liposomes are not in close contact to each other in presence of imipramine or FXT, in the presence of PZQ liposomes are in close contact to each other and stacked structures appear. Addition of Ca^{2+} to the liposomes, in which PZQ is present, leads to heavy aggregation and perturbation of liposome membranes (Fig. 8.3). Single liposomes or liposome borders cannot be observed any more. By contrast, liposomes in presence of imipramine or FXT remain as single liposomes with now close contact to each other.

Our observations had been supported by investigations which had shown that PZQ is able to destabilize liposomes of DPPC by insertion into the bilayer (Schepers et al. 1988). Unfortunately, these experiments were conducted without investigating liposomes of acidic phospholipids and the simultaneous influence of Ca^{2+}.

The aggregation and perturbation of acidic DPPG liposomes in presence of PZQ and Ca^{2+} occurs immediately after addition of Ca^{2+} to liposomes and strikingly

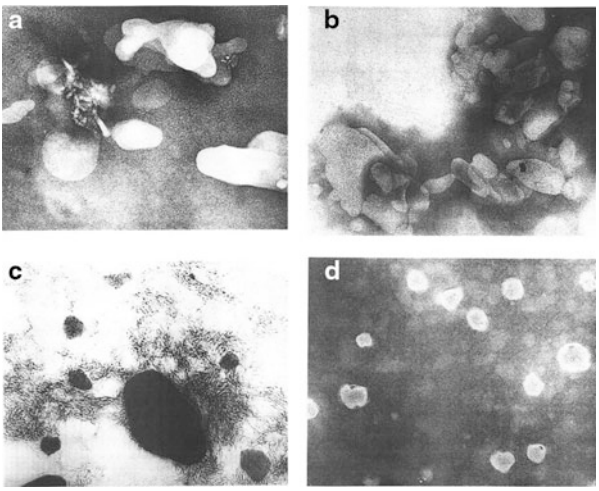

Fig. 8.3 Electron micrographs of dipalmitoylphosphatidylglycerol liposomes in presence of Ca^{2+}-ions without addition (**a**), with PZQ (**b**), with fluoxetine (**c**) or imipramine (**d**). Molar ratio lipid/drug/Ca^{2+} 2:1:1 (magnification ×260,000)

resembles the time course and morphological alterations seen in the schistosomal tegument after PZQ exposure. These in vitro observations then led to further investigations into interactions of phospholipids and PZQ using other experimental techniques.

8.4.8.2 Effects of Praziquantel on Model Membranes

Ca^{2+} ions are generally known to be involved in motility, muscle contraction, and regulation of carbohydrate mechanisms and other cellular processes. Furthermore, maintenance of membrane structure and function are governed by these cations. Therefore, regarding schistosomes, it appears reasonable to assume that those effects of PZQ which can be observed or recorded rapidly (contraction, permeability changes, and tegumental damage), and those processes that can only be measured after minutes or hours (changes in carbohydrate metabolism, serotonin uptake or enzyme activities), are sequelae of the same primary effect: changes in the distribution of Ca^{2+} within the parasite that result from altered membrane properties. This conclusion is supported by the observation that four events—therapeutic efficacy against schistosomes in animal studies, induction of tegumental surface damage, muscular contraction and paralysis, and Ca^{2+} influx—are all maximal in the presence of the levorotatory stereoisomer, while the dextrorotatory stereoisomer of PZQ is virtually devoid of activity. This line of reasoning led to the next step toward understanding the mechanism of action of PZQ, a step that was achieved by studying the interactions of synthetic phospholipids with PZQ, FXT, and other amphiphilic cationic drugs.

The investigations required the use of a model membrane, because tegumental phospholipids of *S. mansoni* can only be prepared in very small amounts. Further, the exact composition of these lipids and their distribution across the schistosomal tegument are not known. The best approximation is derived from the composition of the multilaminate vesicles, which are contained in the tegument and which are thought to be incorporated into the surface of schistosomes; at least 25 % of the total phospholipids of multilamellar bodies of *S. mansoni* are acidic ones: mainly phosphatidylserine (PS) (15 %), phosphatidylglycerol (PG) (8 %), phosphatidylinositol (5 %), and phosphatidic acid. Neutral phospholipids consisting of phosphatidylethanolamine (PE) (25 %) and phosphatidylcholine (PC) (28 %) are the main fraction (McDiarmid et al. 1982). The initial studies were conducted using differential thermal analysis, a method which allows the measurement of stability of phospholipid membranes (e.g., acidic PG) in the presence of different drugs and ions. A second method used was electron microscopy, which reveals possible structural alterations of the model membranes.

The amphiphilic cationic drugs imipramine and FXT increase the fluidity of acidic PG membranes, while PZQ has only a small effect. The Ca^{2+}-induced stabilization is much lower in the presence of the positively charged drugs than in the presence of PZQ, which has no net charge. Electron microscopy reveals that the amphiphilic drugs do not alter the structure of acidic phospholipid vesicles, while PZQ induces a drastic alteration in the organization of acidic phospholipid vesicles, which then appear to be clumped together (Harder et al. 1986).

With respect to the alteration of membrane properties, it is quite obvious that there are important differences between PZQ, which is electrically neutral, and the amphiphilic drugs, which carry positive charges. The latter are able to displace Ca^{2+} ions from membranes and thus reduce the amount of Ca^{2+} ions bound to membranes, while PZQ enhances the permeability of Ca^{2+} ions through membranes (Fig. 8.4). This difference of effect on membrane-associated Ca^{2+} ions could explain why the paralysis induced by these drugs in schistosomes is spastic in the case of PZQ and flaccid in the case of the amphiphilic drugs. Spastic paralysis is provoked by the influx of Ca^{2+} ions, while the inability to contract, which results from reduced binding of Ca^{2+} ions to the membranes, leads to flaccidly paralyzed schistosomes. Under the electron microscope, PZQ appears to induce fusion processes between acidic phospholipid vesicles. Such phenomena are usually associated with, and can be only explained by, changes in the organization of the phospholipid molecules.

To study possible structural alterations of phospholipids that could explain how membranes change their permeability to ions, ^{31}P-nuclear magnetic resonance spectroscopy has been used. This technique allows the study of the effect of drugs, ions, and temperature on the transition between three different phases that a phospholipid can adopt: the bilayer phase, in which biological membranes are normally organized; the isotropic phase (vesicles, inverted vesicles, micellar, cubic or rhombic structures); and the hexagonal phase. The latter consists of hexagonically packed lipidic cylinders in which the polar headgroups are oriented toward an internal aqueous channel of nearly 20-Å diameter. This hexagonal

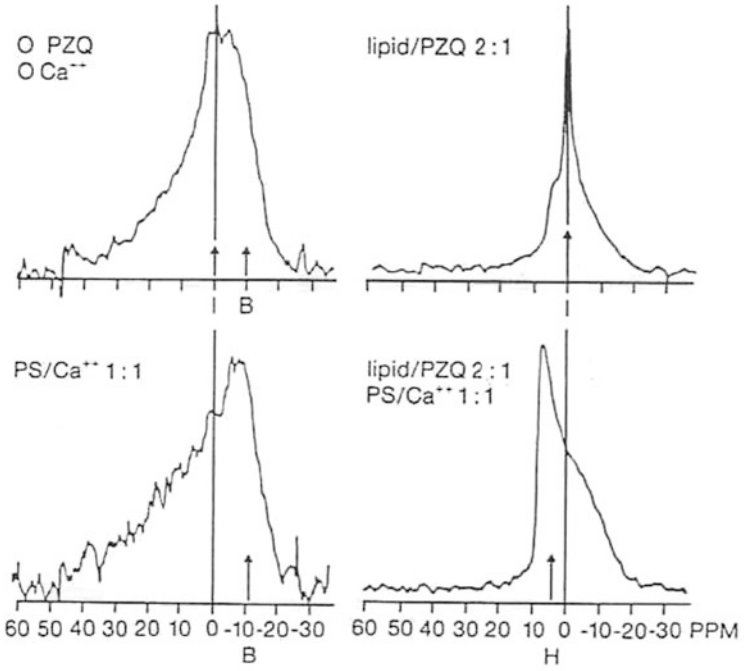

Fig. 8.4 31P-NMR-spectra of mixtures of egg-yolk phosphatidylethanolamine and bovine brain phosphatidylserine (PS) (molar ratio 2:1) without any addition; after addition of praziquantel (PZQ) (lipid/drug molar ratio 2:1); after addition of Ca^{2+} (PS/Ca^{2+} molar ratio 1:1); and after the simultaneous addition of praziquantel and Ca^{2+} (lipid/drug molar ration 2:1, PS/Ca^{2+} molar ratio 1:1) at 25 °C. The *arrows* indicate the position of the signals obtainable from the three phases: *B* bilayer, *H* hexagonal, *I* isotropic

organization of lipid molecules cannot maintain a permeability barrier which is otherwise well maintained by a membrane in the bilayer phase (Cullis et al. 1985a, b). The formation of hexagonal structures is known to facilitate the fusion of membranes, a process which is involved in secretion and is thought to be the basis of the rapid vesiculation and vacuolization occurring in the schistosomal tegument in the presence of PZQ.

A phospholipid dispersion consisting of PE and PS with a molar ratio of 2:1, which corresponds to the assumed composition of tegumental lipids in schistosomes, gives a mixture of isotropic and bilayer signals, i.e., the isotropic and bilayer phases coexist (Fig. 8.4). When Ca^{2+} ions are present, a mixture of hexagonal and bilayer phases exists. The addition of PZQ to the phospholipid mixture (molar ratio 1:1) results in the formation of a pure isotropic phase. The simultaneous presence of PZQ and Ca^{2+} ions gives rise to a complete transition to the hexagonal phase when the experiment is performed at 35 °C. The ratio of lipid to Ca^{2+} is not critical—it can range from 4:1 to 1:1. The degree of transition to the hexagonal phase is less marked when the lipid mixture contains an increased

amount of PE. At a molar ratio of PE to PS of 4:1, there is only a partial transition to the hexagonal phase at 25 °C, while there is a pronounced but not exclusive transition to the hexagonal phase at 35 °C.

These experiments show that PZQ is capable of inducing conformational transitions in artificial phospholipid membranes known to be associated with an increase in permeability to ions under conditions that attempt to simulate the natural situation: a molar ratio of PE to PS of 2:1 (as is presumed to exist in the tegumental lipids of schistosomes), the presence of Ca^{2+} ions, and physiological temperatures of 25 and 35 °C. There is no proof yet that similar transitions occur in natural schistosomal membranes. Electron microscopy after freeze fracture preparation of schistosomal membranes may reveal such changes. However, concentrations of PZQ as low as 3 μM (equivalent to a lipid to drug ratio of 50:1) have still resulted in the formation of hexagonal phases. This is in good agreement with the threshold concentration in serum of 1 μM which has been derived from therapeutic experiments.

In the absence of Ca^{2+}, the amphiphilic drugs FXT and imipramine behave like PZQ. All three drugs induce an isotropic phase when added to the phospholipid mixture. The further addition of Ca^{2+} ions, however, reveals important differences. FXT also induces hexagonal structures but fails to increase membrane permeability. This apparent discrepancy, induction of hexagonal structures without concomitant permeability increase can be resolved, however. FXT is positively charged and imparts this charge to the internal aqueous channel of the hexagonal structures. Thus, cations are repelled and this effectively prevents the permeation of Ca^{2+} ions through the internal channel (Harder et al. 1988). Imipramine, another positively charged amphiphilic drug, induces lamellar structures in the presence of Ca^{2+} ions. Again, positive charges are imparted to the lamellae with the result that Ca^{2+} ions are displaced from the membrane. In order to cause an increase in the ion permeability of the phospholipid membrane, it is not sufficient for a drug to induce hexagonal structures alone. The charge which the drug imparts is important, too. An increase in permeability to positively charged ions is only achieved if the hexagonal phase is induced by an electrically neutral drug like PZQ.

A crucial question is whether PZQ has the same stereospecificity of the effect in the artificial phospholipid system employed as it is known to have against schistosomes in host organisms. It has been found the levorotatory stereoisomer is slightly more effective than the dextrorotatory stereoisomer in inducing the transition of hexagonal phases. This lack of a profound difference in the effects of the two steric forms of PZQ is the reason for postulating the existence of a stereospecific link between membrane and drug. This link is likely to be a receptor protein. Such a receptor has been described for an antischistosomally active benzodiazepine, a compound which induces influx of Ca^{2+} and muscular contraction in a way similar to PZQ (Pax et al. 1978; Bennet 1981). The postulated PZQ receptor of schistosomes remains to be characterized. Such a stereospecific PZQ receptor should also occur in other trematodes and cestodes and should not be found in nematodes and mammals. The occurrence of a receptor could then explain why the

effects of PZQ are essentially restricted to those parasitic helminths possessing a syncytial tegument.

8.4.8.3 Secondary Effects of Praziquantel

There are numerous other effects which only become apparent after several minutes or hours after exposure of PZQ (Table 8.2). These secondary effects include changes in the ionic composition of the parasite, inhibition of various enzyme activities, changes in carbohydrate metabolism (inhibition of glucose uptake, and decrease of glycogen content of cestodes and trematodes), protein and the nucleotide metabolism, and depolarization of the schistosome tegument (Andrews et al. 1983; Andrews 1985).

Many of these secondary processes affected by PZQ can be related to the primary effect of the drug on the tegument (antigen exposure, interference with the uptake of glucose and ATP, interference with the ouabain receptor, and reduction in ATPase content), while others can be related to the sustained contraction (decrease in glycogen and increase in lactate excretion). Antigen exposure and reduction in the content of ATP, one of several membrane-bound tegumental phosphohydrolases of *S. mansoni*, uptake processes, especially of the apparently Na^+-coupled transport of hexoses, could be explained by the interference of PZQ with the ouabain receptor, which represents Na^+/K^+ pump sites. Whether PZQ is directly involved in causing these changes, whether they are mediated via identified physiological lesions, has not been explored. From all the data available, one can conclude that nucleotide metabolism and nucleic acid and protein syntheses are unlikely to be involved in the antischistosomal action of PZQ. PZQ at 10 μM affected the rate of glycolysis of *S. japonicum* only slightly. The ratio of carbohydrate (glucose and glycogen) consumed to lactate produced remained at one.

The ultrastructural effects of PZQ are not limited to the rapidly appearing surface blebs and vacuolization of the tegument. Structural lesions have also been described from the subtegumental parenchyma, the subtegumental and gastrodermal musculature, the vitellaria, and several cellular inclusion bodies of *S. japonicum* and *S. mansoni* recovered from mice treated with PZQ (Andrews et al. 1983; Andrews 1985). The time course of the appearance of most of these changes has not been studied in detail, but it appears that they are slower to develop than the tegumental damage, and they are thus regarded as secondary effects.

Inhibition of nucleoside uptake was shown to be another, up to now unknown PZQ-target in schistosomes (Angelucci et al. 2007). This effect is seen in schistosomes but is absent in mammalian cells in culture. Interestingly, it is a specific pharmacological effect seen exclusively with the active levo-R(-)stereoisomer of the drug and is shared by at least one benzodiazepine having antischistosomal activity. This novel effect acquires significance given that schistosomes cannot synthesize purine nucleosides de novo. A direct relationship between inhibition of nucleoside uptake and the hypothetical action of PZQ on Ca^{2+} channels in schistosomes is not shown up to now. Another explanation could be that

adenosine uptake as a membrane-associated process is impaired by PZQ in a similar way as glucose uptake and lactate excretion.

Ca^{2+} channels have been suggested as the target, although direct interaction of PZQ with their subunits was never demonstrated. Heterologous expression data has implicated an action of PZQ on voltage-operated Ca^{2+} channels, although the relevant in vivo target of this drug has remained undefined over three decades of clinical use (Chan et al. 2012). Overall, the huge amount of broad information available underscore the common view of PZQ-induced disruptions of Ca^{2+} homeostasis in trematodes and cestodes.

8.5 Praziquantel Resistance

Although PZQ is widely used, there is no clinical relevance and evidence for resistance against this drug in schistosomiasis (Doenhoff et al. 2008). However, very low cure rates have been recorded in some studies in Africa (Doenhoff et al. 2008; Wang et al. 2012). Thus, the number of treatment failures is very high. There are arguments that the standard dose of 40–60 mg/kg may be subcurative, or treatment failures may be due to the presence of immature worms, or other failures may be due to incomplete drug intake or to peculiar metabolic conditions of individual patients. It cannot be excluded that some of these treatment failures are due to the real existence of parasites that are genetically drug resistant, but then one would expect that the progeny of resistant worms would gradually expand in the environment, especially in cases of repeated drug pressure (Doenhoff et al. 2008). So far, there is no evidence for this. Schistosomes with a decreased sensitivity to PZQ have shown "resistance" at always modest level and moreover were often unstable and could not be increased by repeated drug pressure (Doenhoff et al. 2008). The same is true for a *S. mansoni* strain that acquired some degree of PZQ insensitivity after repeated drug selection in laboratory mice. It has been suggested that the observed degree of PZQ insensitivity may be due to modifications of drug influx–efflux mechanisms, rather than to modifications of the crucial drug target(s), and some experimental evidence in this direction has been obtained (Doenhoff et al. 2008). In summary, although minor variations of PZQ sensitivity undoubtedly occur, they are not at a level that may threaten clinical efficacy, and there is no evidence that any "PZQ resistance" is spreading in the field (Doenhoff et al. 2008).

Nevertheless, to avoid possible disasters in future, alternative antischistosomal drugs should be developed, especially since PZQ is far from being a perfect drug (Doenhoff et al. 2008). Since PZQ was developed in 1970s, it has replaced other antischistosomal drugs to become the only drug of choice for treatment of human schistosomiasis, due to high efficacy, excellent tolerability, few and transient side effects, simple administration, and competitive cost. PZQ-based chemotherapy is part of global control strategy of the disease and led to the control strategy shifting from disease control to morbidity control, which has greatly reduced the prevalence and intensity of infections (Doenhoff et al. 2008).

As the exact mechanism of action of PZQ is not known today, the mechanism of PZQ resistance in schistosomes also remains unclear. Despite these problems, a new model for the action of PZQ is presented in the following section.

8.6 Model for the Mechanism of Action of Praziquantel

Schistosomes take up PZQ rapidly through the tegument and shortly afterwards the PZQ concentration inside the schistosomes equals that of the medium (Andrews et al. 1983). Bleb-like structures can be seen from the outside after exposure of the drug and are described as protusions of the surface membrane; however, the outer surface of the tegumental membrane appears unchanged and the surface coat remains intact (Becker et al. 1980). This tegumental blebbing is followed by enhanced vacuolization, which develops from the basal region of the tegument. This is undoubtedly the first event observable after PZQ exposure.

The process of vacuolization and ballooning of the tegument continues and eventually parts of the tegument are sloughed off. Similar tegumental alterations are not induced only by PZQ but also occur in schistosomes exposed to many different compositions or compounds (Becker et al. 1980; Andrews et al. 1983). It thus appears that the morphologically visible damage is not related to PZQ but is a general reaction of the schistosomal tegument to noxious agents. However, the PZQ-induced alterations differ in intensity and velocity significantly from that induces by the other compositions or compounds.

Another further important observation is that there are domains in the double-bilayered surface membranes, which show different responses to PZQ reflected by different fluidity behaviors (Lima et al. 1994a, b). These differences in susceptibility to PZQ suggest that membrane composition may be an important factor in PZQ action (Redman et al. 1996).

It therefore seems that the very first effect of PZQ is blebbing and vacuolization in the lower parts of the tegument, but not at the surface. This would make it less probable that Ca^{2+} channels or any enzymes such as adenosine uptake transporters located in the outer tegumental layer serve as the first drug target for PZQ (Redman et al. 1996; Angelucci et al. 2007; Doenhoff et al. 2008). Indeed, it could be shown long time ago that PZQ action was not antagonized by the Ca^{2+} channel blocker D600 (Fetterer et al. 1980), but it was argued that other Ca^{2+} channels, not inhibitable by D600 are functioning (Redman et al. 1996). However, until now effects of PZQ on Ca^{2+} channels were described to be indirect (Doenhoff et al. 2008).

In the new model for the PZQ action, this drug comes in close contact with acidic phospholipids present in the tegumental membranes and domains. In presence of Ca^{2+} ions, the normal structure of the membranes becomes perturbated by PZQ (Fig. 8.5). PZQ induces hexagonal structures, which are permeable to different cations inclusive Ca^{2+} ions. By contrast, imipramine or fluoxetine, two amphiphilic

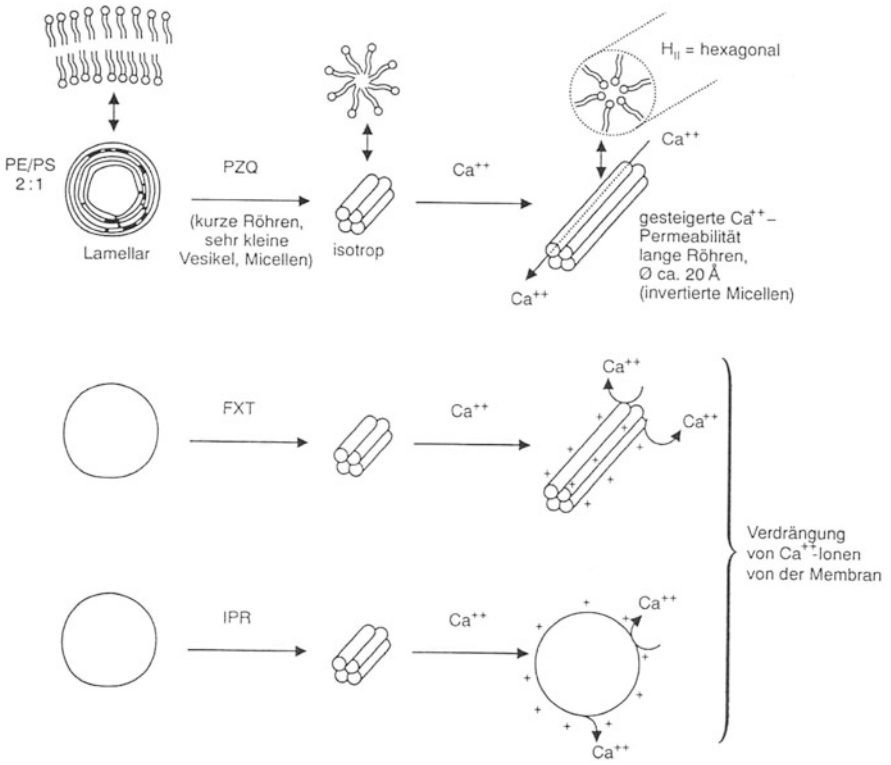

Fig. 8.5 Model for the interaction of praziquantel (PZQ), fluoxetine (FXT), and imipramine (IPR) with phospholipid membranes and Ca^{2+}-ions

cationic drugs, release Ca^{2+} ions from the membranes and leave the bilayer structure intact (Fig. 8.5).

Moreover, synthetic acidic phospholipid membranes get completely perturbated in presence of PZQ and Ca^{2+} ions as shown in Fig. 8.3b. Such an effect would result in complete perturbations of schistosomal tegumental membranes followed immediately by disturbance of homeostasis of Ca^{2+}, K^{+}, and Na^{+} ions and also by disturbance of the integrity of normal enzyme functions (Fig. 8.6).

It is possible that PZQ not only impairs membrane domains by inserting into the phospholipid layers but PZQ may also impair proteins present in these domains. It is reasonable to suggest that PZQ could bind to proteins first and thereafter penetrates into the phospholipid surrounding of the binding protein resulting in the complete perturbation of this part of membrane of the tegumental domain (Fig. 8.6).

This view of PZQ action may also help to explain why after more than 40 years of drug use there is no real resistance against the drug arisen. The PZQ action is nearly instantaneous and directed against distinct domains in the tegument and PZQ may bind here not at one binding site, but at a variety of different proteins and/or

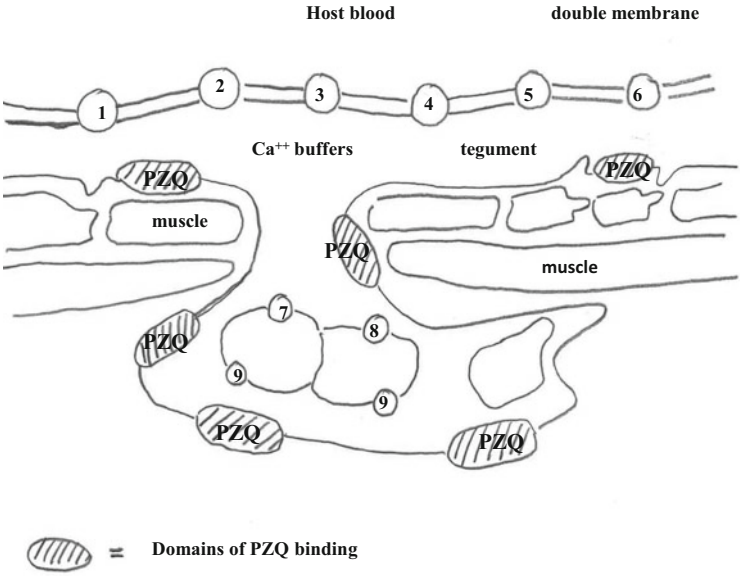

Fig. 8.6 Model for the mechanism of action of praziquantel (PZQ) in schistosomes (adapted from Redman et al. 1996)

phospholipid "receptors" at a site in the worms, which are needed for processes such as sloughing off and shedding membranes and necessary for extrusion of noxious compounds and compositions. This is in line with the view that until now the "real" PZQ protein target has not been found, even after now 40 years after the discovery of this drug.

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Chapter 9
Treatment Methods of Traditional Chinese Medicine for Schistosomiasis and Other Trematode Infections

Zhongdao Wu and Xi Sun

Abstract Disease caused by trematodes infections, such as the *Schistosoma*, *Clonorchis sinensis*, and *Paragonimus westermani* which caused the chronic disabilities, remains a global public health problem in a considerable part of the world. Also, we know vaccines are the mainstay of prophylactic treatment for infection disease. Unfortunately, until now, there is no effective vaccine to anti-schistosomiasis and other trematode infections; treatments of these diseases also rely on the effective drugs. Since praziquantel, a highly effective and safe anti-schistosomal drug, was developed, it has nearly replaced all other antitrematodes agents to become the only useful drug of choice for treatment against these diseases due to high efficacy, excellent tolerability, simple administration, and competitive cost. However, praziquantel also has many unignored side effects such as the drug resistant. Thus, it may need urgent further development for search some other safety and useful antitrematode alternatives. Traditional Chinese medicine commands a unique position among all traditional medicines, which have over 5,000 years of history. Traditional medicine focuses on the use of herbs and traditional Chinese medicine such as *artemisinin* and *artesunate* has performed well in clinical practice and shows potential in the therapy of Schistosomiasis and other trematodes diseases. Here, in this chapter, we attempt to briefly review some constituents and extracts from plants and traditional Chinese medicine, which have the activity against schistosomiasis and infections with other trematodes.

Keywords *Schistosoma japonicum* • Artemisinin • Artesunate • Astragalus • Danggui Buxue decoction • *Bidens bipinnata* • Curcumin

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9.1 Introduction

Schistosomiasis is a notable neglected tropical disease caused by trematodes (trematode worms), which deposit eggs in blood vessels surrounding the bladder or gut of the infected host (Ross et al. 2001). Schistosomiasis remains endemic to at least 76 tropical and subtropical countries. 200 million people have this disease; 120 million of them have symptoms, 20 million have severe illness, and 600 million others remain at risk (Chitsulo et al. 2000). Four species routinely infect the human host and several others rarely do so. The majority of *Schistosoma haematobium* and *Schistosoma mansoni* infections are found in Sub-Saharan Africa. *Schistosoma mansoni* remains endemic in parts of Brazil, Venezuela, and the Caribbean. *Schistosoma japonicum* infection occurs in China, Indonesia, and the Philippines; *Schistosoma mekongi* is found in Cambodia and Laos, along the Mekong River Delta (Patz et al. 2000).

In China, *Schistosomiasis japonica* is the most severe zoonosis infecting about 360,000 people and about 1 % buffalo and/or cattle in endemic regions, particularly in lake/marshland and hilly areas of Hubei, Yunan, Anhui, Jiangxi, and Jiangsu Provinces and mountainous areas of Sichuan and Yunnan province (Hao et al. 2010).

Trivalent antimonial drugs were developed in 1918 and became the first of a long list of somewhat toxic drugs used to treat schistosomiasis in China (Sleigh et al. 1998). In the 1970s, the Bayer AG and E. Merck drug companies developed a new antischistosomal pyrazino–isoquinolin drug known later as praziquantel (PZQ) (Ross et al. 2001). Since then, control of this disease mainly depends on chemotherapy, with praziquantel which is active against all *Schistosoma* species and the recommended drug by the World Health Organization for schistosomiasis treatment at either the community or individual level, has become the exclusive drug because of its low cost and efficacy against the adult form of all *Schistosoma* species. However, praziquantel also has many side effects. Extensive use of praziquantel with concerns about the possibility of drug-resistance development, praziquantel unavailability of an applicable vaccine, and the absence of a reasonable alternative to praziquantel all represent a real challenge. Thus, it may urgently need further investigations for search some other safe and useful antischistosomal alternatives or to incorporate some other alternatives in combination therapies (King et al. 2000; Alonso et al. 2006; Doenhoff et al. 2008; Liang-Xian et al. 2013).

In additional to *S. japonicum*, some other trematodes such as *Clonorchis sinensis* and *Paragonimus westermani* are also very popular in China. *Clonorchis sinensis* is major food-borne parasites in China and relatively heavily transmitted in the Zhujiang River Delta, including Hong Kong and Macao, and Northeast China, where many Korean people live (Chen et al. 2010). The transmission is related to the unhealthy habits of residents who like to have raw fish or half-raw fish. The infection of *C. sinensis* could result in serious liver and biliary system damages, and chronic cases may induce liver cancer (hepatocarcinoma) and bile duct cancer (cholangiocarcinoma) (Fried and Abruzzi 2010). However, human paragonimiasis

is caused by trematodes of the *Paragonimus westermani*, which parasite in the lungs of humans. This disease is mainly endemic in China, Korea, Japan, and some other Asian countries, where people have the habit of eating raw or undercooked freshwater crabs or crayfish, which may be infected with infective Paragonimus metacercariae (Kirino et al. 2009). *P. westermani* caused pulmonary, neurologic, and abdominal diseases by infecting lungs, brains, spinal cords, and other organs in humans and animals including dogs, tigers, cats, pigs, and some other animals (Liu et al. 2008). Like the antischistosomiasis, the most useful effect of anticlonorchiasis and antiparagonimiasis is chemotherapy with antihelminthic drugs such as praziquantel, albendazole, etc.

Traditional Chinese medicine commands a unique position among all traditional medicines because of its 5,000 years of history. Here, this comprehensive chapter shall attempt to briefly review the recent advances in some natural products from traditional Chinese medicine, which have the activity against schistosomiasis and infections with other trematodes.

9.2 Artemisinin and Artesunate

Artemisia annua (or named Sweet wormwood herb) is a herb produced in many areas of China such as Jilin, Liaoning, Hebei, Shanxi, Shandong, Jiangsu, Anhui, Zhejiang, Jiangxi, Fujian, Henan, Hubei, Hunan, Guangdong, Guangxi, Sichuan, Guizhou, and Yunnan. Artemisinin (Figs. 9.1 and 9.2) is a natural product derived from the Chinese herb Artemisia annua, which was first identified by Tu Youyou, a Chinese scientist from the Chinese Academy of Traditional Chinese Medicine (Beijing, China) in 1972 (Tu 1999). Artemisinin was firstly used for antimalarial and Artemisinin, artesunate, and additional derivatives also are the most promising candidate compounds to ease the worldwide malaria burden (Adjuik et al. 2004).

The antischistosomal properties of artemisinin were first reported by Chen et al. in 1980. Two years later, the first report of artemether's activity against younger worm of *S. japonicum* was published, and the artesunate's antischistosomal activity against *S. japonicum* was also announced in the following year by the same group of Chinese (Le et al. 1982, 1983). A ctivity of artemether against *S. mansoni* in mouse was reported by Xiao's and its activity against *S. haematobium* was reported in hamster (Xiao and Catto 1989; Xiao et al. 1995). Meanwhile, artesunate's activities against *S. mansoni*, *S. mekongi*, and *S. haematobium* were noted by many scientists (Araújo and Katz 1999; Borrmann et al. 2001; De Clercq et al. 2002; Utzinger et al. 2002; Inyang-Etoh et al. 2004). Laboratory studies also showed that administration of artemether or artesunate results in reduction of worm glycogen and protein content, inhibition of ATPase activities, inhibition of related enzymes involved in glycolysis, and impacts on worm antioxidant system (Xiao 2005).

Except for the function of artemether and its derivatives against schistosomes (Utzinger et al. 2007; Liu et al. 2012), artemether and artesunate also have been

Fig. 9.1 The plant of *Artemisia annua*

Fig. 9.2 Traditional Chinese medicine of *Artemisia annua*

reported to be important in field application for preventing *S. japonicum* in the high-risk populations in various endemic areas of Southeast China (Song et al. 1997, 2006). The activities of artemether and artesunate to prevent infections with *S. haematobium* and *S. mansoni* have been investigated by Utzinger and N'Goran (Utzinger et al. 2000; N'Goran et al. 2003). Most clinical studies showed that the preventive efficacies of artemether and artesunate could reach up to 65–97 % when administrated with multiple doses of 6 mg/kg body weight by 1- or 2-week intervals during epidemic seasons (Li et al. 2011).

The discovery of the antischistosomal properties of artemether and artesunate and their successful applications in clinical and field trials are bringing us a new

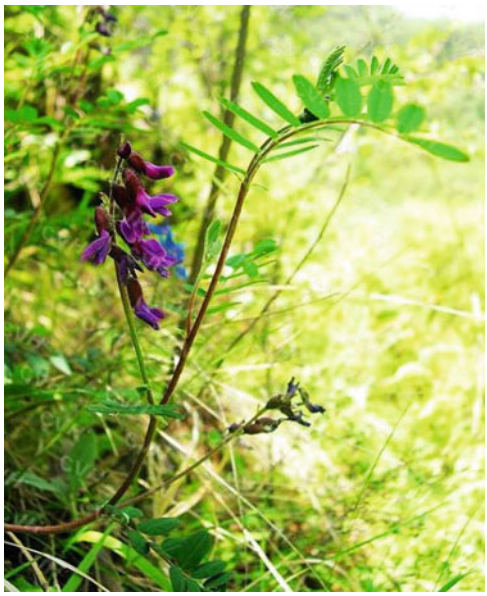

Fig. 9.3 The plant of *Astragalus membranaceus*

sight of antischistosomiasis. So, the discovery of artemisinin and the creation of its derivatives are great achievements of traditional Chinese medicine for the parasitic diseases control—not only for malaria control but also for schistosomiasis control as a preventive treatment.

9.3 Astragalus and Its Combining Forms

Astragalus, a traditional Chinese medicine, produced in North China such as in the provinces of Neimenggu, Shaanxi, Gansu, and Heilongjiang, is an immune regulator and has been clinically used (Figs. 9.3 and 9.4). *Astragalus* species are used as food by the larvae of some Lepidoptera species including the following case-borers of the genus *Coleophora*: *C. astragalella* (feeds exclusively on *A. glycyphyllos*), *C. cartilaginella* (feeds exclusively on *Astragalus*), *C. colutella*, *C. euryaula* (feeds exclusively on *Astragalus*), *C. gallipennella* (feeds exclusively on *A. glycyphyllos*), *C. hippodromica* (feeds exclusively on *A. gombo*), *C. onobrychiella* (feeds exclusively on *Astragalus*), *C. polonicella* (feeds exclusively on *A. arenarius*), and *C. vicinella*. The natural gum tragacanth is made from several species of *Astragalus* occurring in the Middle East, including *A. adscendens*, *A. gummifer*, *A. brachycalyx*, and *A. tragacanthus*. Also, *Astragalus propinquus* (also known as *Astragalus membranaceus*) has a history of use as an herbal medicine and is used in traditional Chinese medicine. Previous study showed that *Astragalus* can significantly increase the phagocytic function of mice macrophages, increase the EPO

Fig. 9.4 Traditional Chinese medicine of *Astragalus membranaceus*

levels of mice serum, promote the production, development, and maturation of the different kinds of blood cells, increase significantly the cell activity of NK cells, and increase the phenotypes and help to mature of mice bone marrow derived dendritic cells (Lu et al. 2007). Recently, its role in antischistosomal compound was also explored. For example, the schistosomicidal effect of PZQ and the efficiencies of vaccines against schistosomes could be strengthened by combining with *Astragalus*. The latter processes the effects of antihepatic fibrosis induced by schistosome eggs. Meanwhile, *Astragalus* is also confirmed to produce a good effect against schistosomula in skin (Zhu et al. 2006; Sun et al. 2008; Wang and Liang 2010).

9.4 Danggui Buxue Decoction

Danggui Buxue Decoction (DBD) is a classical formula of traditional Chinese medicines recorded in endogenous and exogenous agents of diseases in Jin dynasty (AD 1247). DBD consists of Radix *astragali–Angelica sinensis* (ml/ml, 5:1). Extensive studies showed that DBD have function in activating the blood flow and hematopoiesis (Ning et al. 2002) (Figs. 9.5 and 9.6). It has a rather strong effect to improve immune functions and reduces portal pressure. Recently, results showed that DBD can remarkably inhibit the progression of schistosomiasis-derived hepatic fibrosis (Wang et al. 2011). After treatment of the liver fibrosis and portal hypertension (PHT) induced by *S. japonicum*, values of the hyaluronic acid in the angelica roots group was significantly lower than in the control group after 12 weeks. Meanwhile, the LN level was significantly increased. The pathological observation also showed that the liver fibrosis was ameliorated after the treatment (Ding et al. 2004).

Fig. 9.5 The plant of *Radix Angelica sinensis*

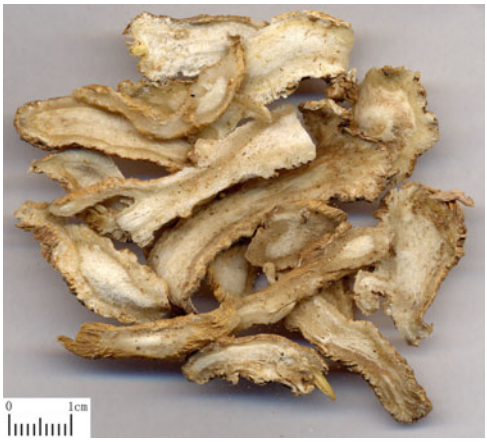

Fig. 9.6 Traditional Chinese medicine of *Radix Angelica sinensis*

9.5 *Bidens bipinnata*

Bidens bipinnata, a species of the composite, is widely distributed in majority of the provinces in China. *Bidens bipinnata* had been traditionally used for many years (Figs. 9.7 and 9.8). It is bitter in taste and has the effects of heat clearing, detoxication, and eliminating stasis to subdue swelling. It has widely used in treatment of hepatitis and malaria. Previous reports showed that it has also anti-inflammatory effects, effects to remove of oxygen-free radicals and the effect of regulation the immunity. It contains many chemical compounds such as flavonoids, enyne, coumarins, organic acids and compound esters, triterpenoids, steroids, and aetherolea. Recently, studies showed that flavones, which are extracted from the leaves of *Bidens pilosa*, have significantly inhibitory and therapeutical effects on hepatic fibrosis in mice with *S. japonicum* being explained by regulating ova and granulomatous immune response, by significantly inhibiting the TGF-b1/Smads

Fig. 9.7 The plant of *Bidens bipinnata*

Fig. 9.8 Traditional Chinese medicine of *Bidens bipinnata*

signal pathway, and by inducing apoptosis of activated HSC in vivo. Thereby the number of activated HSC and content of collagen in liver tissues was reduced (Chen et al. 2004).

9.6 *Curcuma longa*

Curcumin is an extracted natural pigment from the turmeric plant *Curcuma longa*, which is a member of the ginger family (Zingiberaceae) (Figs. 9.9 and 9.10). Curcumin has antioxidant effects, antitumor effects, anti-inflammatory effects, free radical scavenging effects, and an antimicrobial effect. Curcumin has a low toxicity and protects liver function from hepatic fibrosis. The possible mechanisms of curcumin are the raise of activity of SOD, GSH-px and reduction of the content of MDA in liver and serum. Curcumin has therefore the ability to protect liver cells and thus acts against hepatic fibrosis induced by *S. japonicum* in mice. Zhan's results showed that fibrous tissue type I and type III collagen was reduced in curcumin plus praziquantel-treated groups compared to untreated controls and

Fig. 9.9 The plant of *Curcuma longa*

Fig. 9.10 Traditional Chinese medicine of *Curcuma longa*

members of the group, which were just treated with praziquantel alone. Meanwhile, the level of serum ALT, AST, HA, and LN improved obviously in the curcumin plus praziquantel group compared to control group and praziquantel group. In conclusion, curcumin can relieve the liver fibrosis caused by schistosome.

9.7 Other Traditional Chinese Medicines in Treatment of Schistosomiasis

Salvia farinacea or named *Salvia officinalis* is an herbaceous perennial native plant in Mexico and parts of the USA including Texas (Figs. 9.11 and 9.12). This plant requires full or part-sun and will grow to 18″ or more with good soil. This plant attracts butterflies and hummingbirds. *Salvia* injection can improve the kidney perfusion, increase the urine output, and dissipated the ascite being induced by infection with *S. japonicum* (Sy 1992). Paeoniflorin can significantly lower the number of liver egg granuloma, decrease the degree of liver fibrosis and the amount of type-III collagen, and inhibit the development of blood liver fibrosis (Chu et al. 2008) (Figs. 9.13 and 9.14). Yang's reports showed that

Fig. 9.11 The plant of *Salvia farinacea*

Fig. 9.12 Traditional Chinese medicine of *Salvia farinacea*

Fig. 9.13 The plant of *Paeonifloria* (*White Paeony Root*)

Lithospermi flos lonicerae, *Stephania tetrandra*, *Notopterygium* root, *Radix isatidis*, Divaricate Saposhnikovia root, slenderstyle *Acanthopanax* bark, ash bark, Chrysanthemi flos, Chinese thorowax root, and Divaricate Saposhnikovia root 5 g, separately; Pericarpium citri reticulatae and Divaricate Saposhnikovia root 10 g, separately; *Glycyrrhiza uralensis* and *Zingiberis recens* 5 g separately have the significantly inhibitory effect on the function of cercarial penetration

Fig. 9.14 Traditional Chinese medicine *Paeonifloria* (*White Paeony Root*)

through the skin (Jx 2007). Du's results also showed that garlic oil has the ability to kill the cercariae of *S. japonicum* (Du et al. 2011). Meanwhile, some Chinese medicinal formulae also have a killing function on the schistosomes. For example, Wenyanghuoxuetang have the function to treat liver ascites resulting from *S. japonicum* (Xq 2003). Ruangan Sanjie decoction has good effect on depressing and reversing cirrhosis and raising plasma albumin (Hh 2003). Infusions of herba salviae chinensis can lighten the splenohepatomegalia caused by chronic schistosomiasis.

9.8 Traditional Chinese Medicine to Treat of Clonorchiasis

Many traditional Chinese medicines were confirmed to have effects on the treatment of *C. sinensis* infections. In the early 1980s, Chen's reports showed that *artemisinin* and its derivatives, including artemether, have effects on the rats being infected with *C. sinensis*. Artemisinin, at a daily dose of 200 mg/kg for 7 days, and artemether, given at doses of 30–60 mg/kg for 5 days, resulted in worm burden reduction of 100 and 83–100 %, respectively (Chen et al. 1983). Keiser et al.'s work also confirmed that both artemether and artesunate, when administered at a single oral doses of 75 and 150 mg/kg, are highly active against adult *C. sinensis* harbored in rats (Keiser et al. 2006). Recently, a series of animal experiments showed that single 150 mg/kg oral doses of artesunate, artemether, tribendimidine, and praziquantel administered to rats infected with adult *C. sinensis* resulted in mean worm burden reductions of 80.7 % up to 100 %, respectively. Even the dose of 37.5 mg/kg still resulted in a highly significant worm burden reduction for treatments with artesunate, artemether, and tribendimidine (71.4–100 %), but not for praziquantel (20.7 %) (Fan et al. 2005; Xiao et al. 2008).

9.9 Traditional Chinese Medicines to Treat for *Paragonimus westermani*

Artesunate has not only effects on *S. japonicum* and *C. sinensis* but also leads to damages in the other trematodes. Xing's results showed that after treatment of dogs with 5 mg/kg artesunate, adult worms of *P. westermani* were severely destroyed (Xing et al. 2007).

9.10 Conclusions

Compared to the common Western Medicine, Traditional Chinese Medicines have many advantages in the treatment of trematodes. However, most of the mechanisms of the traditional Chinese medicine in the treatment of diseases due to trematodes are not clear. In short, the traditional Chinese medicines in treatment of *S. japonicum* and other trematodes have such clear efficacy and thus are worth to be studied further.

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Chapter 10
Traditional Chinese Treatment of Taeniasis

Xiao-Yi Zou and Bin Ye

Abstract Human taeniasis is infection of adult *Taenia solium* and *Taenia saginata*, but both clinical manifestations are similar. *Taenia* infection was serious and widespread in ancient China. Chinese people had paid long attention to taeniasis in ancient medical books and tried to eliminate the infection by means of traditional Chinese medicine (TCM). Some TCM against taeniasis are summarized referring to both the historical literature and recent modern results of research in this chapter. *Omphalia lapidescens*, *Agrimonia pilosa*, *Semen moschatae*, and *Areca catechu* are involved in the anthelmintic TCM. The origin, preparation, and use of the medical plants, their known chemical components, as well as their possible pharmaceutical functions and the anthelmintic mechanisms were introduced. The dried sclerotes of *Omphalia lapidescens* are ground into powder when used as medicine. Omphalia proteinase is the most effective ingredient in natural *O. lapidescens*. Its proteolysis is able to break down the proteins of the parasitic tapeworms. Experiments have shown that the proteolysis of the parasitic proteins may induce the anthelmintic activity of *O. lapidescens*. *Agrimonia pilosa* is a perennial herb. The clean dried whole plant is prepared for medical use. Each time 30–60 g dried herbs are boiled with water to produce a lotion for orally uptake (The herbal decoction). Agrimophol is the most effective ingredient of *A. pilosa*. The anthelmintic activity of agrimophol may owe more to direct killing effect than through the nervous system way. *Semen moschatae* is the dried seed of *Cucurbita moschata* and is commonly known as pumpkin seed. The pharmacological efficacy of pumpkin seed is not very potent, and thus it is generally combined with *Areca* seed for expelling tapeworms. When the therapy begins, the infected person should eat raw pumpkin seeds 150 g on an empty stomach first, then drink the prepared liquid decoction of *Areca* 300 ml (100 g dried *Areca* seeds are boiled in water to yield 300 ml decoction in advance). Half an hour later, the adult patient should ingest 60 ml of 50 % bitter salt followed

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by plenty of water (1,000–2,000 ml) to finish the treatment. *Areca* seed is the dried seed of *Areca catechu*. Four alkaloids (arecoline, arecaidine, guvacine, and guvacoline) in *Areca* seeds are most important biological components to kill the parasite. A recent study suggested that arecoline might cause nervous paralysis of cestodes as well as inhibit the acetylcholinesterase activity. These findings may explain why *Areca* seeds can paralyze the worms in the small intestine and then expel parasites from host's intestine. In brief, TCM have been proved to be very successful in the fight against parasites, and many of them are still in use today.

Keywords Human taeniasis • Traditional Chinese medicine (TCM) • *Omphalia lapidescens* • *Agrimonia pilosa* • *Semen moschatae* • *Areca catechu* • *Omphalia* proteinase • Agrimophol • Alkaloid

10.1 General Introduction

The infection of adult worms of *Taenia solium* and *Taenia saginata* in human small intestine causes human taeniasis. The clinical manifestations of *T. solium* and *T. saginata* infections are similar. The adult worms irritate the intestine and produce abdominal pain, occasional nausea or vomiting, appetite loss, diarrhea, emaciation, eosinophilia, and weight loss. However, the muscular segments of *T. saginata* crawl and disturb more actively than those of *T. solium* when they pass out of the anus. As well the hooked scolex of *T. solium* may cause greater intestinal disturbance, pain, and inflammatory response than that is caused by *T. saginata*. Furthermore, a remarkable and clinical important aspect is the fact that the *T. solium* larva, named *Cysticercus cellulosae*, may infect humans and result in human cysticercosis,which is potentially a dangerous and life-threatening systemic infection.

Taenia infection was serious and widespread in ancient China. It had been received special and intense attention by Chinese people and had been mentioned in many old Chinese historical books over the last 2,000–3,000 years. The earliest record came from the "*Synopsis of the Golden Chamber*" in AD 217 during Han Dynasty. The "*Synopsis of the Golden Chamber*" is the oldest monograph on febrile and miscellaneous diseases in China. *Taenia* cestodes were recorded in the chapter "contraindication and indication for birds, beasts, fishes and worms." It is stated: If beef is consumed together with white liquor, it causes the birth of "inch-white" worms (He and Fan 1990). Although the knowledge on *Taenia* cestodes were not as precise as today, it had already come very close to the truth that the infections are acquired by means of ingesting raw or undercooked beef or pork containing living cysticercus stages.

"*Clarification of Various Pathogens*" is a monumental ancient Chinese medicinal work on etiology and was compiled in AD 610 by Chao Yuan-fang, who lived at the end of the Sui Dynasty. It is the first huge work in the history of development of traditional Chinese medicine, which describes systematically, scientifically, and thoroughly the causative factors, symptoms, and classification of diseases. *Taenia*

cestodes were named "white worms" in this book and became ranked as one of the "nine worms." The book described "a white worm" by "it is a short whitish worm, and its descendants may grow up and kill man. The worm is about several inches long with a flattened body and looks like a continuous string" (Ding and Ni 1991). Thus, it was clear that ancient Chinese people were aware of *Taenia* cestodes and were able to describe at that time the worm's appearance nearly correctly.

The Chinese people had not only recognized since long the significance of taeniasis, but also made attempts to control the disease by means of traditional Chinese medicine (TCM). TCM is one of the precious treasures in the splendid ancient Chinese culture, which has been handed down from generation to generation. Its acceptance by the population is largely conditioned by cultural factors. Ancient Chinese people often used various herbs for expelling worms. Many TCM therapies against *Taenia* cestodes were documented in Chinese medical historical materials, such as "*Medical Secrets from the Royal Library*" and "*Herbal Classics of the Divine Plowman* (also named *Shennong Bencao Jing*)." The latter is known as "the Canon of Chinese material medicine" being written in the second century BC under the pseudonym of Shennong, the holy Farmer. The traditional Chinese therapeutic remedies include singly used herbs and some detailed prescriptions. For instance, the paragraph of "Some bake *Areca* nut and grind it into powder. Use 7.5 g per serving. Take it with honey and green onion" was cited from "*Shenghui prescriptions*." Most of the documented TCM against taeniasis are different varieties of herbs, e.g., *Areca* nut, pumpkin seed, omphalitis, torreya, and pomegranate peel. These herbs should be used separately or in combination with others.

10.2 Popular TCM Against Taeniasis

10.2.1 Omphalia

The dried sclerote is the underground part of a medicinal fungus *Omphalia lapidescens* (synonym: *Polyporus mylittae* and *Laccocephalum mylittae*), being commonly known as Chinese Lei-Wan, or known under the interesting nickname "thunder ball" because of its appearance (Figs. 10.1 and 10.2). The fungi are collected in spring and summer, but mostly in autumn, were removed from foreign matter and soil, dried in the sun, and were ground into powder when used as herb. This powder-like medicine should be ingested with yellow rice wine or lukewarm water after the meals, 5–7 g each time, three times daily for 3 days. The daily total dosage is 60 g for adults, 30 g for a child with a body weight ranging from 11 to 34 kg.

Although historically used to against many kinds of parasites, including roundworms, cestodes, and hookworms, the anthelmintic mechanisms of *Omphalia* were not very clear until modern pharmaceutical chemical research discovered the components of *O. lapidescens*. The *Omphalia* proteinase or Leiwan proteinase, first

Fig. 10.1 Dried sclerotes of *Omphalia lapidescens*

Fig. 10.2 Sclerotes slices of *Omphalia lapidescens*

found in 1937, is the most important and effective ingredient that was extracted from natural *O. lapidescens* (Jing 1951). The molecular weight is 16,800, the isoelectric point (PI) is 4.4 with amino terminal. It has proteolysis activity to break down the proteins of parasites (Dong and Li 1991). Previous research showed its serious damaging effect on scolex cells of cestodes (Qiu et al. 1986). *Omphalia* is difficult to collect in natural environments because only the dry submerged sclerotium (the underground part of the fungus) is available (Liu 1978). Consequently, the production of this fungus under artificial control by submerged fermentation is much more efficient. Guo et al. (1997) analyzed the fermentation products of *Omphalia lapidescens* Schroet strain and tested the parasiticidal effects on the *Cysticercus* in vitro. When comparing with the natural Leiwan proteinase, it was found that there were no remarkable differences between the Leiwan proteinase extracted from the fermentation products of *Omphalia lapidescens* Schroet strain and the natural Leiwan proteinase. The molecular weight was 16,800, PI was 4.4, and had the amino terminal valine. There were proteolysis activity and parasiticidal effects on *Cysticercus* in vitro. This means that either the molecular structure or the proteolysis activity and parasiticidal effects of the fermentation products of *Omphalia* strain and of the natural Leiwan proteinase are the same.

To investigate the anti-cysticercus effects of the proteinase, 40 cysts of *Cysticercus cellulosae* were separately incubated with proteinase from artificially fermented *O. lapidescens* for 2 h, 4 h, or 8 h and then examined for histological changes by light microscopy and compared with the effects of proteinase from natural dry *O. lapidescens* after similar treatment. The results showed that the *Cysticercus cellulosae* were morphologically and structurally impaired after exposure to the action of proteinase of *O. lapidescens*, and the degree of impairment was proportionally increased with the duration of treatment. The *Cysticercus cellulosae* were equally damaged morphologically and structurally by artificially fermented *Omphalia* and natural *Omphalia*, confirming the anti-cysticercus effect of the proteinase (Zhao et al. 1998).

Further studies by Zhou et al. (2010) discovered that the proteinase is an intracellular metalloprotease, which can kill parasites by degradation of their epidermal and internal proteins. Their experiment showed proteolysis of the parasites caused by the proteinase. Scanning electron microscope (SEM) results showed, in comparison with untreated controls, that the protease-treated worms exhibited a devastating impact. The worm body was largely destroyed by the protease, and only mutilated larvae and tissue residues were present. These experiments had shown that the proteolysis of parasite proteins is related to the anthelmintic activity of *O. lapidescens* (Hu et al. 2010).

Besides proteinases, polysaccharides are other active ingredients that act anti-inflammatorily and immunostimulatorily and can be isolated from natural *O. lapidescens* (Zheng et al. 2011; Wang et al. 2008). Up to now, several more components of *O. lapidescens* have been obtained by chromatography and spectral analysis. These finding include β-sitosterol, oleanolic acid, ergosterol, ergosterol peroxide, tirucallol, stigma-7,22-dien-3β,5α,6β-triol, stigmasterol, 3β-hydroxy-stigmast-5,22-dien-7-one, friedelin, and epifriedelanol (Xu et al. 2011).

10.2.2 Agrimony

Agrimony (also called xi-yang-long-ya grass or xian-he grass) has the scientific name *Agrimonia pilosa*. It is perennial herb with a brawny stem and yellow flowers. The herb was first documented in "*Bencao Tujing*" or "*Commentaries on the Illustrations*" by Su song in the year AD 1062. The whole plant ripens in spring and autumn when it has plenty of buds and luxuriant leaves and branches. Preparation is done by removing any clay, soil, and mud, and then it is processed by drying and cutting before use (Figs. 10.3, 10.4, and 10.5). Boil 30–60 g dried herbs each time with the appropriate amount of water to yield herbal decoction. Then separate and filter out the remnant dregs of the decoction before drinking the liquid medicine.

The most effective ingredient of *A. pilosa* against helminthes is named agrimophol, which is included in different parts of the plant, roots and stems, as

Fig. 10.3 The origin plants of *Agrimonia pilosa* in nature

Fig. 10.4 The living flowers of *Agrimonia pilosa*

well as in buds. The chemical structure of agrimophol has been detected in the late 1970s by Shenyang College of pharmacy (1977) as the following (Fig. 10.6).

Dried plant material is extracted by 85 % ethanol. Then the ethanolic extracts of agrimony are isolated and repeatedly purified by silica gel column chromatography (Zhao et al. 2007). As a result, Apigenin-7-O-methyl glucuronate (I) and Apigenin-7-*O*-butyl glucuronate (II) were obtained from *A. pilosa*. Both extracted compounds

Fig. 10.5 Dried herbs of *Agrimonia pilosa*

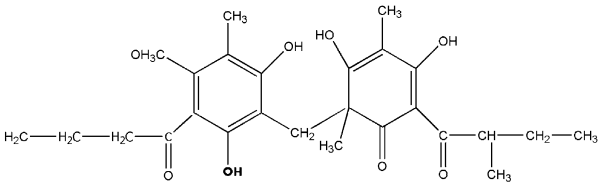

Fig. 10.6 The chemical structure of agrimophol

proved to be active against tuberculosis and could inhibit the growing of *Streptococcus pneumoniae*, *Escherichia coli*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, etc., especially of *Staphylococcus aureus* in vitro. There are some other chemical constituents in *A. pilosa* including hydroxybenzenes, esters, flavonoids, tannins, glycosides, organic acids, essential oils, triterpenoid saponins, etc. They all have extensive pharmacological activities, being antitumor, hypoglycemic, anti-inflammatory, analgesic, hemostasis, antihypertension, and antiarrhythmic, as well as antihelminthic (Hong et al. 2008).

Studies have shown the effects of agrimophol to repel *Taenia* species, *Cysticercus cellulosae*, liver flukes, and so on. It was observed that all parts of the worm, i.e., scolex, neck, and all proglottid segments became totally cramped and were contracted and finally died. This occurred just by touching agrimophol, while the untouched parts remained unaffected and survived. Therefore, it is believed that the anthelmintic activity of agrimophol is due to a direct killing effect and not based on the nervous system. Contact significantly inhibits the metabolism of the parasitic cells (Zhao et al. 2007; Feng and Gui 1980; Shenyang College of pharmacy 1974).

In addition, the herb is also efficient in killing several other parasites. Lai (2005) orally treated Kun Ming mice with different concentrations of single *Gentiana scabra bunge* and *Agrimonia pilosa* and with the combination of the two herbs, respectively. Lai reported that the survival rate of mice in the combination group was boosted up significantly compared to the control group after a treatment

period of 7 days. *Gentiana scabra bunge* in combination with *Agrimonia pilosa* acted also as anti-malaria compounds and enhanced the survival rate of mice infected with *P. berghei*. Further effects of *A. pilosa* were assessed by using various concentrations of the drug against *Trichomonas vaginalis* in in vitro tests. Three Chinese herbs including *A. pilosa, Pulsatilla chinensis*, and *Coptis chinensis* exhibited efficacy against *T. vaginalis*. It is suggested that the three herbs should be tested as new trichomonacidal drugs in clinical treatment (Yan et al. 2010).

10.2.3 Semen moschatae

Semen moschatae is the dried ripe seed of *Cucurbita moschata* and commonly known as pumpkin seed. The seeds are collected in summer and autumn, removed from tissues, washed clean, and dried in sun (Fig. 10.7). It contains cucurbitin, fatty acids (linoleic acid, oleic acid, octadecanoic acid, and glyceride), proteins, vitamins (vitamin A, vitamin B1, vitamin B2, and vitamin C), carotene, phytosterins, and mineral substances, and drives out parasites, lowers cholesterol, prevents oxidation, resists inflammation, reduces hypertension, decreases pressure on the bladder, and urethral system. Pumpkin seeds have a very low toxicity. Their clinical applications are used in taeniasis, schistosomiasis, prostatitis, benign prostatic hyperplasia, bladder stimulus symptoms, and in urolithiasis (Wu et al. 2003). However, the pharmacological function of pumpkin seed is not very strong and in use. They should be combined with other herbs.

10.2.4 Areca *seed*

Areca seed or betel nut is the dried ripe seed of the palm plant *Areca catechu*. The fruits of *Areca catechu* are picked as they ripen, were boiled in water, dried in sun, and were cut to collect the seeds. The harvested seeds have to be removed from pericarps in order to be dried in the sun before being crushed or cut in half for medical use (Figs. 10.8, 10.9, 10.10, 10.11, 10.12, and 10.13).

Comprehensive analyses of the chemical composition of *Areca* seed have been reported. The major constituents of the seed are polysaccharides (17.8–25.7 %), fats (9.5–15.1 %), proteins (6.2–7.5 %), crude fibers (11.4–15.4 %), polyphenols (flavonols and tannins) (11.1–17.8 %), alkaloids (0.5 %), ash (1.1–1.5 %), moisture content (38.9–56.7 %), etc. Of its chemical ingredients, tannins, alkaloids, and some minerals may have biological activities, but adverse effects on tissues were described in Shen and Duan (2009). Among the chemical constituents of *Areca* seed, alkaloids show the most important biologically activity against parasites. The seed has been shown to contain at least six related alkaloids, of which four alkaloids (arecoline, arecaidine, guvacine, and guvacoline) have been conclusively identified

Fig. 10.7 *Semen moschatae*, the dried seeds of *Cucurbita moschata*

Fig. 10.8 The original palm plant *Areca catechu*

in biochemical studies. Arecoline (0.12–0.24 %) extracted from *Areca catechu* is one of the most important alkaloids acting against cestodes. The arecoline hydrobromide is a low-toxic substance and shows slight induced effects in carcinogenesis, mutagenesis, and teratogenesis but has reproductive toxicity at high-dosages application. On the other side, there are good effects on tapeworms in livestock, poultry, and humans at the clinical recommended dosages without reproductive toxicity (Huang and Michael 1989; Green et al. 2002; Ahsana and Shagufta 2000).

A recent study suggested that arecoline might cause nervous paralysis of cestodes (Chu 2001). In another study (Muhammad et al. 2011), *Areca* seed was soaked in aqueous-methanol (70 %) and processed by filtrating, evaporating repeatedly to yield a viscous, dark brown *Areca catechu* crude extract material. Then the follow-up acetylcholinesterase (AChE) inhibitory activity was measured spectrophotometrically in vitro. Muhammad et al. (2011) found that *A. catechu* showed

Fig. 10.9 The fruits on a living plant *Areca catechu*

Fig. 10.10 *Areca catechu*: fresh cut fruits with seeds inside

significant anti-AChE with almost complete inhibition of the enzyme in the AChE assay. There were four compounds (tannic acid, gallic acid, diosgenin, and isoguvacine) of *Areca* seed, which were discovered to be active and responsible for AChE inhibition. Among the four compounds, tannic acid was the principal one, and more potent activity than the others. Experimental findings explained the reason why *Areca* seeds could paralyze the worms in the small intestine, which leads to the result that they are expelled.

Areca seed was generally administered in combination with *S. moschatae* to expel adults of tapeworms. The combination of *Areca* seed and pumpkin seed has a long documentary history in ancient Chinese medical use. The usually joint application is done as follows: Boil 100 g dried *Areca* seeds each time with appropriate

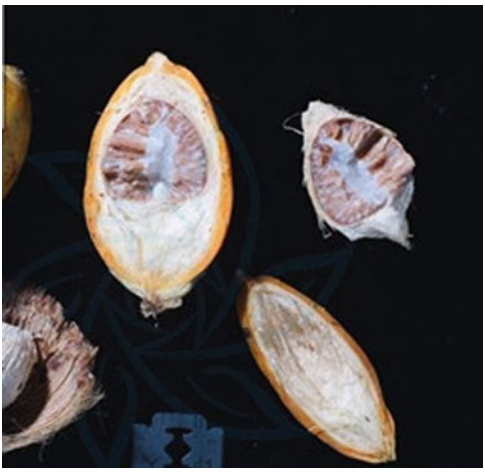

Fig. 10.11 *Areca catechu*: dried cut fruits with seeds inside

Fig. 10.12 *Areca catechu*: the size of dried fruit

Fig. 10.13 *Areca catechu*: broken dried seeds

amount water, filter out the remnant dregs to yield 300 ml decoction in advance. When the therapy begins, the patient should eat raw or uncooked dried pumpkin seeds 150 g each time in the morning on an empty stomach. Forty minutes later, the patient should drink the liquid decoction of *Areca* 300 ml half an hour later, the

adult patient should ingest 60 ml of 50 % bitter salt followed by plenty of water (1,000–2,000 ml) to finish the treatment.

In the joint remedy, diverse effective components in *A. catechu* and *S. moschatae* would act on different parts of the worm. *A. catechu* may act on frontier parts such as scolex and immature proglottids, while *S. moschatae* acts on middle and posterior segments such as mature and gravid proglottids. The whole body of the parasite will become feebler and destroyed by the herb's activity. Bitter salts act as catharsis and contribute to discharge the paralyzed worms from the intestinal tract along with an uptake of plenty of water to prevent dehydration (Feng 1956; Li et al. 2012).

Many investigations have supported the effectiveness of the joint traditional herbs as antihelminth remedies (Xie 2009; Long et al. 2010). For example, Li et al. (2012) treated 175 volunteers with a history of discharging tapeworm proglottids during previous 2 years by *Areca* in combination with pumpkin seeds in Yajiang County during May 2007 to November 2009. Parasite isolates were further examined using multiplex PCR for species identification. 112 of 175 subjects treated in this way expelled whole tapeworms or proglottids start up 5 h after treatment. The efficacy rate over all was 64 %. Among the 112 cases, 93 (83 %) discharged whole tapeworms including their scolices. As common side effects dizziness and gastrointestinal discomfort occurred, which, however, disappeared after expulsion of worms.

Tian et al. (2002) studied the immature, mature, and gravid proglottids of *T. solium*, which had been expelled after a mixed *Areca* and pumpkin seeds meal by help of the transmission electron microscopy and found that there were no injuries in the tegument and parenchyma. The microtrichiae of the surface of the distal cytoplasm were intact. There were not seen swellings, vacuoles, or degeneration of organelles. The circular and longitudinal muscles in the upper facial parenchyma were not disarranged. The parenchymal cells and the supporting cells in the deeper parenchyma showed their normal structure. It was concluded that paralysis was the main repellent mechanism when uptake combining *Areca* and pumpkin seeds was preceded.

10.3 Conclusions

In brief, medicinal plants, such as TCM, are small but important parts of the biological heritage of the Earth. The use of TCM is based on the belief that existed for thousands of years before the development and spread of modern medical products. With more than 5,000 years of Chinese history and as a part of the Chinese culture, China has accumulated a long and rich experience in the use of medicinal plants. Chinese herbal medicines have been playing a critical role in fighting against various diseases and saving human's health. Experience over the past thousands of years has proven its efficiency with rather low side effects or environmental pollution, which are the main problems caused by modern

Table 10.1 The common TCM against taeniasis

Scientific name	Common name	Effective ingredient	Usage
Omphalia lapidescens	*Omphalia*, Chinese Lei-Wan, Thunder ball	*Omphalia* proteinase	The medical powder should be taken with yellow rice wine or water after meals, 5–7 g each time, three times daily
Agrimonia pilosa	Agrimony, Xiyanglongya grass or Xianhe grass	Agrimophol	Boil 30–60 g dried herbs each time with water to yield herbal decoction, drink the liquid medicine
Semen moschatae	Pumpkin seed	All the ingredients together cause the common effect	*Areca catechu* is generally administered in combination with *S. moschatae*. Boil 100 g dried *Areca* seeds each time to yield 300 ml decoction in advance. First eat raw pumpkin seeds 150 g each time on an empty stomach, then take the decoction, half an hour later, drink 60 ml of 50 % bitter salt followed by 1,000–2,000 ml water
Areca catechu	*Areca* seed	Alkaloids (arecoline, arecaidine, guvacine, and guvacoline)	

chemotherapeutic agents. Therefore, TCM should be also considered in the fight against parasites in modern time. The TCM against taeniasis are summarized in Table 10.1.

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Chapter 11
Sparganosis in China

Yan Chen and Bin Ye

Abstract Sparganosis is the term used for human's infection of plerocercoid larvae of genus *Spirometra*. Most sparganosis cases in China are caused by *Spirometra mansoni*, a parasite of carnivores. A higher prevalence is in Asian countries, especially in China, Vietnam, Japan, and Korea. Sparganosis has become an important food-borne disease in China. The cases have been reported continually because of many causes, such as change of our diet habit, deficiency of knowledge on sparganosis, transportation development, enhancement of parasitic adaptability, and food supervisory defect. Since the first human infection was discovered by Patrick Manson in 1882, more than thousands cases have been reported in China. Human is an accidental host in its life cycle, becoming infection of plerocercoid larvae by contacting with or ingestion of the first, second intermediate hosts, or a paratenic host. The human sparganosis is divided into subcutaneous sparganosis, ocular sparganosis, buccal cavity sparganosis, cerebral sparganosis, and visceral sparganosis. Diagnosis depends on pathological examination after surgical removal of the larvae. Surgical removal is the first choice of treatment. Although praziquantel is often used to kill the parasite, its curative effect against plerocercoid seems different in clinical practices. Up to date, treatment of sparganosis with traditional Chinese medicines has not been in practices. A traditional Chinese medicine, the dried sclerote of *Omphalia lapidescens*, named as "thunder ball" (the words translated from Chinese words "*Lei*" and "*Qiu*," "*Lei*" means thunder, "*Qiu*" means ball) was used to treat the animal experimental infection of plerocercoid, which revealed certain efficacy for treating the infected mice in vivo. The in vitro experimental results demonstrated that thunder ball can cause extensive

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tissue damages in plerocercoids; more severe ultrastructure damages of plerocercoid were found by thunder ball than praziquantel. Avoiding ingestion of raw fleshes of frog or snake is the best prevention.

Keywords Sparganosis • *Spirometra mansoni* • Plerocercoid • Traditional Chinese medicine • Thunder ball

11.1 Introduction

Sparganosis is an infection of the plerocercoid larvae of genus *Spirometra*. About 40 species of *Spirometra* in Pseudophyllidean tapeworm have been reported in the world, namely *Spirometra mansoni*, *S. mansonoides*, *S. erinacei*, *S. ranarum*, *S. decipiens*, *S. houghtoni*, *S. proliferum*, and *S. serpentis*. However, a number of molecular identifications and phylogenetic analyses suggested that most species of *Spirometra* were synonyms of each other recently. For example, *S. erinacei* has more than 40 synonyms, *S. mansoni* was included also. In China, four species in genus *Spirometra* have been reported in literatures: they are *S. mansoni*, *S. decipiens*, *S. houghtoni*, and *S. serpentis*.

More than thousands cases of sparganosis have been reported from most countries of the world, but a higher prevalence of infection is in Asian countries, especially in China, Vietnam, Japan, and Korea. Most cases of sparganosis in China are probably caused by *S. mansoni* or *S. erinacei*, a parasite of carnivores, while most sparganosis cases are caused by *D. mansonoides* in North America.

11.2 Structure of Plerocercoid

The plerocercoid larva is a wrinkled, whitish, ribbon-shaped organism, with a few millimeters in width and several centimeters up to 30 cm in length. The plerocercoid length seems to be related to its host and parasitic time (Fig. 11.1). The anterior end is capable of invagination and has two sucking grooves that are present in the scolex of the mature worm. The body of larva is characterized by a stromal network of smooth muscle. In general, plerocercoid of *S. mansoni* is described as a larger and more delicate than that found in the West world.

Xu et al. (2012) observed the surface structures of plerocercoids from the frog *Rana nigromaculata* by scanning electron microscopy and found that the anterior and posterior ends of the plerocercoids were slightly swollen and without segmentation under scanning electron microscope (Fig. 11.2). Annular furrows ran irregularly along the body. The anterior end was drawn inside and had a deep dorsoventral hollow, around which, the plasma membrane displayed lip bulge. The posterior end was similar to the anterior end in the shape, but the fissure in its central part was comparatively narrower and shallower than the hollow in the

Fig. 11.1 The *Spirometra* plerocercoids removed from patient (*left*) and experimental mouse (*right*) (Xu et al. 2012)

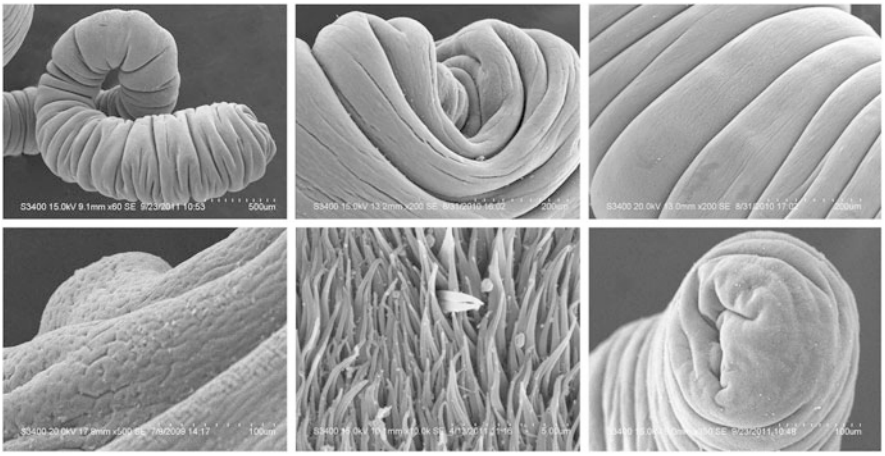

Fig. 11.2 Plerocercoids from the frog *Rana nigromaculata* observed by SEM. *Upper left*: lateral view of anterior segment; *upper middle:* lateral view of anterior end; *upper right*: annular furrows on tegumental surface; *below left*: pits and grooves on plasma membrane; *below middle:* microtriches of anterior segment; *below right*: face view of posterior end

anterior end. There were thousands of pits and grooves on the body surface, unequal in size. The whole body surface of the plerocercoid was densely covered by sharp spine-like microtriches.

Tang et al. also found that the walls of plerocercoids consisted of tegument and parenchyma under transmission electron microscope (Xu et al. 2012). Thorn-shape microtriches were distributed over the outer surface of the tegument. Matrix zone had a lot of the granular discoidal bodies, vesicles, mitochondrias, and endoplasmic reticulums. Most of mitochondrias were near the basal membrane. Parenchyma zone consisted of muscular layer, tegument cells, parenchymal cells,excretory system,and so on. Many cytoplasmic pathway of tegumentary cell, which stretches into muscular layer, suggested that the tegument was the main absorptive site of nutrients (Fig. 11.3).

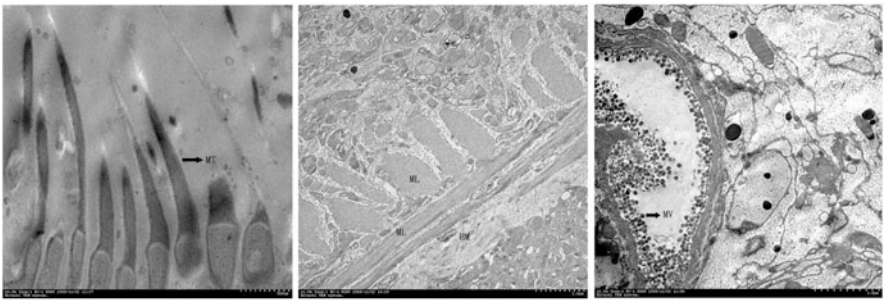

Fig. 11.3 Plerocercoids from the frog *Rana nigromaculata* observed by TEM. *Left*: sagittal section of tegumental microtriches (MT). *Middle*: tegument of plerocercoid, showing basal membrane (BM), muscular layer (ML). *Right*: excretory duct of plerocercoid, showing bead-like microvilli (MV)

11.3 Life Cycle

The adult *Spirometra* lives in the small intestine of the definitive host—a dog, cat, raccoon, or other mammal. The ovoid eggs measure about (52–76) μm × (31–44) μm and have a lid-like operculum at one end. When released through the uterine pore, the shelled embryo is at an early stage of development, and it must be deposited in water for development to continue. Completion of development to coracidium takes from 2 to 5 weeks, depending on the temperature. Emerging through the operculum, the ciliated coracidium swims randomly about, it may attract the attention of copepods (crustaceans of the genus *Cyclops*, the first intermediate hosts). Soon after being eaten, the coracidium loses its ciliated epithelium and immediately begins to attack the wall of midgut with its six tiny hooks. Once through the intestine and into the crustacean's hemocoel, it become parasitic procercoid larvae in about 3–11 days, absorbing nourishment from the surrounding blood. There are often 20–25 procercoids in a copepod's hemocoel. The procercoid has an elongate, undifferentiated mass of parenchyma with a cercomer at the posterior end. The second intermediate hosts (any of several species of amphibians) consume the copepods. The larvae penetrate the intestinal tract of the second intermediate host, where they become plerocercoid larvae and proliferate to the subcutaneous tissues and muscles. Some reptiles, mammals, and birds are paratenic hosts of *Spirometra*. The second intermediate host is eventually eaten by a definitive host predator, such as a dog or a cat, and the cycle begins again. Humans are accidental hosts in the cycle, becoming infected with the plerocercoid larvae by contact with or ingestion of the first, second intermediate hosts, or paratenic host. The larvae migrate to the subcutaneous tissues in humans; however, no development takes place and the human is not capable of transmitting the disease. Plerocercoid can live up to 10–20 years in the human host.

11.4 Pathogenesis

The mechanism of pathogenesis is unclear. In the case of mammals, including humans, present evidence suggests that pathogenesis may be caused in part by the host's immune reactions. Cellular immunity plays an important role to resist the infection of plerocercoids. CD8+ T cells are important immune cells. According to the dynamic cytokines detection, Jiang et al. thought that the Th1 immune response is dominant in the early infection when mice are infected by plerocercoids. Then, it changes for Th2 response. The shifting of Th1/Th2 immune response could affect the finale of plerocercoid infection (Jiang et al. 2009).

The plerocercoids lack any trace of a digestive tract and therefore must absorb all required substances through their tegument. The tegument is covered by microtriches. The microtriches serve to increase the absorptive area of the tegument, but they also may injure their parasitic positions. In addition, a number of phenomena, apparently depending on interaction of certain molecules with the glycocalyx, have been reported. Yang (2004) purified specific 31/36 ku antigenic molecules of plerocercoids from snakes and mice and analyzed their monosaccharide compositions. The result showed that glucose and mannose concentrations were two- to threefold higher in the 31/36 ku molecules purified from snakes than those from mice. This result implies that antigenic glycoproteins of plerocercoids from snakes might be modified in mammalian sparganosis with respect to their carbohydrate composition. However, the biological significance of carbohydrate changes in the infection of mammalian hosts is not well known, and it also remains the relationship with larvae infectivity, migration, and pathogenesis (Yang 2004; Sun et al. 2001).

The early migratory stages in the development of the plerocercoid are asymptomatic, but when it has reached its final site and begins to grow, its presence elicits a painful inflammatory reaction in the surrounding tissues. Plerocercoids parasitize mainly subcutaneous and muscle tissues in mice, including humans. The organs of mice infected with plerocercoids will show different degree of histopathological changes such as, fat tissue shows severe degeneration and necrosis, and fibrous tissue shows moderate proliferation of enveloping plerocercoids, with a large number of neutrophilic mainly acute and chronic inflammatory cell infiltration, and so on, while the larvae apparently do not become encysted (Tang and Chen 2011, Fig. 11.4).

11.5 Immune Reaction of the Host

Recently many studies show that humoral immunity and cellular immunity play an important role to resist the infection of plerocercoids. The serum anti-plerocercoid antibodies (IgG) in mice infected with different doses of plerocercoids could be detected at 2 wpi (Li et al. 2010). There was a preponderance of IgG4 among IgG. Proteins of plerocercoid (Mr: 31–36 ku) could react specifically with IgG and IgG4

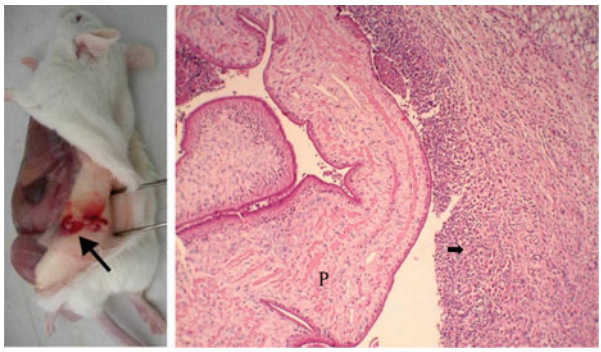

Fig. 11.4 Plerocercoids in subcutaneous tissues of mice. *Left*: a parasite in subcutaneous tissues. *Right*: section of subcutaneous tissues of the experimental group mouse in the fourth week (HE, ×40), showing still marked neutrophil infiltration within the inflammatory cystic wall (*arrow*) enveloping the plerocercoid (P) tissue

of the infected mice sera. In the infected mice, level of CD4+ T cells was increasing constantly from 2 to 5 weeks, then, it was significantly lower than that in control and recovered at week 10. The level of CD8+ T cells was higher than that in control and reached to a peak at week 4. The level of IFN-γ was higher than that in control at weeks 3–10 and reached to a peak at week 4. The level of TNF-α, IL-4, and IL-10 reached to the highest at week 4, week 7, and week 8, respectively. The results showed that cellular immunity played an important role to resist the infection of sparganum. CD8+ T cells were important immune cells. According to the dynamic cytokines detection, the Th1 immune response was dominant in the early infection when mice were infected by sparganum. Then, it changed for Th2 response. The shifting of Th1/Th2 immune response influences on the finale of the sparganum infection.

11.6 Symptoms

The manifestations are dependent on the following variables: the number of plerocercoids, the location of plerocercoids, the stage of development, involvement, and involution of the plerocercoid, and the intensity of host immune inflammatory response. The human sparganosis is divided into following five types.

Subcutaneous sparganosis: The plerocercoid invades most commonly subcutaneous tissue or muscle, especially in the abdominal wall, chest, limbs, breast, groin, labia, or scrotum. Eight hundred and thirty-six cases have been reported in China since 1949–2010 with 297 of them (35.53 %, 297/836) presenting with skin involvement. The typical feature is a slowly migrating subcutaneous nodule. Erythema, pruritus, swelling, turning red, and local tenderness may develop as well.

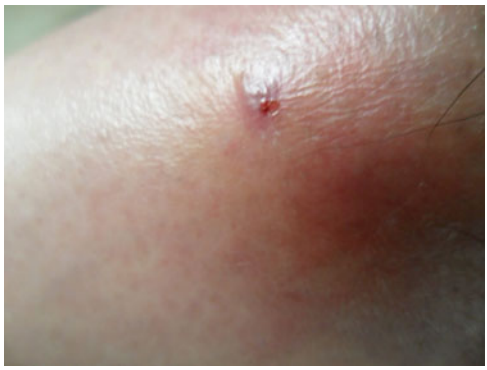

Fig. 11.5 The erythematous skin leg of a patient with sparganosis

Ocular sparganosis (34.09 %, 285/836): The usual symptoms are periorbital itching, pain, epiphora, chemosis, and ptosis. An asymptomatic subconjunctival mass is the most common clinical sign. In addition to these of symptoms, ocular sparganosis can cause similar symptoms of orbital cellulitis, exophthalmos, and expose cornea ulcer. If the worm invades orbit, it leads to severe inflammation or blindness.

Buccal cavity sparganosis (16.39 %, 137/836): The subcutaneous or mucosa nodules are the typical features, when the plerocercoid invades the subcutaneous or mucosa tissue of buccal cavity, or genital region. The nodules range from 0.5 to 3 cm. The nodules usually are itchy, swollen, red, and are often accompanied by painful edema.

Cerebral sparganosis (12.44 %, 104/836): Cerebral sparganosis is a rare parasitic infestation of the central nervous system (CNS, including the brain and the spinal cord), usually producing chronic active and recurrent granulomatous inflammation leading to eventual degeneration and atrophic changes of the white matter or formation of small punctuate parenchymal calcifications. Lesion of the cerebral sparganosis involve most commonly brain parenchyma including subcortical white matter and basal ganglia followed by intraventricular region and meningobasal area. Recent increasing reports of cerebral sparganosis from various parts of the world are ascribable to the developments in sero-immunologic and radiologic studies. Most reported cases of cerebral sparganosis were discovered by chronic recurrent or active inflammatory response of the larvae. The clinical manifestations of cerebral sparganosis can be asymptomatic or it can cause widely varied symptoms, such as seizure, hemiparesis, headache (increased intracranial pressure), ischemic cerebrovascular disease, dementia, and signs of spinal nerve root or cord compression. Among these, seizure and headache are the most frequent ones (Fig. 11.5).

Visceral sparganosis (1.56 %, 13/836): In human the worm might have penetrated through the intestinal wall, migrates into the tissues around the major vessel, and then reaches the visceral organs such as liver, lung, or intestine. The plerocercoids parasitized in liver, lung, or intestine may cause different symptoms.

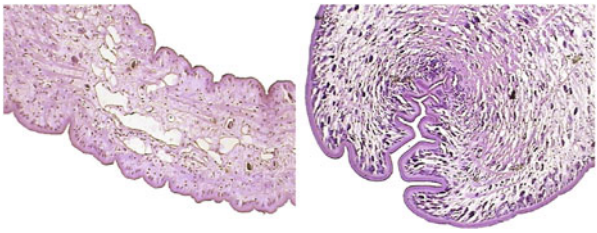

Fig. 11.6 The sections of plerocercoid. *Left*: longitudinal section of plerocercoid (HE, ×200), showing calcareous body, excretory canals, and smooth muscle fibers in loose parenchyma. *Right*: longitudinal section of plerocercoid (HE, ×200), showing the anterior end

About 20 cases from Florida, Taiwan, Paraguay, and Venezuela, human infections with a peculiar budding type of larva known as *Sparganum proliferum* have been reported. These larvae may occur almost anywhere in the body, and the branched proliferating larvae may break up into segments capable of further independent development. Infection with this form of parasite is extremely serious, since many thousands of worms can result, with the infected organs becoming honeycombed.

11.7 Diagnosis

Diagnosis is made following surgical removal of the worms, which on section are seen to possess a rather homogeneous parenchyma in which are scattered the laminated, intensely basophilic calcareous corpuscles characteristic of cestode tissue (Fig. 11.6). With the exception of the forms with scolex armature, it is impossible to distinguish the species of plerocercoids found in humans by examining their morphology. Recently a number of authors have utilized PCR technology (PCR, PCR-RAPD, PCR-RFLP, and PCR-SSCP) and partial and complete ribosomal DNA (such as 18*S rDNA*, 28*S rDNA*, *ITS1*, and *ITS2*) and mitochondrial DNA sequences (such as mtDNA and *mCO*I) to resolve species of *Spirometra* phylogenetic relationships within intraspecies and interspecies. Li et al. (2011) realize that *cox*1 is more suitable than *ITS* in studying intraspecific genetic polymorphism which is considered from the bootstrap values, and *ITS* might be used as genetic marker for the species identification.

If such biopsy and excision procedures are not feasible, the anti-plerocercoid ELISA, DIGFA, or ELIB test may be used. ELISA test is a very sensitive test for detection of sparganosis when the diagnosis is in doubt. In surgically proven cerebral sparganosis, both serum and CSF ELISA tests showed high sensitivity and concordance rate in diagnosing cerebral sparganosis, negative conversion was noted in patients with successful surgical removal. However, it is difficult to say whether the decrement in the serum was due to the treatment or to a natural course of human immune response to the plerocercoid. The DIGFA showed high

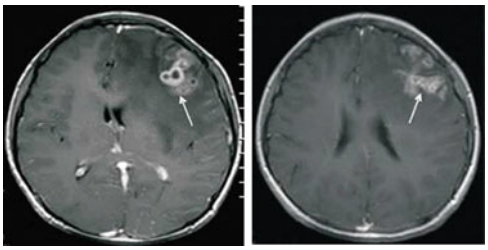

Fig. 11.7 MRI and follow-up MRI patterns of cerebral sparganosis. *Left*: conglomerated *ring-shaped* or *beaded tortuous-shaped* enhancements (T1WI). *Right*: changes of lesion shape 5 months later (enhanced T1WI)

sensitivity and specificity in detecting specific antibodies in sera from infected patients. However, there are high cross reactions with antibodies in sera from other helminth patients. One worm burden could induce high levels of antibodies. The results of DIGFA were similar to those of routine ELISA, but DIGFA was more handy and rapid without any specific instrumentation. In addition, the serologic diagnosis by ELISA or DIGFA could be a useful tool in the epidemiologic study of human sparganosis in a susceptible population, as well as in individual diagnosis (Gong et al. 2006; Wang et al. 2006, 2008).

In theory, a preoperative diagnosis could be made by identification of exposure history and a painful, migratory, subcutaneous nodule. Sparganosis usually presents as a single nodule, while other cestode infections such as cysticercosis typically present as multiple nodules. Preoperative diagnosis, however, is rare. CT and MRI scans are especially useful for diagnosis of cerebral sparganosis, as they reveal lesions in the brain. Through a retrospective analysis of 17 cases of cerebral sparganosis in children, Gong et al. found a number of characteristic signs that could be used in the future to diagnose cerebral sparganosis without performing an excision or tissue biopsy. The most characteristic finding of enhanced MRI is the conglomerated ring-shaped, beaded tortuous-shaped, or serpiginous tubular-shaped enhancement and changes in location and shape of lesions in the follow-up (Fig. 11.7). Kim et al. found the differential features between live and degenerated worms on CT images. In patients with cerebral sparganosis, live worm infection is highly suggested if there is a focal amorphous hyperattenuated lesion on the precontrast CT with a surrounding mass effect, irregular nodulotubular enhancement extending to the pial surface and change of the enhancing lesion on the follow-up images.

These findings suggest that clinical history, ELISA, and either MRI or CT scans could be sufficient to make a cerebral sparganosis diagnosis. It is important to note, however, that these lesions are sometimes mistaken for tuberculosis lesions.

Fig. 11.8 Plerocercoids in the intermediate and paratenic animal hosts. *Left*: plerocercoids in frog legs; *right*: plerocercoids in a snake

11.8 Epidemiology

11.8.1 Distribution

Sparganosis is endemic or potentially endemic in 48 countries, and although rare, about 1,400 cases have been described in Asia, Africa, Australia, South America, and the USA. The majority of cases occur in Southeast Asia and Eastern Africa. The highest numbers of cases occur in China, Vietnam, Korea, and Japan. So for, 1,056 cases (since 1927–2009) have been reported in China. The majority of cases occur in Guangdong, Fujian, Jilin, and Hunan provinces of China.

11.8.2 Host

Definitive hosts of *Spirometra* include dogs, cats, and wild carnivores, while humans are accidental hosts. First intermediate hosts include copepods and other freshwater crustaceans, while second intermediate hosts include amphibians. Paratenic hosts include reptiles, birds, and mammals. Many species of copepod, amphibian, and carnivore have been reported to serve as first intermediate hosts, second intermediate hosts, or paratenic hosts of *S. mansoni*, respectively, in China.

Frogs, snakes, and pigs are important intermediate and paratenic hosts; the infective rates of frogs, snakes, and pigs are 3.17–77.6 %, 7.7–30.8 %, and 0.2–44 %, respectively, in China (Fig. 11.8).

11.8.3 Transmission

The parasite is transmitted to humans in three different ways. First, humans may acquire the infection by drinking water that is contaminated with copepods housing *Spirometra* larvae. Second, humans may acquire the infection by consuming the raw flesh of one of hosts, such as amphibians, reptiles, birds, or even mammals such as pigs and dogs. For example, humans consume raw snakes or tadpoles for medicinal purposes in some Asian cultures; if the snakes or tadpoles are infected,

the larvae may be transmitted to humans. Third, humans may acquire the infection by placing raw poultices of the second intermediate hosts or paratenic hosts on open wounds, lesions, and/or the eyes for medicinal or ritualistic reasons. If the poultice is infected with plerocercoid larvae, the human also may become infected. In their retrospective study of 104 cases of sparganosis, Wu et al. (2007) found that 36 patients (34.62 %, 36/104) had eaten raw or uncooked frog or snake that was infected with plerocercoid, 20 patients (19.23 %, 20/104) had applied an animal's flesh as a poultice to an open wound, and 26 patients (25.00 %, 26/104) had drunk contaminated water (the remaining 22 patients [21.15 %, 22/104] had no known history of being infected).

To observe the effect of different physicochemical factors on the infectivity of plerocercoids, the muscle samples with plerocercoids taken from frogs are treated with different temperature (−20, 4, 37, and 56 °C) or different concentration of ethanol (20, 30, 40, 50, and 60 %) for 1, 2, or 3 h, or soaked in 100 % ginger juice, vinegar (total acid concentration of 4.5 %), or soy sauce (containing 19.3 % NaCl) for 3, 6, 12, and 24 h. The plerocercoids are used under each condition and fed to ten mice averagely (five larvae/mouse). Another 20 plerocercoids with frog meat are comminuted for 3 min and then fed to ten mice. One week later, the mice are sacrificed to collect the parasitic plerocercoids and the number of positive mice and plerocercoids are recorded. Treatment with −20 °C or 60 % ethanol for 2 h, soy sauce for 6 h, and vinegar for 24 h can destroy the infectivity of plerocercoids in 1 cm^3 frog muscle. The plerocercoids comminuted for 3 min will not lose infectivity completely to mice. It suggests that drinking "frog juices" is not safe (Tang and Chen 2011).

11.9 Treatment

Surgical removal of one or a few plerocercoid larvae is often the best treatment. Praziquantel is apparently effective when administered at a total dose of 120–150 mg/kg body weight over 2 days, however, praziquantel has had limited success.

Traditional Chinese medicines have been used against cestodes in past centuries in China. These Chinese drugs found to have any effect against taeniasis were *Areca catechu*, *Cucurbita moschata*, *Omphalia lapidescens*, *Agrimonia pilosa*, *Rangoon creeper*, etc. Chinese drugs have many advantages, such as safe and effective. Because there are many drug target spots, it is difficult for them to cause resistance; however, complicated chemical components, unknown pharmacological functions, and toxicity will restrict their use in clinical medicine.

Recently studies on traditional Chinese drugs suggest that the effective element of *A. catechu* or *C. moschata* is arecoline or cucurbitin, respectively. Arecoline or cucurbitin can cause adult muscle paralysis, but there are a few differences in their influence regions.

A traditional Chinese medicine, named as thunder ball, or"*Lei-Qiu*" in Chinese, was used to treat the animal experimental infection of plerocercoid. Thunder ball is a word translated from Chinese words "*Lei*" and "*Qiu*" because "*Lei*" means thunder, and "*Qiu*" means ball in Chinese langue. Thunder ball is the dried sclerote of *O. lapidescens*. The fungus is collected in autumn, removed from foreign matter and soil, and dried in the sun. The proteinase (molecular weight: 16,800; PI: 4.4; amino terminal: valine; content: 3 %) extracted from *O. lapidescens* is an active ingredient against parasites; it may kill tapeworms, roundworms, or hookworms. Thunder ball powder must be given daily for 3 days with lukewarm water. The daily dose is 15–21 g (7.5–10.5 g twice daily for 3 days) for adults.

For the purpose of finding an anthelmintic with high efficacy, low toxicity, and low cost, especially from the Chinese traditional medicines, 40 cysticercus cellulosae were separately incubated with proteinase (1.5 mg/ml or 2.5 mg/ml) from artificially fermented *O. lapidescens* for 2, 4, or 8 h and then examined for histological changes and compared with those of proteinase from natural dry *O. lapidescens*. Cysticercus cellulosae was morphologically and structurally impaired after exposure to the action of proteinase, and the degree of impairment was proportionally paralleled to the duration of treatment. There were no remarkable differences between the proteinase obtained by artificially fermented and that of natural *O. lapidescens* (Guo et al. 1997; Zhao et al. 1998).

To observe the effect of thunder ball powder on adult worms of *Hymenolepis nana*, ICR mice are treated with different concentrations (5, 15, 30, or 45 %) of thunder ball in vitro (Chen et al. 1999). The thunder ball could cause extensive tissue damages in vitro, including contracture of worm; agglutination, fusion, fracture, and abscission of microthrix; and edema, bulge, erosion, and hole of plasma membrane. The damages became heavier with the drug concentration increasing and the test time extending (Chen et al. 1999).

Up to date treatment of sparganosis with traditional Chinese medicines has not been in clinic practices. Some laboratory trials of treatment of sparganosis with traditional Chinese medicines were under way in China. Xu observed the effect of thunder ball on ultrastructure, infectivity, and therapeutic action of plerocercoids removed from frog *R. nigromaculata*, the plerocercoids larvae were treated with different concentrations of thunder ball in vitro and in vivo (Xu et al. 2012; Tang et al. 2010; Chen 2013). Thunder balls are the dried sclerotes of a fungus *O. lapidescens* (Fig. 11.9). The fungi are collected in autumn, removed from foreign matter and soil, and dried in the sun. The proteinase (molecular weight: 16,800; PI: 4.4; amino terminal: valine; content: 3 %) extracted from thunder ball is an active ingredient against parasites, its parasiticidal function may be related to resolving proteins of tapeworms.

Under observation by transmission electron microscopy, it was found that the plerocercoids cultured in 40 mg/ml thunder ball for 12 h demonstrated a fracture and abscission of microtriches; break of plasma membrane; excretion of calcareous corpuscles; rupture of muscle fibers; obvious damage of tegument and parenchymal cells. The organizations of plerocercoids cultured in 80 mg/ml thunder ball for 24 h were damaged seriously, and the structures were not distinguished completely

Fig. 11.9 Thunder balls, the slices and dried sclerotes of *Omphalia lapidescens*

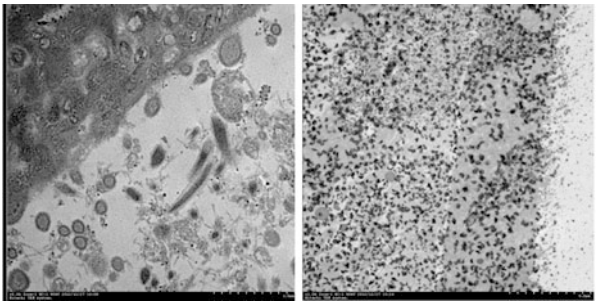

Fig. 11.10 Plerocercoids observed by transmission electron microscopy. *Left*: abscission of microtriches; break of plasma membrane; excretion of calcareous corpuscles (plerocercoid cultured in 40 mg/ml thunder ball for 12 h). *Right*: the plerocercoid cultured in 80 mg/ml thunder ball for 24 h, its structures were damaged seriously

(Fig. 11.10). Although the damages caused by exposure to thunder ball were similar to praziquantel in many ways, it caused more severe damages than praziquantel.

Thunder ball demonstrated certain parasiticidal effects on the mice infected artificially with plerocercoids in vivo. After having been cultured in 40 mg/ml of thunder ball for 4, 12, 24 h or in 80 mg/ml of thunder ball for 4 h, the plerocercoid larvae were fed to experimental mice; it was found that the infective percentage of plerocercoids was 32.5 % (13/40), 20 % (8/40), 5 % (2/40), and 5 % (2/40), respectively, considerably lower than those of the control group without culture with thunder ball ($P < 0.05$). None of 16 mice fed with plerocercoids cultured in 80 mg/ml for 12 or 24 h was infected. The survival rate of plerocercoids from infective mice treated with 400, 800, or 1,600mg/(kg.d) for 3 days is 37.5 % (15/40), 35 % (14/40), and 40 % (16/40) respectively, considerably lower than that of the control group (72.5 %, 29/40) ($P < 0.05$). The infectivity of plerocercoids was reduced more seriously with the drug concentration increasing and the test time extending.

11.10 Prevention

Because sparganosis is an important food-borne disease, the health education about sparganosis and the importance of food sanitation should be implemented in all rural endemic areas.

In areas of endemic infection, strategies should warn people against ingesting the raw flesh of the second intermediate hosts or paratenic host, such as frogs, snakes, pigs, and against using them as poultices. People should be advised of the dangers of drinking water from ponds and ditches, which may contain infected copepods.

11.11 Conclusions and Perspectives

Sparganosis is one of the food-borne zoonotic parasitic diseases in China and other Asian countries such Vietnam, Japan, and Korea. Its transmission results from the local people's diet habit, deficiency of knowledge on sparganosis, and food supervisory defect. Human is an accidental host in its life cycle, becoming infection of plerocercoid larvae by contacting with or ingestion of the first, second intermediate hosts, or a paratenic host. The infection of plerocercoid may cause subcutaneous, ocular, buccal cavity, cerebral, or visceral sparganosis. Diagnosis depends mainly on pathological examination of the plerocercoid larvae. Surgical removal is the first choice of treatment. Praziquantel is often used to kill the parasite. The dried sclerote of *O. lapidescens* (thunder ball or "Qiu-Lei") was used to treat the animal experimental infection of plerocercoid, which revealed certain effects against the parasite. Avoiding ingestion of raw fleshes of frog or snake is the best prevention. For preferable control of sparganosis, the complex means should be conducted in following aspects to enhance the health education about sparganosis; to enhance the management of food sanitation; to enhance the cultivation of professionals; to increase studies of sparganosis in all aspects; and to improve the techniques of diagnosis and treatment.

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Chapter 12
Treatment of Echinococcosis with Traditional Chinese Medicines

Hui Cai and Bin Ye

Abstract Human cystic Echinococcosis (CE) and alveolar Echinococcosis (AE), caused by infection with the larval stage of *Echinococcus granulosus* and *E. multilocularis*, respectively, remain endemic in some western regions of China. Traditional Chinese medicines (TCM), with its long history and peculiar characteristics, have good efficacy for some difficult and complicated disease. Recent researches proved that some TCM alone or combined with other chemical drugs were effective and safe in the treatment of echinococcosis. This chapter summarizes the literature concerning the treatment of hydatidosis with TCM and its extract, including tetrandrine, matrine, artemisinin and its derivatives, "*Xiao-bao*" liquid and its preparations, *Peganum harmala* and its extracts, Tibetan medicines, etc. Some researches demonstrated that the combination of TCM and other chemical drugs for hydatidosis had a strong inhibitive action to hydatids, which revealed an important clinical significance. More researches for treatment methods of TCM for hydatidosis should be conducted to improve the efficacy of special therapy.

Keywords Hydatidosis, cystic echinococcosis • Cystic echinococcosis • Alveolar echinococcosis • Traditional Chinese medicine • Tetrandrine • Matrine • Artemisinin • *Peganum harmala* • Tibetan medicines

12.1 Introduction

Echinococcosis is one of the most lethal parasitic zoonoses and remains a public health problem of worldwide importance. The larvae of four species of genus *Echinococcus* (*Echinococcus granulosus*, *E. multilocularis*, *E. oligarthrus*, and

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E. vogeli) can infect humans. In China, the two major species of medical and public health importance are *E. granulosus* and *E. multilocularis*, whose larvae can cause cystic echinococcosis (CE) and alveolar echinococcosis (AE), respectively (Lorca et al. 2007; McManus et al. 2003). Both CE and AE are highly endemic over large areas of north and northwest China, mainly distributed in the provinces of Qinghai, Gansu, Sichuan, Ningxia, Xinjiang, Inner Mongolia, Tibet, and Yunnan (Craig 2006; Ito et al. 2003; Li et al. 2008).

Until the 1980s, surgery was the only option for treatment of AE and CE (Organization 1996). New sensitive and specific diagnostic methods and effective therapeutic approaches against echinococcosis have been developed in the past years. Several new therapeutic strategies which are aimed at controlling CE and AE are under development in China. Traditional Chinese medicines (TCM) are used against parasitic diseases by Chinese peoples in the past thousand years. Recent researches proved that some TCM were effective and safe in the treatment of CE and AE.

12.2 The TCMs for Echinococcosis

12.2.1 Tetrandrine

Tetrandrine is the main component of the alkaloid extracted from the root tuber of *Stephania tetrandra S. moore* (Figs. 12.1 and 12.2). The chemical constitution of tetrandrine is bisbenzylisoquinoline, and the molecular formula is $C_{38}H_{42}N_2O_6$ (Fig. 12.3). The root tubers of *S. tetrandra* are collected in autumn, and sunning to semi-dry after peeling the rough skins, then cutting into sections and continue drying. The pharmacological studies indicate that tetrandrine is a kind of calcium blocker. It exhibits inhibitory effects on various types of diseases such as hypertension, pneumosilicosis, and arthrophlogosis (Cai et al. 2011). It was also found that tetrandrine had the activities of antiparasite when it was tested to kill the larvae of *E. granulosus and E. multilocularis*. Bao collected *E. granulosus* protoscolices and treated them with different concentrations of albendazole and a combination of the two drugs. It was found that tetrandrine had an obvious killing effect on the protoscolices, and the combination of tetrandrine and albendazole improved efficacy against the protoscolices (Bao et al. 2003a).The in vivo experiments demonstrated that a synergistic effect of tetrandrine and albendazole against *E. granulosus* was also reported when the two drugs were used to treat the experimental mice infected with *E. granulosus*. According to the criteria of cyst wet weight, inhibition rate, and histopathological changes of cyst both microscopically and electron-microscopically and pathological grading, it was found that both tetrandrine and albendazole could inhibit the growth of hydatid cysts, and the combination of them revealed a best effect (Bao et al. 2003b). Some researches (Dou et al. 2003; Li et al. 2006) showed that tetrandrine could inhibit the growth of

Fig. 12.1 Stem, leaves, and root tuber of *Stephania tetrandra*

Fig. 12.2 Root slices of *Stephania tetrandra*

E. multiocularis metacestodes in vitro and the results were similar to those obtained by Bao's study. Chen's experiment indicated that tetrandrine had an inhibiting effect on *E. multilocularis*. The combination of tetrandrine and albendazole had strong inhibitive action to *E. multilocularis*, which suggested that it could strengthen albendazole's effect on *E. multilocularis*, and the two drugs had synergistic action (Chen and Shi 2002). The mechanism of tetrandrine and albendazole against *E. multilocularis* infection in mice was studied also. It was found that tetrandrine could influence trace elements (Ca^{2+}, Cu^{2+}, Zn^{2+}, and Fe^{2+}) in AE tissue, and IgE, IL-2, hyaloplasm acid content of therapeutical groups were changed significantly compared with control groups. The authors thought that the growth

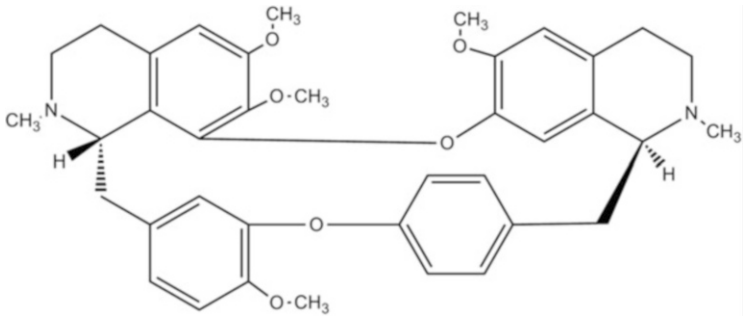

Fig. 12.3 Chemical structure of tetrandrine

inhibition of *E. multilocularis* in mice might be related to tetrandrine's blocking to calcium channel in cell membrane and enhance immunity (Bao et al. 2003c; Chen et al. 2003; Dou et al. 2004).

12.2.2 Matrine

Matrine is an alkaloid isolated from the root of *Sophora flavescens* (Leguminosae) (Figs. 12.4, 12.5, and 12.6). Its molecular formula is $C_{15}H_{24}N_2O$ (Fig. 12.7), a kind of tetracyclic quinolizidine alkaloids, and a relative molecular mass of 248.37. The roots of *S. flavescens* are collected in September or October in third year after planting and prepared by washing and drying or fresh cutting and drying. Matrine has functions of anti-inflammation, anti-parasite, anti-arrhythmia, and anti-fibrosis of liver cell (He et al. 2011). Yin found that matrine had an inhibitive effect on mice infected with *E. multilocularis*, and the combination of matrine and albendazole had more potent effect on *E. multilocularis*, which suggested that matrine can strengthen the effect of albendazole on AE (Yin et al. 2009; Zhang et al. 2006). The assessing of the immune status and metabolic reaction of hepatic enzymes on mice with AE after treatment with matrine and albendazole was tested. Zhang (Zhang and Jing 2006) found that matrine might strengthen the immunity of the mice and demonstrated an inhibitory effect on the growth of *E. multilocularis* in vitro and in vivo. Yin pointed out that there were definite alterations in the immune function and changes in redox enzymes and transaminase in the liver tissue of mice infected with hydatid cysts after being treated with matrine and albendazole and that matrine improved remarkably liver function comparing with treating of albendazole (Yin et al. 2005).

Fig. 12.4 Leaves of *Sophora flavescens*

Fig. 12.5 Root of *Sophora flavescens*

12.2.3 Artemisinin and Its Derivatives

Artemisinin, an ancient Chinese herbal remedy for fever, was isolated in 1972 from *Artemisia annua* (Fig. 12.8). The parts above root can be used as herb medicine after cutting and drying in the sun. Artemisinin and its derivatives, including artemether, arteether, and artesunate, have unique structure and high efficiency with low toxicity (Fig. 12.9). In recent years, artemisinin and many of its derivatives were reported to possess some other effects such as anti-inflammatory, immunomodulation, and antitumor activities besides antiparasitic actions against *Plasmodium falciparum* and schistosomes (Ding et al. 2010; Meshnick et al. 1993). In Pu's study (Pu et al. 2005), the mice infected with CE were divided into five groups, and four experimental groups were treated with different concentrations of artesunate and with both artesunate and albendazole. It was found that all

Fig. 12.6 Root slices of *Sophora flavescens*

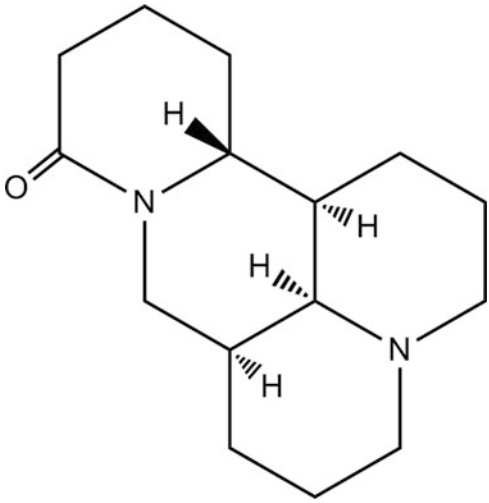

Fig. 12.7 Chemical structure of matrine

medication groups had obvious effect against growth of the cysts, and the combination of artesunate and albendazole showed much higher superiority to the single medication. Compared with the mice in control group, the spleen index and the level of IL-4 and IgE in serum decreased significantly in mice from the medication groups (Pu et al. 2005). Pu pointed out that artesunate could enhance the immunity of the mice with hydatids and improve albendazole's impact on hepatic function of mice (Pu et al. 2006). It was also found that artemisinin combined with albendazole was successful in inhibiting the growth of recurrent CE cysts but lacked ability to prevent recurrence (Zhang et al. 2010). Li's results demonstrated that albendazole and artemether had a dose- and time-dependent effect of anti-*E. multilocularis* (Li et al. 2011). Dihydroartemisinin and artesunate exhibited promising results in the in vitro treatment of *E. multilocularis* and *E. granulosus* larval stages, while

Fig. 12.8 The stems and leaves of *Artemisia annua*

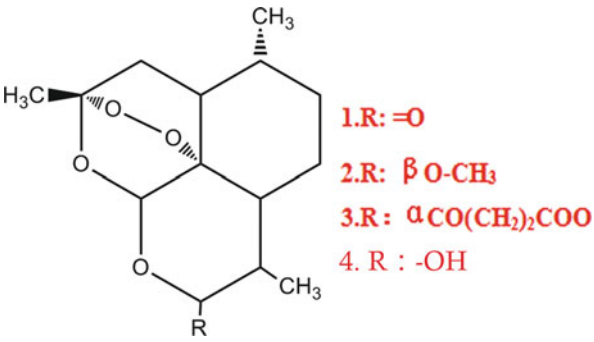

Fig. 12.9 Chemical structures of artemisinin and its derivatives (*1*. artemisinin; *2*. artemether; *3*. artesunate; *4*. dihydroartemisinin)

6 weeks of in vivo treatment of mice infected with *E. multilocularis* metacestodes had no effect (Spicher et al. 2008). However, combination treatments of both drugs with albendazole led to a substantial but statistically no significant reduction in parasite weight compared to results with albendazole alone (Spicher et al. 2008).

12.2.4 Xiao-bao *Liquid and Its Preparation*

Xiao-bao stock liquid is a complex solution of Chinese medicines, mainly comprised of areca, ornphaia, snake slough, ground beetle, honeycomb, and gangolin scales. The Chinese word "*Xiao-bao*" means removing or reducing of hydatid cyst. In order to achieve a better anti-hydatidosis effect of the stock liquid of *Xiao-bao*, the other complex forms of the medicine were produced as well, such as *Xiao-bao* powder, *Xiao-bao* capsule, and complex *Xiao-bao* tablets. According to the results of mice infected with *E. multilocularis* in experiments, 20 % of the murine hydatid cysts revealed damaging of walls after cysts being immersed in the *Xiao-bao* stock liquid (Jiang 2002; Jiang et al. 2000). Jiang's results indicated that *Xiao-bao* powder had an apparent effect to inhibit the proliferation and growth of

alveococcus in the early and advanced stage of murine alveococcosis (Jiang 1995). It was also found that water and alcohol extracts of *Xiao-bao* pills could inhibit the growth of hydatid cyst in mice by inhibiting the proliferation of cells and germinal layers of cysts and causing the degeneration of laminated layer and extensive damage of cell inner structures (Jiang et al. 1996; Liu et al. 1996). Tian reported that the therapeutic effect of complex *Xiao-bao* tablets in the treatment of liver hydatid disease was significant (Tian et al. 2008). The treatment methods of *Xiao-bao* pills combined with albendazole for hydatidosis have also been explored experimentally. The early and advanced stages of mice intraperitoneal alveococcus infection were respectively treated with albendazole and *Xiao-bao* powder. It was found that three groups for treating early-stage infected mice had the effects of inhibiting alveococcus proliferation and growth, while the effects of *Xiao-bao* powder was less active than albendazolem. The ultrastructure of alveococcus showed more severe damage in the mice treated with the combined drug of albendazole and *Xiao-bao* powder (Jiang 1991; Yu 1992). The results of mice experiment and clinical trial indicated that *Xiao-bao* capsule exhibited good efficacy with very low toxicity. The clinical observation of combined treatment for nine cases of liver CE and 1 case of lung CE was summarized in Jiang's chapter (Jiang 2011). Jiang reported that the ultrasonic comparison of pre- and post-treatment showed a difference, the mixed echo turned to heterogenous, later homogeneity became gradually with occasional multiple calcification, indicating a cure stage (Jiang 2011). Some clinical reports revealed good curative effects of *Xiao-bao* pills combined with albendazole for hydatidosis (Jiang 1982, 1986; Jiao et al. 1990a; Li and Jiao 1989). In Jiao's study, the complex *Xiao-bao* tablets were used to treat 204 patients with hydatidosis. According to the criteria of the clinical signs, medicophysics-imaged examinations and observations of the morphological and internal structural changes in hydatid cysts, the significant differences were found between the patients given the complex *Xiao-bao* tablets and patients receiving *Xiao-bao* pills or albendazole by Chi squared test analysis (Jiao et al. 1992). The hydatid cysts, which were obtained from patients treatment failure by *Xiao-bao* pills combined with albendazole, were detected by histopathological examination and electron microscopy. Serious structural damage of protoscolices and complete destruction or even disappearance of laminated layer and germinal layer was observed, which demonstrated that combined using *Xiao-bao* pills and albendazole was effective in the treatment of hydatidosis (Jiao et al. 1990a, b).

12.2.5 Peganum harmala and Its Extracts

Peganum harmala is a perennial herbaceous plant of Zygophyllaceae, which grows in semiarid rangeland (Fig. 12.10). The pharmacological compounds of *P. harmala* are several alkaloids, which are found especially in the seeds (Fig. 12.11) and the roots. These alkaloids include β-carbolines such as harmane, harmine, harmaline (identical with harmidine), harmalol (Fig. 12.12), and quina-zoline derivatives:

Fig. 12.10 *Peganum harmala*

Fig. 12.11 *Peganum harmala* seeds

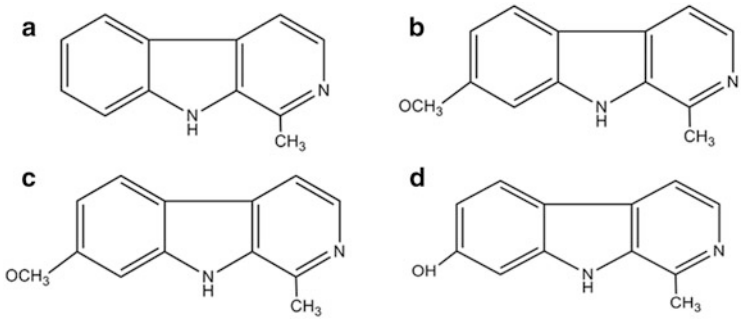

Fig. 12.12 Chemical structures of *P. harmala* alkaloids [(**a**) harmane; (**b**) harmine; (**c**) harmaline; (**d**) harmalol]

vasicine and vasicinone. The alkaloidal content of unripe seeds is less than ripe ones. *P. harmala* has very important biological activity, especially in the aspect of antineoplastic, the action on nervous system, and the action on cardiovascular system (Mahmoudian et al. 2002). It was also found that *P. harmala* had the activities of antiparasite when it was tested to kill the larvae of *E. granulosus and E. multilocularis*. Kang reported the therapeutic effect of *P. harmala* seed against

cystic hydatid and alveolar hydatid (Kang et al. 1993). Through the experiment, it was confirmed that the *P. harmala* seed was more effective on the Echinococcosis than other anti-parasitic herbs, its effective active ingredients were alkaloid harmine and harmaline. The *P. harmala* seed solution was made and used to treat cases with the liver hydatid disease. The effective rate was 75 % and two cases were cured (Yang et al. 1993; Yao et al. 1995). It was found that the effect of *P. harmala* on inhibiting development of hydatid in mice was amount to albendazole, and the combination chemotherapy of *P. harmala* and albendazole was more effective than either agent alone (Ming et al. 2001; Xue et al. 1993).

12.3 Tibetan Medicines

Tibetan medicine, with its long history and peculiar characteristics, is an important part of TCM. Tibetan medicine has good efficacy for some difficult and complicated disease (Gan 2001; Loizzo et al. 2009). The Tibetan medicines, such as native copper and *Corydalis stricta*, are used to treat hydatidosis.

Native copper is pyrite mineral belonging to sulfide iron ore with molecules of FeS_2. The mineral material is processed by washing, drying, smashing, or by forging, or quenching in vinegar, then drying in sun, and crushing. It has better character of activating blood circulation to dissipate blood stasis and is generally used in bruises, fracture (Fig. 12.13). In Cao's study, Tibetan drugs and pure metal copper were used for the treatment of secondary hydatid disease in mice, in which it was observed that a great damage in cyst wall of hydatid and other organ of mice (Cao et al. 2006; Cao and Shi 2004; Cao and Zhao 2008). Although the Tibetan drugs and pure mental copper had effect on the murine secondary hydatid disease, they should not be used alone. Cao found that IL-2, IL-6, IL-10, and IgE could be used as indicators of the treatment for hydatidosis with Tibetan drugs (Cao et al. 2010).

The alkaloids are extracted from the epigeal part and root of *C. stricta*. The plant is collected in autumn and processed by washing and drying or is collected in summer and dried in sun. It was used to treat the massive hemoptysis due to pulmonary tuberculosis, nocturnal emission, carbuncle toxin, and tinea (Fig. 12.14). *C. stricta* might be a promising drug for hydatidosis (Chen et al. 1985a, b; Ye et al. 1985a, b, 1990), because the pharmacological and toxicological tests demonstrated that it had the characteristics of low toxicity, little side-effect, and marked curative effectiveness. After the treatment of *C. stricta*, the ultrastructural changes of germinal layer of human *E. granulosus* cyst and protoscolices were observed, which seemed clearly that *C. stricta* had a profound intracellular effect on *E. granulosus* cysts and protoscolices in humans and might be a promising drug for human hydatid disease (Chen et al. 1986, 1987).

Other Tibetan medicines, such as *Sophora moorcroftiana* and *Jiu-wei-niu-huang-san* (a complex medicine comprised of nine ingredients in Chinese, including *artificial bezoar*, *safflower*, *meconopsis*, *aristolochia manshuriensis*, *Swertia bimaculata*, *common vladimiria root*, edible corydalis, *herpetospermum*

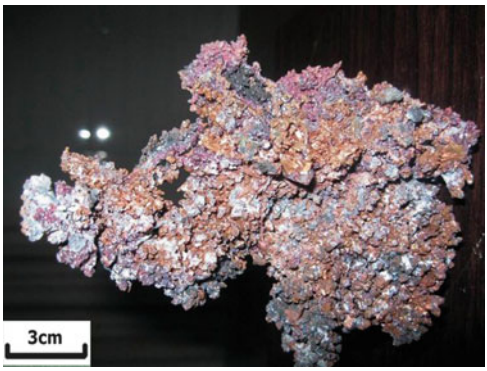

Fig. 12.13 Native copper

Fig. 12.14 Herb of common corydalis

pedunculosum, etc.), showed anti-hydatidosis effect in vitro and in vivo (Bao et al. 2005; Ye et al. 1987). Some clinical reports demonstrated that the treatment of Tibetan medicine for hydatidosis had good curative effects (Cai 1998; De 2001; Ma 2000).

12.4 Other Chinese Herbal Medicines

Wu-lei-he-ji, a complex medicine comprised of five ingredients in Chinese, includes *Radix linderae*, *omphalia*, *pumpkin seeds*, *betelnut*, and *Isodon ternifolius*. The complex medicine was used in treatment of 120 patients of hydatidosis and found that 62 patients were cured after taking it for 90 days, and 42 cases were cured after taking it for 120–150 days (Liu et al. 2004).

It was found that Chinese herbal medicines had a good synergism with classic anti-hydatid drugs. *Rhizoma chuanxiong* is collected at the late May or early June in the second year after planting and is processed by collecting roots and oven-dry (Fig. 12.15). The effective component from *R. chuanxiong* is tetramethylpyrazine, an amide alkaloid with several bioactivity elements from total rhizome chuanxiong alkaloids (Fig. 12.16). Wang's results indicated that tetramethylpyrazine had a

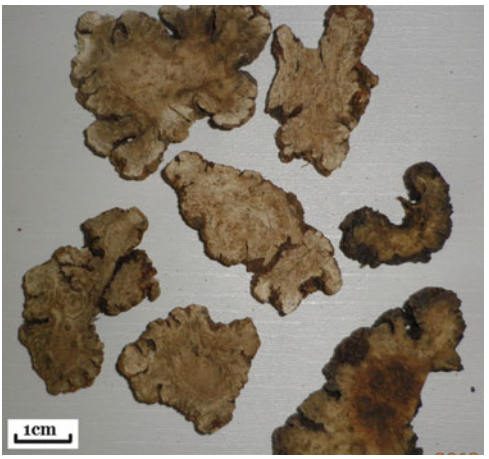

Fig. 12.15 Root slices of *Rhizoma chuanxiong*

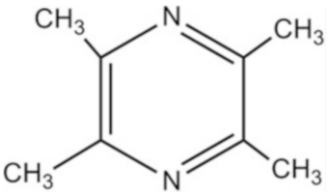

Fig. 12.16 Chemical structure of tetramethylpyrazine

synergia action with albendazole against *E. multilocularis*, and tetramethylpyrazine could significantly improve hepatic functions of the mouse infected with *E. multilocularis* (Wang et al. 2008).

12.5 Conclusions and Perspectives

TCM is widely used in clinic practices in China, and many TCM products have been used in treating hydatidosis. According to the literatures, the TCM and its extracts that are utilized to therapy for hydatidosis include tetrandrine, matrine, artemisinin and its derivatives, *Xiao-bao* liquid and its preparations, *P. harmala* and its extracts, Tibetan medicines, etc. (Table 12.1). These TCM prescriptions to treat hydatidosis have curative effects, and the combination of TCM and other chemical drugs for hydatidosis have strong inhibitive action to hydatidosis, which have important clinical significances. The active compositions in most of Chinese herbs are still not clear, and it is difficult to explain the functional mechanism of TCM by the theory of modern biomedicine. Although TCM has good effect and low toxicity in treating hydatidosis, there are still many researches to explore the effective fractions and components of TCM, and investigate the mechanisms against hydatids, and more clinical studies are needed to confirm the effects of TCM treatment.

Table 12.1 The main traditional Chinese medicines for echinococcosis

Name of TCM	Functional component	Clinical application	Use dosage/test dosage
Stephania tetrandra	Tetrandrine	N/A[a]	50 mg/(kg day) × 90 days for CE and AE of mice
Sophora flavescens	Matrine	N/A	50 mg/(kg day) × 90 days for AE of mice
Artemisia annua	Artemisinin and its derivatives	N/A	25 or 50 mg/(kg day) × 90 days for AE and CE of mice
Xiao-bao liquid	Areca, ornphaia, snake slough, ground beetle, honeycomb, gangolin scales	Yes	38 or 56 mg/(kg day) × 30 days for CE of mice
Xiao-bao powder		N/A	The mice were fed with a mixture of Xiao-bao powder and food in the ratio of 1:4 for 90 days
Xiao-bao capsule		Yes	500–1,000 mg/day × 90 days for AE and CE of human
Complex *Xiao-bao* tablets		Yes	2.0 g, po, tid × 30 days for CE and AE of human
Xiao-bao pills		Yes	28 mg/day × 60 days and 56 mg/day × 4 days for CE of mice 20 mg, po, tid × 30 days for CE and AE of human
Peganum harmala seeds	Harmane, harmine, harmaline (identical with harmidine), harmalol, etc.	N/A	N/A
Native copper	Unknown	N/A	200 mg/(kg day) × 30 days or 90 days for CE of mice
Corydalis stricta	Alkaloid	Yes	
Sophora moorcroftiana	Alkaloid	N/A	50 mg/(kg day) × 90 days for CE of mice
Jiu-wei-niu-huang-san	*Artificial bezoar*, *safflower*, *meconopsis*, *aristolochia manshuriensis*, *Swertia bimaculata*, *common vladimiria root*, edible corydalis, *herpetospermum pedunculosum*, etc.	N/A	15 mg/(kg day) × 30 days for CE of mice
Wu-lei-he-ji	*Radix linderae*, *omphalia*, *pumpkin seeds*, *betelnut*, *Isodon ternifolius*	Yes	*Radix linderae* 10 g, *omphalia* 10 g, pumpkin seeds 30 g, betelnut 30 g, Isodon ternifolius 30 g in addition, 1 bag/day, qd, for 90 days a course of treatment of human AE and CE
Rhizoma chuanxiong	Tetramethylpyrazine	N/A	25 mg/(kg day) × 60 days for CE of mice

[a]N/A: not available

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Chapter 13
Treatment Methods of Traditional Chinese Medicine for Infection with *Ascaris lumbricoides* and Other Nematodes

Hejun Zhou

Abstract Intestinal nematodes, especially the *Ascaris lumbricoides*, *Trichuris trichiura*, the hookworms, and the tapeworms, are the most common infections worldwide affecting the most deprived communities. According to new WHO estimates, about 883 million children worldwide require preventive chemotherapy for these diseases, of whom more than 300 million suffer from severe morbidity. Some drugs such as benzimidazole have the function on the treatment of these infections. However, benzimidazole also has some unignored side-effects. Thus it may urgently need further development for search of some other safe and useful anti-nematode alternatives. Traditional Chinese medicines, such as Fructus quisqualis, *Caloglossa leprieurii*, Fructus meliae toosendan, Semen arecae, dried pumpkin seeds, and *Semen torreyae* all have good effects on intestinal nematodes infections. Based on experimental studies, some of these traditional Chinese medicines, like toosendanin and quisqualic potassium, can paralyze *Ascaris lumbricoides* and other nematodes, which was reported that the effect of anti-nematodes was equal to mebendazole and albendazole. After all, these traditional Chinese medicines are simple and safe to use, easy to promote, which command a unique position in anti-intestinal nematodes. Here this comprehensive review attempt to briefly review in some natural products from traditional Chinese medicine which have the activity against ascariasis and infections with other nematodes.

Keywords *Ascaris lumbricoides* • *Trichuris trichiura* • Hookworms • Tapeworms • Nematodes infection • Fructus quisqualis • *Caloglossa leprieurii* • Semen arecae • Dried pumpkin seeds • Semen torreyae • Traditional Chinese medicine

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13.1 Introduction

Intestinal worms, especially the *Ascaris lumbricoides*, *Trichuris trichiura*, and the hookworms, are the most common infections worldwide affecting the most deprived communities (Knopp et al. 2008; Petersen 2003). Recent estimates suggest that *A. lumbricoides* infects over 1 billion people, *T. trichiura* 795 million, and hookworms (*Ancylostoma duodenale* and *Necator americanus*) 740 million (Brooker 2010). According to new WHO estimates, about 883 million children worldwide require preventive chemotherapy for these diseases, of whom more than 300 million suffer from severe morbidity (Friedman et al. 2012). Intestinal worms produce a wide range of symptoms including intestinal manifestations (diarrhea, abdominal pain), general malaise, and weakness, which may affect working and learning capacities and impair physical growth. Hookworms cause chronic intestinal blood loss that results in anemia (Craig and Ito 2007).

Drugs for the treatment of intestinal nematodes are benzimidazole, which are efficient, broad spectrum, and few side effects (McKellar and Scott 1990). Albendazole is one of the best drugs against intestinal nematodes in Benzimidazoles, and the cure rate of ascariasis, hookworm, trichuriasis, and stercoralis is 84.6–100 %, 87.5–100 %, 100 %, and 50 % separately (Bethony et al. 2006; Hu 1995). Moreover, mebendazole, levamisole, pyrantel, and hydroxyl pyrimidine are also good anthelmintic drugs. However, in recent years, the imidazole drugs have a severe allergic reaction, and the emergence of resistant, which should attract attention (Hu 1995; Franchi et al. 1999).

The traditional Chinese medicine usage is simple and safe to use, easy to promote in treatment of nematode parasites (Jiyou 1955). As recorded in "Pharmacology" by Wangjunmo, toosendanin, an active ingredient in Chinaberry, can paralyze *Ascaris*, and this effect is the same with quisqualic potassium, an extract of the water-soluble portion from the nucleoli of quisqualic (Zhang et al. 1959). Arecoline, an effective ingredient of the betel, can paralyze the tapeworms, not only pork tapeworms but also beef tapeworms, and if used in conjunction with the pumpkin seeds, the effects will be enhanced (Li et al. 2007). Moreover, the aqueous extracts of Omphalia can kill segments of tapeworms (Okabe 1972; Watkins 1958). After all, the occurrence of side effects of these traditional Chinese medicines is rarely heard, and some of the medicines can be used as snacks.

13.2 Fructus quisqualis

Fructus quisqualis, a traditional Chinese medicine, is the fruit of *Quisqualis indica* L., which could be found in Southeast Asia, India and South China. It is reported that Fructus quisqualis was firstly used as an anti-ascariasis medicine by Guo shijun in Northern Song Dynasty in China (Shang 2007). And from then on, Fructus quisqualis was considered as a commonly used anti-ascariasis drug. Cui

et al. (1994) reported that the effect of anti-ascariasis Fructus quisqualis is equally to mebendazole and albendazole (Chen 2009). In an anti-ascariasis clinical experiment which include 900 patients from 7 to 10 years old, 500 of who took Fructus quisqualis (100 g/person) only, while 200 of who took mebendazole (2 pieces/day) and albendazole (2 pieces/day) only, separately. After 3 days, the ascarid eggs disappeared in 382 (76.4 %) patients in Fructus quisqualis team, while 154 (77 %) in albendazole team and 148 (74 %) in mebendazole. And the ascarid eggs decreased in 102 (20.4 %) patients in Fructus quisqualis team, while 43 (21.5 %) in Albendazole team and 52 (26 %) in Mebendazole (Cui et al. 1994). After all, Fructus quisqualis tasted good, which is welcomed by children. However, overdosage of *Fructus quisqualis* also has some adverse effects like hiccup, dizziness, nausea, emesis, and diarrhea (Shen et al. 2008).

The modern pharmacological studies show that the main component of anti-ascariasis of Fructus quisqualis is potassium quisqualate which could selectively activate central nervous (Martin et al. 2002; Rice and Nicholson 1990). Except for the function of *Fructus quisqualis* against ascariasis and enterobiasis, *Fructus quisqualis* also has been reported in field application for treating headache, fever, and wound infection in Southeast Asia (Lauritzen et al. 1988). Two components, punicalagin and punicalin, of *Fructus quisqualis* were reported to have the function of anti-AIDS virus and protect liver from damage (Lin et al. 2001; Nonaka et al. 1990) (Fig. 13.1).

13.3 Caloglossa leprieurii

Caloglossa leprieurii is the body of *Caloglossa leprieurii* (Mont) J.Ag, which was firstly reported as anti-ascariasis drugs by Xueminzhao in "Compendium of Materia Medica Supplements" in 1891 (Chunbo et al. 1964; Li et al. 1987). *Caloglossa leprieurii* could be cooked or used as *Caloglossa leprieurii* decoction that decocted from dry *Caloglossa leprieurii*. Few adverse effects were reported as mild abdominal discomfort, abdominal pain, diarrhea, vomiting, loss of appetite, and dizziness (Rongyuan et al. 2004; Xing 2008).

According to an observation of more than 1,000 patients, 84–88 % of who discharged lumbricus, more than 60 % of the cases discharged lumbricus within 24 h after taking *Caloglossa leprieurii*. However, the ascariasis couldn't be drained completely so that *Ascaris* eggs were detected in 93 patients after 3 weeks, though the number of *Ascaris* eggs decreased. These results suggested that one medication cannot achieve radical purposes (Chang-Chuin 1965). To strengthen the anthelminthic and anti-lumbricus efficacy of *Caloglossa leprieurii*, levamisole, which has good "anti-lumbricus" efficacy while with some adverse effects, is added to make compound *Caloglossa leprieurii* powder. A clinical study of 375 patients has shown that 363 (96.8 %) patients discharged *Ascaris* worms and 340 patients were found exclusive of eggs. Most important, few adverse effects were found in the study.

Fig. 13.1 Traditional Chinese medicine: Fructus quisqualis

The *Caloglossa leprieurii* decoction could induce paralysis of the worm but not kill it. And this "anti-lumbricus" effect was attributed to digenic acid, a component in *Caloglossa leprieurii*, that could eliminate the worm by decomposition the ferrugination respiratory enzyme contained in parasites gastrointestinal epithelial cells (Li et al. 1987). Furthermore, low concentration of digenic acid could increase the neuromuscular tension of earthworm specimens, while high concentration of digenic acid caused paralysis of earthworm, which could be antagonized by L-proline (Murakami et al. 1953) (Fig. 13.2).

13.4 Toosendanin

Toosendanin is an active substance, existing in the fruits (Fructus toosendan), leaves, and cortexes (cortex meliae) of *Melia toosendan* (TSN) (Choi et al. 2012), which has antifeedant effects and stomach poisoning effects (Chen et al. 1995). Toosendan is produced in China, Gansu, Hubei, Sichuan, Guizhou and Yunnan provinces, but the best quality comes from Sichuan (Lu 1997).

The anti-ascarid effects of Fructus toosendan and cortex meliae have long been documented in Chinese medicine documents like: "Lei Gong's Treatise on the Preparation" in Tang dynasty, and "Compendium of Materia Medica" in Ming dynasty (Xiong and Chen 2006). After taking the decoction of Fructus toosendan and cortex meliae, about 70 % of the patients could be cured. The compatibility of toosendanin with Fructus quisqualis or betel nuts can enhance its de-worming role (Hong and Hong 1991).

Modern pharmacological studies show that the main component of anti-ascariasis efficacy in Fructus toosendan and cortex meliae is toosendanin that

Fig. 13.2 Traditional Chinese medicine: *Caloglossa leprieurii*

could block the conduction of the nerve center, destruct the intestinal tissue in a variety of detoxification enzymes and respiratory metabolism of ascarid, weeken the digestion and absorption of the ascarid, and induce the worms to antifeeding resulting in the death of the worms (Li et al. 2008; Wang and Wen 1959). The formula of toosendanin is C30H38O11, and the molecular weight is 574.63. Twenty-five mg of toosendanin is included in one tablet, treatment can be done by 250 mg taken once by adult, while 50–100 mg by 2–4-year-old, 100–150 mg by 4–8-year-old, 150–200 mg by 8–16-year-old, and 200–250 mg by >16-year-old (Wang and Wang 1959).

Compared to santonin, a commonly used anti-ascariasis drug, the efficacy of toosendanin is slow and long-lasting, so that the time of discharging the worms starts a bit later (about 24–48 h) and the expelled worms are still alive (Shi and Wu 1963).

In addition to its role of an anti-roundworm drug, toosendanin is also effective against botulism. Toosendanin could antagonize the effect of nerve repression induced by botulinum at the neuromuscular junction. Toosendanin can also be used in agriculture to prevent Lepidoptera pests such as *Pieris*, borer, cabbage armyworm, and beet armyworm (Tang et al. 2002; Wang et al. 2000; Xing 1987) (Fig. 13.3).

13.5 Semen arecae

Semen arecae is the seeds of *Areca catechu* Linn. which can be commonly found in the warm regions of humid tropics of Asia and Africa (Zhou et al. 2007). The anti-parasitic effect of Semen arecae was firstly reported in "Drug recorded by

Fig. 13.3 Traditional Chinese medicine: Fructus meliae toosendan

Lidangzhi" in Three Kingdom Periods (Niang-Ningxia 2004). Semen arecae is a broad-spectrum anti-parasitic drug that can act against *Clonorchis sinensis*, *Schistosoma* species, and some intestinal nematodes. The cure rates of Semen arecae of infections with *Fasciolopsis buski* is about 47.2–90 %, with *Trichuris* is above 65 %, with *Ascaris* is about 40–68 %, with hookworms is above 55 % (some experiments are above 91 %, but some others are about 32 %), however, its effects on *Enterobius vermicularis* is not good (Chen and Fang 2007). Furthermore, it will service better to use Semen arecae together with dark plum, licorice, $MgSO_4$, or (and) cushaw seed (Li et al. 2007).

In vitro experiments showed that Semen arecae decoction could induce pinworm (*Enterobius*) paralysis, and 50 % water–acetone extracts of Semen arecae has a strong killing activity on ascarid larva. After all, experiments also showed that a 30 % dilution of the Semen arecae decoction could induce stiffness of the dog's short tapeworm or even its death in 40 min, while 1–2 % Semen arecae extract led to paralysis of the pork tapeworm, of the cattle tapeworm, and of the short tapeworm (Chang et al. 2001).

Modern pharmacological studies showed that the main component of Semen arecae is arecoline which induces paralysis of intestinal nematodes. Straight chain fatty acids, especially the lauric acid (dodecanoic acid) from Semen arecae has strong killing effects on roundworms. However, excessive use of arecoline will cause some adverse effects like salivation, vomiting, urination, drowsiness, and convulsions, which could be treated by atropine (Li et al. 1992) (Fig. 13.4).

13.6 Dried Pumpkin Seeds

Dried pumpkin seeds are the seeds of pumpkin, which is used for treatment of prostatic hyperplasia in Europe and in China for a long history (Carbin et al. 1990). It was mentioned in "Compendium of Materia Medica" in China and also recorded

Fig. 13.4 Traditional Chinese medicine: Semen arecae

in Pharmacopoeia in German, that it could be used as a treatment for urinary incontinence, the sensitivity of bladder disease, and prostate hypertrophy. After all, dried pumpkin seeds can also be used to treat parasitic diseases such as ascariasis, enterobiasis, pork taeniasis, and schistosomiasis (Huang et al. 2005).

Good effects can be achieved by the combination use of dried pumpkin seeds and betel nut on pork tapeworms—the cure rate is higher than 90 %. Experiments showed that dried pumpkin seeds and betel nut could paralyze the tapeworm, but betel nut has effects on the scolex and immature proglottids of tapeworm, while dried pumpkin seeds attack the fecundity proglottids in the middle and the rear section. In addition, such pharmaceutical compositions also may expel hookworms and roundworms. Moreover, powder containing honey, dried pumpkin seeds can expel ascarids. Powder containing sugar, dried pumpkin seed can prevent schistosomiasis (Xu 1988).

However, overdose of dried pumpkin seeds can produce temporary pathological damage of the liver, lung, and kidney and decrease the levels of glycogen and fat in liver, which, however, may return to normal rates after stopping the use of dried pumpkin seeds (Wenyin 2011) (Fig. 13.5).

13.7 Semen torreyae

Semen torreyae is the dried ripe seed of the yew plants *Torreya grandis* Fort, which is common in South China. Semen torreyae has a special aroma which is a very attractive appetizer and can be added to delicious food (Chen et al. 2000; Xu 1999). Besides its use in dried stage as a delicious food, *Torreya grandis i*s also an

Fig. 13.5 Traditional Chinese medicine: dried pumpkin seeds

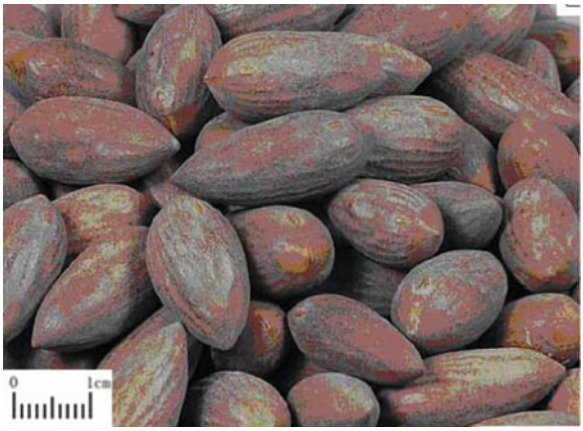

Fig. 13.6 Traditional Chinese medicine: Semen torreyae

expensive Chinese herbal medicine that can effectively help to get rid of intestinal parasites like tapeworms, hookworms, pinworms, roundworms, or *Fasciolopsis buski*. The deworming effectiveness of Semen torreyae is the same as that of the Rangoon creeper fruit. These two traditional Chinese medicines taste good and thus are very welcomed by children and patients (Chang 2010; Hu 2012) (Fig. 13.6).

13.8 Semen pharbitidis

Semen pharbitidis was first documented in "MingYiBieLu" in Chinese as one of the traditional Chinese medicines that could be used in the treatment of ascariasis and taeniasis and of some other helmithiasis (Ao and Weiqun 2003). In a clinical trial,

Fig. 13.7 Traditional Chinese medicine: Semen pharbitidis

Semen pharbitidis was confirmed to have effects in the treatments of enterobiasis, too. All 35 patients were cured at a dose of 5 g Semen pharbitidis which was mixed in cakes (1:10) (Anjian 1991). In combination with betel nut, Semen pharbitidis showed a better anthelmintic effect in a clinical trial of 312 patients infected with nematodes. Semen pharbitidis in combination with betel nut, at the dose of 60 mg/kg body weight, resulted in worm burden reductions of 77.7 % in ascariasis, of 60.0 % in hookworm infections, 80 % of whipworm cases, and in 95.6 % of infections with *Fasciolopsis buski*. An in vitro experiment showed that the combination of Semen pharbitidis and betel nut could paralyze the worms (Ai-rong and Chen 2002) (Fig. 13.7).

13.9 Conclusions

Compared to common western medicine, Traditional Chinese Medicines (TCM) have many advantages in the treatment of nematodes. However, most of the mechanisms of the use of Traditional Chinese Medicine in treatment of the nematodes are not clear. In short, the use of Traditional Chinese Medicine in the treatment of infections of intestinal nematodes has a proven efficacy and is worth to be studied more in detail.

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Chapter 14
Angiostrongylus cantonensis in China

Jie Wei and Zhongdao Wu

Abstract *Angiostrongylus cantonensis* was first discovered in 1934 by Professor Chen Xintao and has become an important emerging pathogen causing human angiostrongyliasis. Rats are permissive host, and mice and human are non-permissive host. The adult worms live in the right ventricle and pulmonary arteries of rats. However, worms can't develop to adult worm and the IV and V stage worm live in brain of mice and human. Human infect this disease by eating raw or undercooked snails or slugs, paratenic host such as prawns or contaminated vegetables, and water that contain the infective larvae. *A. cantonensis* has spread from its traditional endemic regions of the Pacific islands and Southeast Asia to the American continent including the USA, Caribbean islands, and Brazil. During the past few years, major outbreaks of human angiostrongyliasis have been reported in mainland China, Taiwan, Thailand, Ecuadorian, French, Germany, India, and Jamaica. Additionally, sporadic cases in travelers who have returned from endemic areas have been reported. Thousands of cases of human angiostrongyliasis have been documented worldwide. The main clinical manifestations of human angiostrongyliasis are eosinophilic meningitis and ocular angiostrongyliasis. In adult patients, the common symptoms were headache, neck stiffness, paresthesia, vomiting, and nausea. The treatment of this disease includes supportive treatment, corticosteroid therapy, and combined therapy with corticosteroids and anthelminthics. However, it is pity that some patients have sequela after treated. Therefore, some new drugs like Chinese herbal medicine have been studied for therapy angiostrongyliasis. The basic research is very important for better solution of angiostrongyliasis, so more and more study are in process, which involve

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mechanism of brain inflammation, new drugs finding, protein and gene sequence and analysis, new diagnosis method exploring, etc. Whatever, persuading people not to eat raw or undercooked intermediate and paratenic hosts is the most effective method for prevention and control angiostrongyliasis.

Keywords *Angiostrongylus cantonensis* • China • Finding history • Molecular biology • Clinical aspects • Geographical distribution • Population distribution • Route of infection • Epidemiological factors • Diagnosis • Chemotherapy • Prevention • Inflammation mechanism • Geographical strain • Development of new drugs

14.1 Introduction

The nematode *Angiostrongylus cantonensis* was discovered in the pulmonary arteries and hearts of domestic rats in Guangzhou (Canton), China, by Chen Xintiao in 1934 (Chen 1935). *A. cantonensis* is a metastrongyloid nematode that normally lives in the right ventricle and pulmonary arteries of rats, and rats are definitive (permissive) hosts (Bhaibulaya 1975). While many species of rats can carry patent infections, the Norwegian rat (*Rattus norvegicus*) and the black rat (*Rattus rattus*) are considered the most important definitive hosts. In wild populations of rats, *A. cantonensis* infections induce little disease, as expected for an efficient parasite (Bhaibulaya 1975; Maizels and Yazdanbakhsh 2003; Fernando 2001). Dogs, humans, horses, Australian native mammals (e.g., possums, macropods, macrobats), and birds (e.g., Tawny frogmouths), and various zoo animals are nonpermissive "accidental" hosts that are infected after ingesting third-stage larvae (L3) in intermediate hosts (molluscs) (Bhaibulaya 1975; Kliks and Palumbo 1992) or transport hosts (such as planarians, frogs, fish, and crustaceans). In the past ten years, major outbreaks were reported in endemic regions, especially in mainland China. So far, more than 2,800 people has been reported to infect *A. cantonensis* (Wang et al. 2008). Therefore, angiostrongyliasis is not only endemic disease but also the severe public health problem and worth paying attention to. Morera and Céspedes (1970) described *Angiostrongylus costaricensis* causing an abdominal eosinophilic ileocolitis as a new human disease. Several cases are asymptomatic or show light symptoms. However, in cases showing severe symptoms, the disease is often characterized by acute abdominal pain related to lesions in the ileocolic region, with presence of intense eosinophilia, eosinophilic infiltration of the intestinal wall, eggs in the submucosa, and nematodes in the mesenteric arteries. Therefore, the characteristic of angiostrongyliasis induced by *A. cantonensis* is eosinophil infiltration in CNS.

14.2 Finding History of Angiostrongyliasis in China and Other Countries

Angiostrongylus cantonensis was discovered in the pulmonary arteries and hearts of domestic rats in Guangzhou (Canton), China, by Chen Xintiao in 1934 (Chen 1935). *A. cantonensis* is a rat lungworm, which occasionally causes human angiostrongyliasis with the main clinical manifestation of eosinophilic meningitis. The first human case of angiostrongyliasis was reported in Taiwan in 1945 (Rosen et al. 1961). In 1984, He et al. reported the first human case of *A. cantonensis* infection in the mainland of China (He et al. 1984). After that, infection cases were reported continuously. So far, angiostrongyliasis cases were reported in about 10 provinces or cities, involving Guangzhou, Hongkong, Wenzhou, Shanghai, Beijing, Tianjin, Heilongjiang, Liaoning, Hainan, Yunnan, and Fujian (Wang et al. 2010). In past decade, some outbreaks of angiostrongyliasis happened in China. In 2004, China's Minister of Technology has defined angiostrongyliasis as emerging infectious disease.

Thailand is one of the major source of human angiostrongyliasis. At least 1,337 cases of human angiostrongyliasis have been reported. The high infective rate among Thai population is associated with custom of eating raw or undercooked snails (*Plia* spp.) with alcohol, which is especially popular among young adult men (Cross and Chen 2007; Schmutzhard et al. 1988). Since two cases of eosinophil meningitis induced by *A. cantonensis* were reported in Hawaii in 1962 (Rosen et al. 1962, 1967), the parasite has been found in the Pacific islands and southeast Asia. The first case of human angiostrongyliasis in the Caribbean islands was reported in Cuba in 1973 (Pascual et al. 1981). Some surveys reported increasing numbers of human *A. cantonensis* infection in Costa Rica and Jamaica (Slom et al. 2002; Vazquez et al. 1993; Lindo et al. 2004). There was a group of 23 US travelers and about half of them occurred eosinophilic meningitis after returning from Jamaica in 2000 (Slom et al. 2002).

14.3 Biology of Angiostrongyliasis

14.3.1 Life Cycle

As a zoonotic pathogen, *A. cantonensis*, a rat lungworm, is endemic in south Asia, the Pacific islands, Australia, and the Caribbean islands. The life cycle of this nematode involves rats as the definitive host, molluscs as intermediate hosts, and crustaceans (prawns and land crabs) (Fig. 14.1), predacious land planarians (flatworms in the genus *Platydemus*), frogs, and monitor lizards as paratenic (transfer or transport) hosts. Human beings acquire *A. cantonensis* after eating intermediate or paratenic hosts or vegetables that contain the infective larvae (the third stage) of the worm. Once swallowed, the infective larvae are digested from

Fig. 14.1 The intermediate hosts of *A. cantonensis*

those vectors and invade intestinal tissue, causing human enteritis, before passing through the liver (Yii 1976). Cough, rhinorrhoea, sore throat, malaise, and fever can develop when the worms move through the lung (Cross 1978). Finally, the larvae reach the central nervous system in about 2 weeks and cause eosinophilic meningitis.

The major pathological changes of human angiostrongyliasis occur in the brain (Eamsobhana and Tungtrongchitr 2005; Chotmongkol et al. 2006; Sonakul 1978). According to autopsy studies, the external surface and spinal cord are generally normal, and gross hemorrhage is not commonly seen. Infiltration of lymphocytes, plasma cells, and eosinophils is commonly reported in the meninges and around intracerebral vessels (Eamsobhana and Tungtrongchitr 2005; Sonakul 1978).

Cellular infiltration around living worms is not prominent, but dead worms were usually surrounded by a granuloma, an increase in the number of eosinophils, and sometimes Charcot–Leyden crystals (Eamsobhana and Tungtrongchitr 2005). The physical lesions of tracks and micro-cavities caused by movement of the worms can be found in the brain and even in the spinal cord. The larvae can also move to the eyes and cause ocular angiostrongyliasis with visual disturbance such as diplopia or strabismus in many patients (Punyagupta et al. 1975; Sawanyawisuth et al. 2006).

14.3.2 Morphology

A. cantonensis larvae got from lung tissue of *P. canaliculata* by means of micro-anatomy usually appeared to be intact. Few refractive granules were observed in very early stage L1. At early-stage L1, refractive granules began to emerge in the larval body, and in later-stage L1 and L2, they had increased and even obscured the expanded gut (Fig. 14.2) (Lv et al. 2009c). A clear line emerged at the esophagus–intestine junction, dividing the larval body into an anterior section with few refractive granules and a posterior section dense with granules. Later, just before the second molting, the line blurred, and the amount of refractive granules decreased (Fig. 14.3) (Lv et al. 2009c). Some big refractive granules

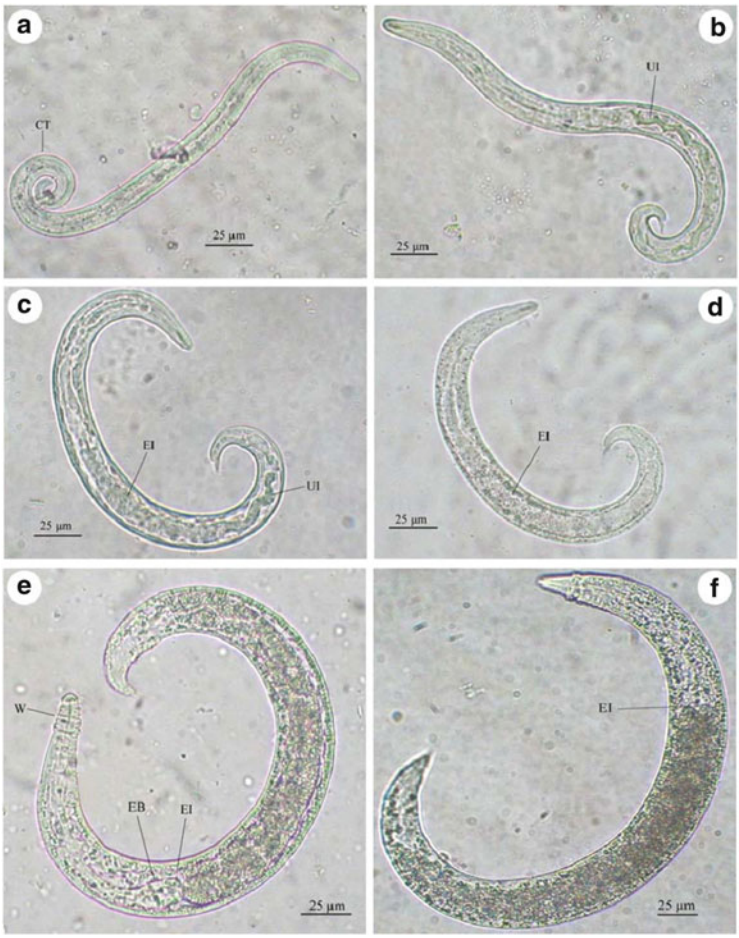

Fig. 14.2 The image of first-stage larva of *A. cantonensis*. (**a**) Very early first-stage larva (L1) of *A. cantonensis* recovered from fresh rat feces. The larva moves with a coiled tail (CT). (**b**) Early L1 of *A. cantonensis* recovered from *P. canaliculata* on day 3 postinfection. Unexpanded intestine (UI) presents strand-like. (**c**) Early L1 of *A. cantonensis* recovered from *P. canaliculata* on day 5 postinfection. The intestine is subdivided into two segments: expanded intestine (EI) at the anterior part and unexpanded intestine (UI) at the posterior part. (**d**) Mid-stage L1 of *A. cantonensis* recovered from *P. canaliculata* on day 7 postinfection. The expanded intestine (EI) replaces the unexpanded intestine observed at an earlier stage. (**e**) Late L1 of *A. cantonensis* recovered from *P. canaliculata* on day 11 postinfection. The intestine becomes obscure due to refractive granules, while the esophagus bulbus (EB) anterior to the esophagus-intestine (EI) line is clear, with wrinkles (W) appearing at the anterior end. (**f**) Late L1 of *A. cantonensis* recovered from *P. canaliculata* on day 15 postinfection. The body size has increased approximately to that of L2. The esophagus–intestine (EI) line is clear due to heterogeneous distribution of refractive granules

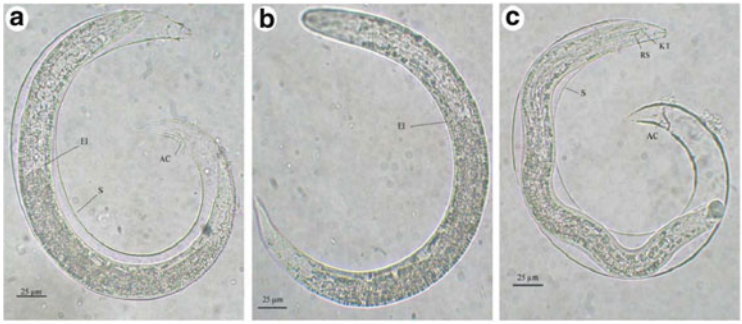

Fig. 14.3 The second-stage of *A. cantonensis*. (**a**) Second-stage larva (L2) of *A. cantonensis* with one sheath. The anus cuticle (AC) of L1 is completely molted and presents on the sheath (S). The esophagus–intestine (EI) line appears clear. (**b**) L2 of *A. cantonensis* without sheath. The larval body is not destroyed, and the esophagus–intestine (EI) line still appears clear. (**c**) L2 of *A. cantonensis* with expanded knob-like tips (KT) and rod-like structure (RS). The developed larva shows capability of movement and the esophagus–intestine line has disappeared, whereas the anus cuticle (AC) of L1 is well presented on the sheath (S)

could still be seen in the posterior section of early-stage L3. Late-stage L3 were transparent, and the anus, excretory pore, and esophagus–intestine junction could easily be identified (Fig. 14.4) (Lv et al. 2009c).

The larvae in the cranial cavity were mainly at the stage of L4 and L5, which were slender, thread-like roundworm, with a circular mouth in the anterior part. The heads of both sexes were similar, but the tails were different. The female was pointed with the vulva in front of the anus, but the male was broad with copulatory bursa and long spicules. The gastrointestinal tracts and genital tubes were clearly seen inside the coelomic cavity through the translucence body (Fig. 14.5) (OuYang et al. 2012). Adult worms were recovered from the cardiopulmonary systems of rats. These worms had features characteristic of *A. cantonensis*, including size (males measured 14–15 mm in length; females 24–26 mm in length), body shape, and prominent dark intestine (Fig. 14.6a) (Lindo et al. 2002). The long copulatory spicules in the male worms measured approximately 1.2 mm (Fig. 14.6b) (Lindo et al. 2002). The eggs were got from lung of rats after they were infected 8 weeks (Fig. 14.7) (Gu et al. 2008).

14.4 Molecular Biology of *A. cantonensis*

It is not incompletely known for the molecular characteristics of *A. cantonensis*. The expressed sequence tags (ESTs) of *A. cantonensis* were analyzed in order to get more insight to its genomic expression patterns. About 1,277 ESTs of *A. cantonensis* in NCBI databases. The result showed that there were 60 ESTs had no match to any of the proteins and gene sequences in the published databases,

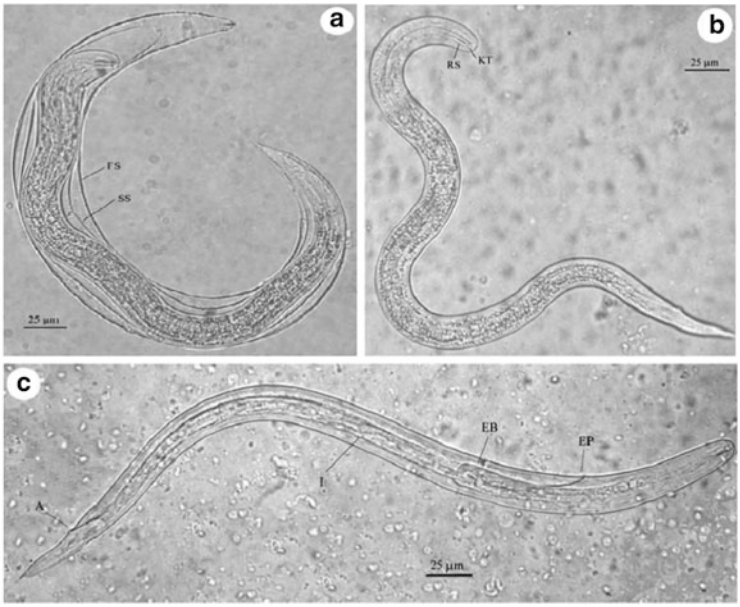

Fig. 14.4 The third-stage larva of *A. cantonensis*. (**a**) Third-stage larva (L3) of *A. cantonensis* with two sheaths. The outer one is the first sheath (FS) produced during the first molting, whereas the inner one is the second sheath (SS) produced during the second molting. (**b**) Typical L3 of *A. cantonensis* with expanded knob-like tips (KT) and rod-like structure (RS), but no sheath. (**c**) Mature and transparent L3 of *A. cantonensis*. Clearly visible structures include excretory pore (EP), esophagus bulbus (EB), intestine (I), and anus (A)

Fig. 14.5 The fourth stage and fifth stage of *A. cantonensis* from rat and mice brain. Images show the intracranial larvae obtained from rats. *A. cantonensis* larva was a kind of thread-like, semi-transparent nematode with a simple circular mouth (*arrowhead*) in the anterior part and a tail (*arrow*) with a copulatory bursa (male) or a vulva (female)

and 695 ESTs score more than 80. According to the function, the identified 695 ESTs could be grouped into 13 categories related to metabolism, cellular development, immune evasion, host–parasite interactions, and so on. Among them, 65 (9.4 %) were proteases and protease inhibitors, represented 19 potential proteases and protease inhibitors genes; 42 (6.0 %) were allergens or antigens,

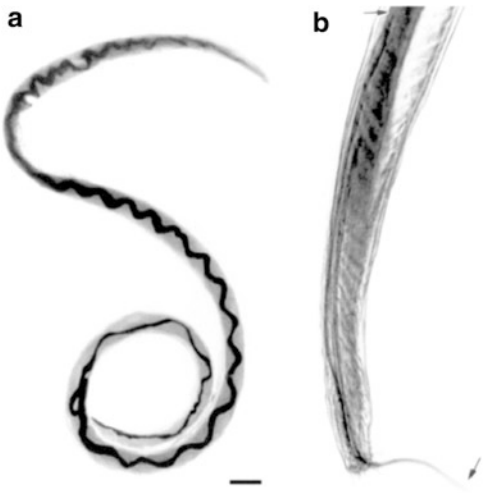

Fig. 14.6 Adult *A. cantonensis* recovered from rat lungs. (**a**) Adult female worm with characteristic barber-pole appearance (anterior end of worm is to the top). Scale bar = 1 mm. (**b**) Tail of adult male, showing copulatory bursa and long spicules (*arrows*). Scale bar = 85 μm

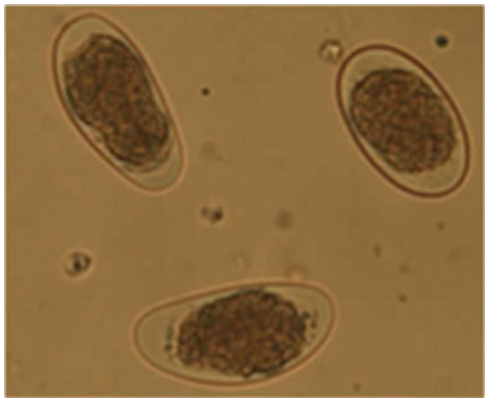

Fig. 14.7 The eggs of *A. cantonensis*

represented 15 potential antigens/allergens genes (Fang et al. 2010). It is also excepted that the whole genome of *A. cantonensis* is sequencing by Sun Yat-sen University. The information substantially expand the available genetic information about *A. cantonensis* and should be a significant resource for *A. cantonensis* gene research.

In addition, a cDNA library of *A. cantonensis* fourth-stage larvae was constructed, and ~1,200 clones were sequenced. Bioinformatic analyses revealed 378 cDNA clusters, 54.2 % of which matched known genes at a cutoff expectation value of 10^{-20}. Of these 378 unique cDNAs, 168 contained open-reading frames encoding proteins containing an average of 238 amino acids. Characterization of the functions of these encoded proteins by Gene Ontology analysis showed enrichment in proteins with binding and catalytic activity. The observed pattern of enzymes involved in protein metabolism, lipid metabolism, and glycolysis may reflect the central nervous system habitat of this pathogen (He et al. 2009).

There are many genes of *A. cantonensis* having been cloned and analyzed. They are cysteine protease inhibitor (Liu et al. 2010), matrix metalloproteinase (Sun et al. 2012), cathepsin B-like cysteine proteinase (Cheng et al. 2012), protein disulfide isomerases (Liu et al. 2012), novel gene encoding 16 kDa protein (Li et al. 2012a), cathepsin B (Han et al. 2011), galectin-10 (Liu et al. 2013), etc. These work establish the foundation for researching the vaccine against *A. cantonensis* and searching drug target of angiostrongylsis.

MicroRNAs (miRNAs) are endogenous, small, noncoding RNAs that play key roles in gene expression regulation, cellular function and defense, homeostasis, and pathogenesis. A study term determine and characterize miRNAs of female and male adults of *A. cantonensis* by Solexa deep sequencing. A total of 8,861,260 and 10,957,957 high quality reads with 20 and 23 conserved miRNAs were obtained in females and males, respectively. No new miRNA sequence was found. Nucleotide bias analysis showed that uracil was the prominent nucleotide, particularly at positions of 1, 10, 14, 17, and 22, approximately at the beginning, middle, and the end of the conserved miRNAs (Chen et al. 2011c). MicroRNA of *A. cantonensis* third- and fourth-stage larvae and the brain microRNA of infected mice have been sequenced by Sun Yat-sen University. Undoubtedly, these study will establish the foundation for the research of *A. cantonensis* molecular biology and angiostrongyliasis mechanism.

14.5 Epidemiology

14.5.1 Epidemiology of A. cantonensis *Worldwide*

Human *A. cantonensis* infection has evidently increased the public attention worldwide due to outbreaks and also because more and more sporadic cases are being reported in Western tourists in recent years. Over 2,800 cases of human angiostrongyliasis had been documented in approximately 30 countries (Wang et al. 2008). However, there are no doubts, many more cases unreported due to lack of awareness of this parasite within the medical community. During 2008–2012, an additional 120 cases were reported (Table 14.1).

14.5.2 Epidemiology of A. cantonensis *in China*

China has become one of the major countries where cases of human angiostrongyliasis increased significantly in the past decade. Therefore, much more efforts have been made to investigate the prevalence of *A. cantonensis* in this country. In mainland China, the first case of human angiostrongyliasis with eosinophilic meningitis was reported in 1984 in Guangdong province. Outbreaks of human

Table 14.1 Cases of human angiostrongyliasis reported science 2008

Regions	Cases	References
China	100	Chen et al. (2011b), Deng et al. (2011), Lv et al. (2009a), Zhang et al. (2008)
Thailand	8	Sawanyawisuth and Kitthaweesin (2008), Sinawat et al. (2008)
India	1	Paul and Pammal (2008)
French	1	Malvy et al. (2008)
Germany	1	Luessi et al. (2009)
Jamaica	1	Mattis et al. (2009)
Ecuadorian	8	Dorta-Contreras et al. (2011)

infection with *A. cantonensis* have been reported with increasing frequency in mainland China in recent years, possibly caused by the growing popularity of eating exotic foods such as raw and undercooked snails. One outbreak of human *A. cantonensis* infection with 65 cases of eosinophilic meningitis was observed in Wenzhou, Zhejiang province, in 1997 (Zheng et al. 2001). An outbreak of five human cases occurred in Liaoning province in 1999 (Lin and Wang 2004) and three outbreaks with a total of 30 cases were reported in Fujian province in 2002 (Yang et al. 2004; Ye et al. 2008; Lin et al. 2003). Two outbreaks with 34 human cases occurred in Yunnan province in 2003 and 2005 (Zhou et al. 2009). Unfortunately, these outbreaks do not seem to have drawn sufficient public attention to the threat of this parasite in China, a situation that contributed to a larger outbreak of some 160 cases in Beijing in 2006. The same exposure pattern was also reported in two outbreaks with a total of 17 human infections in Taiwan in 1998 and 2001 (Tsai et al. 2001a, b) (Fig. 14.8). From the epidemiological survey, the west-central region of Guangdong Province in China is the natural focus of *A. Cantonensis* and there were 180 positive samples of IgG antibody against *A. cantonensisin* 1,800 serum samples of the residents, with a positive rate of 10 % (Chen et al. 2011a).

In China, *Pomacea canaliculata* and *Achatina fulica* are main vectors for human infection (Wang et al. 2007). *P. canaliculata*, native to South America, was introduced to Taiwan and the mainland of China in the 1980s. *P. canaliculata* has replaced the African giant snail, *A. fulica*, as a major intermediate host and has become the main source of human infection both in Taiwan and mainland China. A retrospective study of published prevalence of *A. cantonensis* in mainland China revealed that 22 of 32 species of wild mollusk species (69 %) are infected with the parasite (Lv et al. 2008). *Achatina fulica* has been recorded with the highest rate and intensity of infections, followed by slugs (*Vaginulus* spp.) and *Pomacea canaliculata*. The rates and intensities of infections in terrestrial snails and slugs are higher than in freshwater mollusks. This was confirmed by a recent national survey conducted in China (Lv et al. 2009b). *P. canaliculata* and *A. fulica* were found in 11 and 6 provinces, respectively. Out of 11,709 *P. canaliculata* snails examined, 6.8 % were infected with *A. cantonensis*. Of 3,549 *A. fulica* snails examined, 13.4 % were infected with *A. cantonensis*. The infection prevalence among terrestrial snails was 0.3 %. A total of 5,370 terrestrial slugs were dissected,

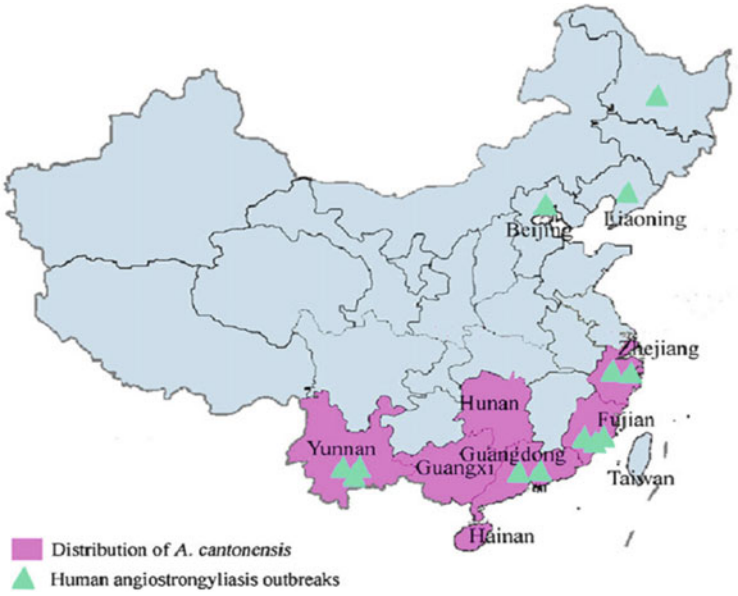

Fig. 14.8 The distribution of *A. cantonensis* and its outbreaks in China. The endemic regions of *A. cantonensis* are marked in *purple* and those with outbreaks of human *A. cantonensis* are marked with *green triangles* (Wang et al. 2012)

revealing an infection prevalence of 6.5 %. The prevalence among the other fresh water snails was 0.05 %. In Guangdong province, during 2008–2009, specimens from 510 snails (144 *P. canaliculata*, 306 *A. fulica*, and 60 *Bradybaena despecta*) were digested with pepsin for isolation of *A. cantonensis* larvae. Prevalence rates of *A. cantonensis* in *P. canaliculata*, *A. fulica*, and *B. despecta* were 8.3 %, 2.0 %, and 5.0 %, respectively (Qu et al. 2011). After that, the followed survey reported that a total of 795 *A. fulica* snails and 734 *P. canaliculata* snails were collected and the average infection rates of these two species were 13.96 % (111 of 795) and 1.50 % (11 of 734), respectively, in 2008–2010 (Yang et al. 2012). However, a recent study demonstrated that *P. canaliculata* had an average infection rate of 21 %, significantly higher than that of *A. fulica* (10 %) in Shenzhen, Guangdong province (Zhang et al. 2008). *P. canaliculata* has replaced *A. fulica* playing an important role in the epidemiology of *A. cantonensis* in recent outbreaks of human angiostrongyliasis (Lv et al. 2008).

The retrospective study also revealed that 11 of 15 wild rodent species in mainland China are infected with *A. cantonensis*. *Rattus norvegicus* is the most frequently identified host with a generally higher prevalence and intensity of infection compared with other rodents. This was consistent with a national survey that found 32 of 711 rats infected with *A. cantonensis* (31 *R. norvegicus* and 1 *R. flavipectus*) (Lv et al. 2008). In Guangdong, China, researchers captured 288 rats of seven species (257 *R. norvegicus*, 13 *R. flavipectus*, 7 *R. Losea*,

6 *R. rattus*, 3 *Bandicota indica*, 1 *R. rattus alexandrinus*, and 1 *Mus musculus*) and rats were examined for adult *A. cantonensis* nematodes in pulmonary arteries and right heart cavities. The result showed that among the 288 rats examined, 27 (9.4 %) from three species (25 *R. norvegicus*, 1 *R. losea*, and 1 *M. Musculus*) were infected with *A. cantonensis* adults in their cardiopulmonary systems during 2008–2009 (Qu et al. 2011). From 2008 through 2010, a total of 430 rats were captured and 23 rats, from two species, were infected, with an average infection rate of 5.35 % and the infection rate was calculated to be 9.09 % (20 of 220) for *R. norvegicus* and 15.00 % (3 of 20) for *R. flavipectus*, respectively (Yang et al. 2012). Interestingly, *A. cantonensis* was also found in nonhuman primate, equine, and canine species. *A. cantonensis* was discovered in paratenic host frog species (*Hylarana guentheri*, *Rana limnocharis*, and *Rana plancyi*) and toads (*Bufo melanostictus*), but has not yet been identified in freshwater shrimp, fish, crabs, or planaria in published studies (Lv et al. 2008). *A. cantonensis* was not found in any of 652 paratenic hosts collected during a national survey that included frogs, shrimps, crabs, toads, and fish (Lv et al. 2009b).

14.6 Clinical Aspects

The term human angiostrongyliasis refers primarily to eosinophilic meningitis/meningoencephalitis and ocular angiostrongyliasis, which are the major clinical features of *A. cantonensis* infection in human beings. However, a rare and extremely fatal cases were reported (Sawanyawisuth et al. 2009). The incubation of this disease is highly variable, ranging from 1 day to several months, depending on the number of parasites involved (Yii et al. 1976; Chen et al. 2006; Zheng et al. 2001; Punyagupta et al. 1970). In an outbreak in Beijing, China, the incubation period of 128 (80 %) of 160 patients was 7–35 days (He et al. 2007). In an outbreak in Wenzhou, Zhejiang, China, clinical symptoms appeared in 40 (62 %) of 65 patients on days 6–15 after infection (Xue et al. 2000).

In adult patients, the common symptoms were headache (95 %), neck stiffness (46 %), paresthesia (44 %), vomiting (38 %), and nausea (28 %). Headache, mainly caused by increased intracranial pressure or the direct damage of the larvae, was intermittent, frequent, and severe at first; after repeated lumbar puncture, it became less frequent and less severe and eventually resolved (Yii 1976; Punyagupta et al. 1975). Neck stiffness was usually mild and lasted for a short period. Nuchal rigidity was less common but has been reported in severe cases (Slom et al. 2002; Chau et al. 2003). Paresthesia, which usually persisted for less than 2 weeks and occurred in a variety of anatomical locations (usually in the extremities) was expressed as pain, numbness, itching, or a sensation of worms crawling under the skin (Yii 1976). Vomiting and nausea were probably related to increased intracranial pressure and usually disappeared after the first lumbar puncture. Although few adult patients with visual disturbance or diplopia were reported in China, this symptom was noted in 184 (38 %) of 484 patients in Thailand and 11 (92 %) of

12 patients in the USA (Slom et al. 2002; Punyagupta et al. 1975). Thirty-two percent of adult patients had fever, which was mostly low grade; however, approximately 10 % of these had high-grade fever ranging from 38 °C to 39 °C (Yii 1976; Punyagupta et al. 1975). In addition, the clinical features of human ocular angiostrongyliasis in 35 patients involve visual loss (94.3 %), fundus change (34.3 %), eye redness and pain (17.1 %), eye floater (8.6 %), and blindness (5.7 %) (Diao et al. 2011).

In children, stiff neck and paresthesia were less common than in adult patients, whereas reports of nausea and vomiting were higher. Of 94 (82 %) pediatric patients with nausea and vomiting, 56 % had projectile vomiting, which usually disappeared within 1 week (Yii 1976). Additionally, rates of fever, somnolence, constipation (76 %), and abdominal pain were higher in children than in adults. In adults or children with heavy infections, coma and death can occur (Yii 1976; Chotmongkol and Sawanyawisuth 2002). Especially, children have lower immunity than human, the rate of death increased, and the autopsy found adult worm in pulmonary artery (Li et al. 2001).

14.7 Diagnosis

14.7.1 Parasite Diagnosis

Human angiostrongyliasis is confirmed by detection of *A. cantonensis* in patients. However, the detection rate is frequently low (Punyagupta et al. 1975; Yii 1976). The diagnosis is, therefore, primarily based on clinical symptoms and medical history. The typical clinical manifestation of human angiostrongyliasis is eosinophilic meningitis. However, other causes for this clinical presentation must be considered (Lo and Gluckman 2003). Medical history of eating intermediate or paratenic hosts of *A. cantonensis* critical for diagnosis. The detection of eosinophils and brain lesions are also helpful for diagnosis. Eosinophils account for a large portion of white cell counts in blood and CSF in *A. cantonensis* infections (Yii 1976; Tsai et al. 2001a). MRI and CT have been used to detect damage in brain for differential diagnosis of *A. cantonensis* from other parasites. After administration of Gd-DTPA, multiple round or oval contrast-enhanced nodules, diameter ranging from 3 to 10 mm, were seen on T1WI, and the resolution of lesions in the pia mater was faster than that in parenchyma of the brain (Fig. 14.9) (Jin et al. 2005; Ogawa et al. 1998; Hasbun et al. 2001).

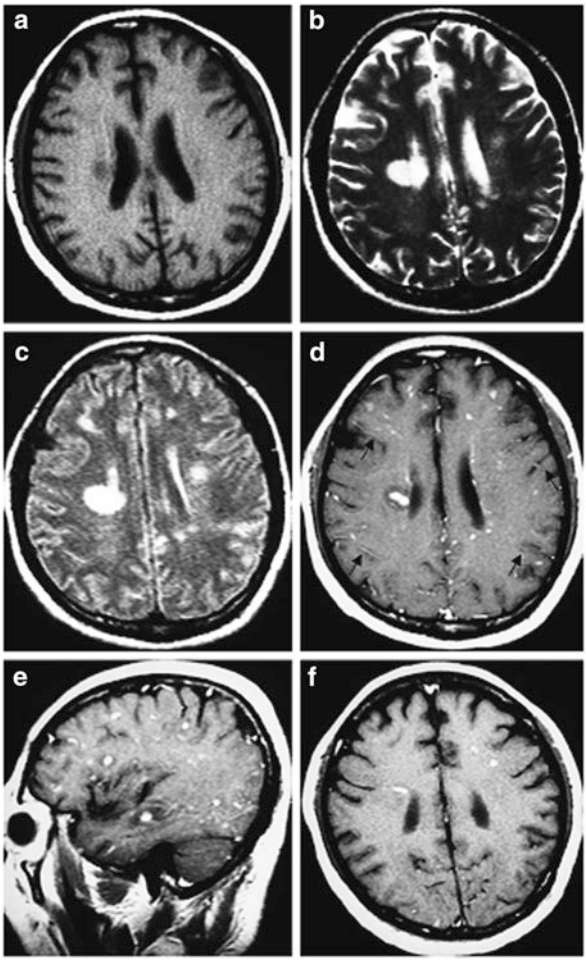

Fig. 14.9 Brain MR images obtained at 7 weeks (**a**–**e**) and 11 weeks (**f**) after ingestion of snails. Some lesions presented as (**a**) hypointense on a unenhanced axial T1WI (TR 500 ms; TE ms) and (**b**) hyperintense on a transverse T2WI (TR 4,000 ms; TE 100 ms). (**c**) More lesions were revealed on a corresponding transverse FLAIR image (TR 5,000 ms; TE 110 ms; TI 2,000 ms). Diffuse contrast-enhanced round or oval nodules of different sizes were shown on (**d**) a transverse T1WI (TR 500 ms; TE 15 ms) and (**e**) a sagittal T1WI (TR 500 ms; TE 15 ms) after gadolinium administration. The nodular lesions mentioned above disappeared or diminished on a 5-week (**f**) follow-up transverse T1WI (TR 500 ms; TE 15 ms) after gadolinium compared with (**d**). Note that pia enhancement (*arrow*) shown on (**d**) completely resolved in (**f**)

14.7.2 Immunological Detection

To effectively diagnose and manage *A. cantonensis* infection, serological tests such as enzyme-linked immunosorbent assay (ELISA) have been developed to detect the antigens of or antibodies against *A. cantonensis* in serum or cerebrospinal fluid. The detection of circulating antigens in serum or CSF provides a rapid confirmation of infection. Monoclonal antibodies (mAbs) against parasite-specific antigens detect circulating antigen with relatively high specificity and reasonably good sensitivity (Eamsobhana and Yong 2009). Recently, several mAbs against the excretory/secretory (ES) proteins have been developed (Chen et al. 2010). The mAbs against an ES protein of 55 kDa have the highest specificity and sensitivity. The detection rate of antigen in the sera of angiostrongyliasis patients was 100 % and cross-reactions to normal sera or the sera of patient s with other parasitic infection, such

as clonochiasis, fasiolopsiasis, ancylostomiasis, anisakiasis, or schistosomiasis were not found (Huang et al. 2010). In addition, antigens from *A. cantonensis* can also be detected in sera by immuno-PCR (Chye et al. 2004). Human antibodies to *A. cantonensis* may be generated after infection. Several specific *A. cantonensis* antigens such as 29 kD, 31 kD, 32 kD, and 66 kD have been identified for immunodiagnosis of the presence of such antibodies (Maleewong et al. 2001; Nuamtanong 1996; Bessarab and Joshua 1997).

14.8 Control

14.8.1 Chemotherapy

Human angiostrongyliasis displays two main forms of clinical presentation: eosinophilic meningitis and ocular angiostrongyliasis. For eosinophilic meningitis, effective supportive treatments are repeated by lumbar puncture and analgesics (Punyagupta et al. 1975; Yii 1976). Corticosteroid therapy has been effective in human angiostrongyliasis. Patients were given a 2-week course of prednisolone (treatment group), 60 mg/day, and compared with those given placebo (control group). The results indicated that a 2-week course of prednisolone was beneficial in relieving headache in patients with eosinophilic meningitis (Chotmongkol et al. 2000). Anthelminthics, such as albendazole and mebendazole, have been used to treat this disease at 15 mg/kg/day or identical placebo for 2 weeks in attempts to more effectively relieve symptoms and reduce their duration. The mean duration of headache was reduced significantly by using albendazole alone (Jitpimolmard et al. 2007). The combination of corticosteroids and anthelminthics has been commonly used for treatment of human angiostrongyliasis. Patients were given a 2-week course of prednisolone, 60 mg/day, and mebendazole, 10 mg/kg/day. Treatment for 2 weeks with the combination regimen of prednisolone and mebendazole is safe and beneficial in relieving headaches in patients with eosinophilic meningitis (Chotmongkol et al. 2006; Wang et al. 2008). Currently, some Chinese herbal medicines display efficacy for treating angiostrongyliasis in animal studies but have not been used in humans (He et al. 2011; Wan and Weng 2004; Shih et al. 2007; Lai et al. 2008; Lai 2006). Surgery is required to remove worms from the eyes of patients with ocular angiostrongyliasis.

14.8.2 Prevention

Because of its worldwide distribution, it is impossible to eliminate *A. cantonensis* from the environment. However, it is possible to avoid or reduce human infection by blocking the transmission pathway of this parasite. The simple method is to

persuade people not to eat raw or undercooked intermediate and paratenic hosts in endemic regions. Epidemiological surveys indicate that most cases of human angiostrongyliasis would be avoided in this way. Also some rare cases caused by eating contaminated vegetables can be avoided by effective washing. However, the difficulty for prevention is that most people have no or limited know ledge of the worm and are totally unaware of the danger of consuming it. Therefore, one of the most effective measures would be the spread of knowledge regarding *A. cantonensis* and its potential for damage to the health of the general population, especially in remote and poor areas of endemic regions. Another approach is persuading people to abandon their habit of eating raw snails and paratenic hosts. Travelers heading to endemic regions must know the dangers of eating raw mollusks and raw vegetables with unknown sources and should avoid these foods. For physicians in both nonendemic and endemic regions, it is necessary to be aware of the existence of these worms, their symptoms, and modes of transmission to suspect and diagnose *A. cantonensis* infection in humans promptly (Wang et al. 2012).

14.9 Basic Research

In China, there are some research groups from universities or institutes involving in basic reaearches about *A. cantonensis*, such as Sun Yat-sen University and National institute of parasitic diseases Chinese Center For Disease Control and Prevention (NIPD). The research topics involves genetics of differential isolated strains, mechanism of pathogenesis and inflammation induced by the worm, development of new drugs, molecular biological study (showed in the part of molecular biology of *A. cantonensis*), etc.

14.9.1 Different Geographic Strain Study

He Han-jiang et al. study the biology, genetics, and virulence of *A. cantonensis* isolated from Guangdong, Fuzhou, Haikou, Hekou, and Wenzhou in China. Phylogenetic analysis revealed that the combined CO1 and ND4 mtRNA sequences were able to distinguish *A. cantonensis* isolates from these geographical regions. According to CO1 and ND4 sequences and phylogenetic analysis, there are two geographical origins probably. One geographical origin includes Guangzhou, Haikou, and Fuzhou strains, another geographical origin consist of strains from Wenzhou and Hekou. To compare virulence of *A. cantonensis* isolates from Guangzhou, Haikou, and Fuzhou, the rate of death, change of weight, worm recovery, neurological function points, and leukocyte counts are detected in infected BALB/c mice. The result showed that the mice infected by L3 from Guangzhou were not different from Haikou. However, these indexes were lower

in the mice infected with the worm from Fuzhou region. Therefore, the difference of virulence in *A. cantonensis* matched up to genes variation. (This part is not published.)

14.9.2 Immunity Reaction of Angiostrongyliasis

The study of immunity against *Angiostrongylus cantonensis* infection is attempted in animal model. Researches suggest that systemic and local Th2 cytokine responses, especially those involving IL-5, are predominant in *A. cantonensis*-nfected mice and that IL-5 is an important cytokine underlying the innate resistance of the mouse against *A. cantonensis* (Sugaya et al. 1997). The levels of interleukin 5 (IL5), IL10, and IL13 in the cerebrospinal fluid (CSF) were markedly higher in 30 patients with eosinophilic meningitis associated with angiostrongyliasis (EOMA) than in the controls (Intapan et al. 2008).CCR3 is a major chemokine receptor that is abundant on the surface of eosinophils and is responsible for their activation and chemotaxis. CCR3 recognizes many chemokines, including CCL11, CCL5 (RANTES), and CCL3 (MIP-1α). Mice received an intraperitoneal injection of anti-CCR3 monoclonal antibody (mAb) (50 μg) at 10 days postinfection (dpi); the levels of CCL11 (eotaxin) in the peripheral circulation and the expression of the Th2-type cytokine interleukin-5 and eosinophil count in the brains were significantly reduced (Chuang et al. 2010). Another important cytokine is IL-33. IL-33 protein and ST2L messenger RNA (mRNA) transcripts in the brains were upregulated during *A. cantonensis* infection and that both splenocytes and brain mononuclear cells became IL-33 responsive and produced interleukin 5 and interleukin 13. Furthermore, administration of IL-33 to *A.cantonensis*-infected mice enhanced ST2L expression and cytokine production, which indicated that IL-33 produced in the brain may function as an inflammatory mediator in eosinophilic meningitis induced by *A. Cantonensis* (Peng et al. 2013).

The types of cells involved in the BBB include astrocyte, microglia, and endothelial cells. When the worm invade into the brain, BBB is damaged. *A. cantonensis* larvae extracts can induce apoptosis of brain astrocytic cells and brain microvascular endothelial cells and increase the permeability of the BBB in vitro (Hu et al. 2012). Microglia is considered to be the key immune cell in the central nervous system like macrophage. Soluble antigen of the fourth larva of *A. cantonensis* can induce mice microglia activation and produce IL-5, IL-13, and eotaxin which are related to eosinophil (Wei et al. 2013).

14.9.3 Development of New Drugs

For improving the effect of therapy for angiostrongyliasis, many new drugs have been researched. The results showed that the combination of albendazole and

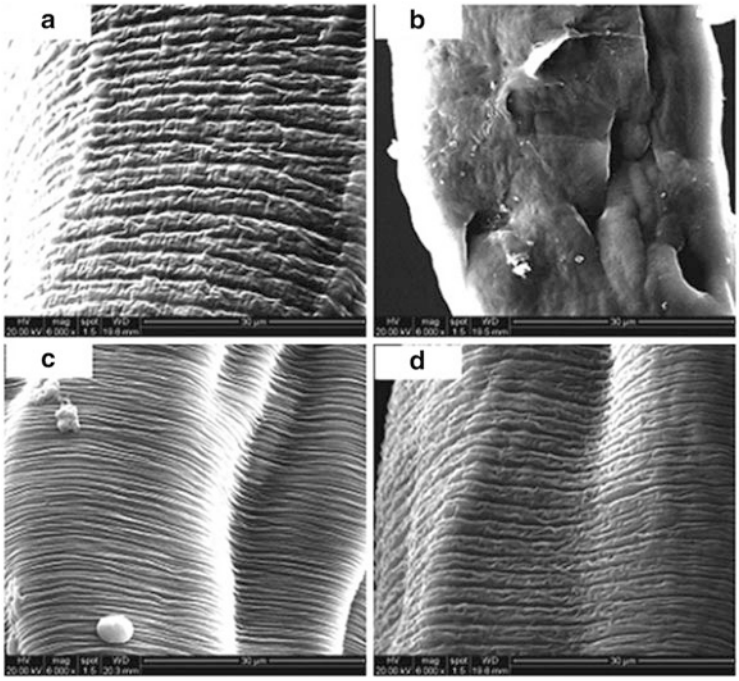

Fig. 14.10 Scanning electron microscopy images showing results after treatment with edible oil (**a**), tribendimidine early treatment (**b**), tribendimidine late treatment (**c**), and albendazole treatment (**d**). Images show larvae, taken from the control group, with clear and regular epidermal folds (**a**). The epicuticule of the larvae from tribendimidine early treatment group was damaged and incomplete (TBD7) (**b**). The epidermal fold is fuzzy and its structure is not clear on the larvae from the tribendimidine late treatment group (TBD14) (**c**). The epidermal fold is clear and regular on larvae from albendazole-treated mice (**d**)

baicalein increased the survival time, decreased body weight loss, neurological dysfunction, leucocyte response, eotaxin concentration, and MMP-9 activity, so the combination of albendazole and baicalein was more effective than either drug administered singly (He et al. 2011). In addition, albendazole combined with a marine fungal extract (m2-9) increased body weight, reduced worm burden, improved learning ability, memory and action, decreased neurological dysfunction and leucocyte response in these mice, and m2-9 is a natural product with potentially significant therapeutic value for angiostrongyliasis and is worthy of further study (Li et al. 2012b). Tribendimidine, a broad-spectrum anti-helmintic drug developed in China, is a derivative of amidantel. The study showed that a strong efficacy of tribendimidine against *A. cantonensis* and provided suitable alternative treatments to further explore its potential use in treatment of human angiostrongyliasis. These drugs also can induce worm surface damage (Fig. 14.10) (Wang et al. 2013).

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Chapter 15
Dengue Fever in China

Yu Wu, Xiaoying Zheng, and Zhongdao Wu

Abstract Dengue fever is an acute infectious disease caused by dengue viruses and transmitted by Aedes mosquitoes. All four serotypes of DENV have caused outbreaks in China. *Aedes albopictus* is the major vector in China. Dengue fever is a severe, flu-like illness that affects infants, young children, and adults, but seldom causes death. Dengue can be diagnosed by isolation of the virus, by serological tests, or by molecular methods. There is no specific treatment and effective vaccines available for dengue fever. The only method to prevent dengue fever is to control vector mosquitoes.

Keywords Dengue fever • Dengue virus • *Aedes albopictus* • *Aedes aegypti* • China

15.1 Introduction

Dengue fever (DF) is an acute infectious disease caused by dengue viruses and transmitted by Aedes mosquitoes. Before 1970, only nine countries had experienced severe dengue epidemics. This disease is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South-east Asia, and the Western Pacific. The American, South-east Asia, and the Western Pacific regions are the most seriously affected. WHO currently estimates over 2.5 billion people—over 40 % of the world's population—are now at risk from dengue, and 50–100 million dengue infections worldwide every year. In recent years, transmission has increased predominantly in urban and semi-urban areas and has become a major international public health concern (http://www.who.int/topics/dengue/en/).

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Severe dengue (previously known as Dengue Hemorrhagic Fever) was first recognized in the 1950s during dengue epidemics in the Philippines and Thailand. Today, severe dengue affects most Asian and Latin American countries and has become a leading cause of hospitalization and death among children in these regions.

There are four distinct, but closely related, serotypes of the virus that cause dengue (DEN-1, DEN-2, DEN-3, and DEN-4). Recovery from infection by one provides lifelong immunity against that particular serotype. However, cross immunity to the other serotypes after recovery is only partial and temporary. Subsequent infections by other serotypes increase the risk of developing severe dengue.

15.2 Epidemiology

Dengue is a category 2 notifiable infectious disease in China. *Aedes albopictus* is the major vector to transmit the dengue virus between humans in China. Dengue epidemics have occurred mainly in the southern regions of the country, primarily in Guangxi province, Guangdong province, Fujian province, Zhejiang province, and Hainan province (Fig. 15.1). All four serotypes of DENV have caused outbreaks in China. Large outbreaks were caused by DENV-4 in 1978 in Guangdong province, DENV-3 in 1980 in Hainan province, and DENV-2 in 1986 in Hainan province (Wu et al. 2010; Gao et al. 2010; Zhao et al. 1981; Li et al. 1986; Qiu et al. 1991).

In the early 1940s, DENV was epidemic on the southeastern coast of China and the middle and lower reaches of the Yangtze River (Cui 1983). In 1978, the first outbreak of DF caused by DENV-4 in mainland China occurred in Foshan Guangdong province (Zhao et al. 1981). The outbreak affected seven neighboring counties, lasted 8 months, and resulted in 22,122 cases and 14 deaths. Since then, dengue outbreaks were recorded sequentially in Hainan, Guangxi, Fujian, and Zhejiang provinces. A dengue outbreak involving 13 cities and counties occurred in Hainan province in 1980, during which DENV-3 was isolated from acute-phase sera and adult *Ae. aegypti* (Li et al. 1986). This outbreak caused 437,468 cases and 64 deaths (Qiu et al. 1993). In 1985–1986, severe dengue was reported in Hainan province. This outbreak was caused by DENV-2 and produced considerable morbidity and high mortality, with 113,589 cases and 289 deaths (Qiu et al. 1991, 1993). Since the 1990s, dengue epidemics have frequently occurred in Guangdong province, Guangxi province, and Fujian province.

The outbreaks of dengue fever in China usually resulted from the introduction of the virus by infected travelers and refugees from various areas of southeastern Asia where dengue is endemic. For example, an outbreak of DENV-1 in Zhejiang province in 2004 was associated with a traveler from Thailand (Xu et al. 2007). This outbreak caused a total of 82 reported cases. Epidemiological investigation of

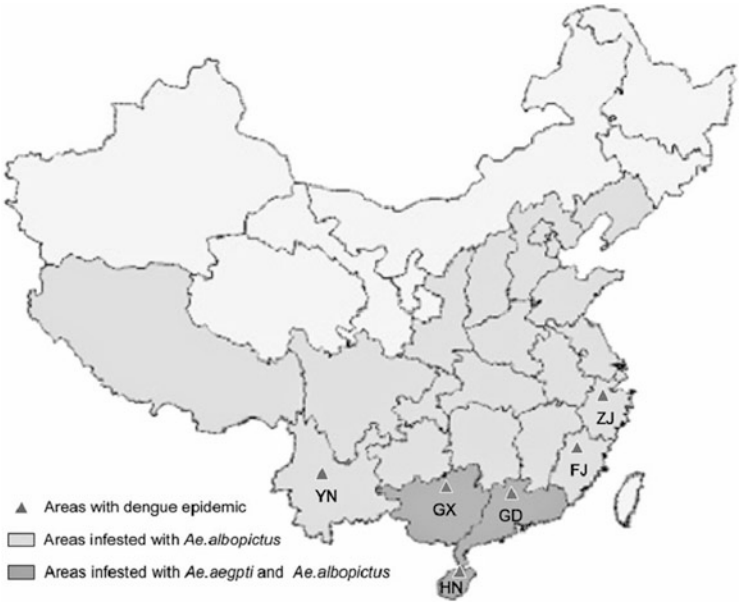

Fig. 15.1 Approximate distribution of dengue and *Aedes* mosquitoes in mainland China. ZJ: Zhejiang Province; FJ: Fujian Province; GD: Guangdong Province; GX: Guangxi Province; HN: Hainan Province; YN: Yunnan Province (from Wu et al. 2010)

cases reported from Guangdong showed that cases reported from 1990 to 2006 were mostly imported or occurred in local epidemics initiated by imported cases (Luo et al. 2002; Liang et al. 2007). Nationwide in China, the number of reported cases has varied considerably, ranging from 40 in 2005 to 1,044 in 2006.

The mosquito species *Aedes aegypti* and *Aedes albopictus* are vectors of DENV in China. *Aedes aegypti* is the vector in coastal areas and is mainly distributed in Hainan province and found sporadically in Guangdong province and Guangxi province (Group 1982). *Aedes albopictus* is the vector in inland regions and is widespread in mainland China. This species is distributed from Liaoning province in the north to Shanxi province in the northwest and from Tibet in the southwest to the southern reaches of China beyond the Yangtze River (Fu 1982).

The epidemic season for DF occurs mainly in the rainy, hot summer, and autumn, which correlates with the seasonal periodicity of the vectors. Most cases are reported from March to November with a peak in July to September, while Hainan province has cases throughout the year with a peak in July to October (Yi 2002). Statistical analysis of incidence rates of age groups in different years showed that, in general, the highest incidence was in the 10- to 39-year-old group (Kan et al. 1997).

This pattern of dengue transmission in Guangzhou, Guangdong Province has been repeated every year: beginning with importation from Southeast Asia on May; July is the early stage of indigenous cases epidemic, which then increases from

August to October, climbs to the peak on September and falls obviously on November, and ends in December because of cold weather in the winter (Wu et al. 2010). It is documented that the prevalence of virus is associated highly with the breeding activity of the Aedes mosquitoes. *Aedes albopictus* is the predominant species in South China. It can breed in various small containers or plants that hold accumulated water (such as tree holes, bamboo stems, or leaf axils) that are found in gardens or backyards. The large amount of rainfall from July to September increases the breeding places of the mosquitoes. The emergence of large numbers of larvae causes drastic expansion of the mosquito populations and greatly increases the probability of DF epidemic.

In Guangzhou, dengue was epidemic in worse environment with high density of residence, amounts of accumulated water, where residents like to raise water plants in vases or plants in flowerpots with the tray. In summer the density of *Aedes albopictus* is very high there. For 3–5 years interval dengue would outbreak again in these areas.

The dengue cases were categorized into either imported cases or indigenous ones based on the virus origin of the infected person. So far, DF has not been confirmed to be an endemic infectious disease in China. But some evidences show *Ae. albopictus* is the reservoir host of Dengue virus.

Between 2005 and 2010, Adult females and larvae (developed to adult in lab) from *Ae. albopictus* were collected from 11 districts of Guangzhou, where DF occurred currently or previously, and screened for dengue virus by one step SYBR Green I real time RT-PCR. A total of 1,172 pools of *Ae. albopictus* (14,750 mosquitoes) was analyzed. Five samples were found to be infected with dengue virus serotype I and the results were confirmed by sequencing (Li et al. 2008; Cai and Yao 2011).

DENV-2 can survive in desiccated eggs of infected *Ae. albopictus* and also be detected in F1 progeny. Guo et al. collected three gonotrophic cycles (GC) eggs. Vertical transmission was not observed in the first gonotrophic cycle. The total positive rates of GC2 and GC3 progeny were 26.7 % and 27.8 %, respectively. DENV-2 can be transmitted at least to the fourth generation after the female *Ae. albopictus* infected and there was a tendency of increased virulence from parent generation to offspring generation. So *Ae. albopictus* has a potential role in the natural circulation of the virus (Song et al. 2005).

The healthy virgin females mated with vertically infected males, the healthy virgin mosquitoes and their progenies were infected, but the healthy virgin males were not infected after mating with vertically infected females. The female mosquitoes can gain DENV-1 virus via sexual route and transmit it to the progenies, but the males cannot (Jiang et al. 2006).

In addition to detecting virus in the mosquito vectors, a study reported that DENV RNA has been detected using RT-PCR in the brain tissue of Rousettus leschenaultia, a fruit bat collected in Hainan province (Zhang et al. 1998). Moreover, antibodies to DENV were detected in Rousettus leschenaultia collected in Yunnan province during a study of dengue fever in the region (Zhang et al. 1999).

15.3 Clinical Manifestation

Dengue fever is a severe, flu-like illness that affects infants, young children, and adults, but seldom causes death. Dengue should be suspected when a high fever (40 °C/104 °F) is accompanied by two of the following symptoms: severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands, or rash. Symptoms usually last for 2–7 days, after an incubation period of 4–10 days after the bite from an infected mosquito.

Severe dengue is a potentially deadly complication due to plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment. Warning signs occur 3–7 days after the first symptoms in conjunction with a decrease in temperature (below 38 °C/100 °F) and include severe abdominal pain, persistent vomiting, rapid breathing, bleeding gums, fatigue, restlessness, and blood in vomit. The next 24–48 h of the critical stage can be lethal; proper medical care is needed to avoid complications and risk of death.

15.4 Diagnosis

Dengue can be diagnosed by isolation of the virus, by serological tests, or by molecular methods (http://www.cdc.gov/dengue/clinicallab/laboratory.html) (Fig. 15.2). Diagnosis of acute (ongoing) or recent dengue infection can be established by testing serum samples during the first 5 days of symptoms and/or early convalescent phase (more than 5 days of symptoms). Acute infection with dengue virus is confirmed when the virus is isolated from serum or autopsy tissue specimens, or the specific dengue virus genome is identified by reverse transcription-polymerase chain reaction (RT–PCR) from serum or plasma, cerebrospinal fluid, or autopsy tissue specimens during an acute febrile illness. Methods such as one-step, real-time RT–PCR or nested RT–PCR are now widely used to detect dengue viral genes in acute-phase serum samples. This detection coincides with the viremia and the febrile phase of illness onset. Acute infections can also be laboratory confirmed by identification of dengue viral antigen or RNA in autopsy tissue specimens by immunofluorescence or immunohistochemical analysis, or by seroconversion from negative to positive IgM antibody to dengue, or demonstration of a fourfold or greater increase in IgG antibody titers in paired (acute and convalescent) serum specimens.

Patients who have IgM antibodies to dengue detected in their serum specimen via an IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) and had either

1. A negative RT–PCR result in the acute phase specimen or
2. Did not submit an acute phase specimen, are classified as having a *recent probable dengue infection*. This is due to the fact that IgM antibodies for dengue may remain elevated for 2–3 months after the illness. The elevated IgM observed in a sample could be the result of an infection that occurred 2–3 months ago. In addition, there is cross reactivity with other flaviviruses including West Nile virus (WNV), St. Louis encephalitis virus (SLE), Japanese encephalitis virus (JEV), and yellow fever virus (YFV). The provider should review the patient's past medical history, recent travel history, and vaccination record (especially yellow fever vaccination) to determine the likelihood that the current acute febrile illness is due to an infection with dengue virus.

Often times both an acute and convalescent phase specimens are needed to make a diagnosis of dengue infection. This is especially true for those who submit a day 5 acute specimen because the virus and IgM antibodies may be at undetectable levels. So if a patient with suspected dengue infection submits a late acute phase specimen that is negative (e.g., by RT–PCR and MAC-ELISA), and they do not submit a convalescent specimen, they are classified as a laboratory-indeterminate case.

15.4.1 Immunological Response to Dengue Infection

The acquired immune response following a dengue infection consists of the production of IgM and IgG antibodies primarily directed against the virus envelope proteins. The immune response varies depending on whether the individual has a primary (first dengue or other flavivirus infection) versus a secondary (had dengue or other flavivirus infection in past) dengue infection. In general, diagnosis of dengue is dependent on the phase of the infection. The general timeline of a primary infection from virus isolation or identification to IgM detection followed by IgG detection is as follows.

A primary dengue infection is characterized by a slow and low titer antibody response. IgM antibody is the first immunoglobulin isotype to appear. Anti-dengue IgG is detectable at low titer at the end of the first week of illness and slowly increases. In contrast, during a secondary infection, antibody titers rise extremely rapidly and antibody reacts broadly with many flaviviruses. High levels of IgG are detectable even in the acute phase and they rise dramatically over the proceeding 2 weeks. The kinetics of the IgM response is more variable. IgM levels are significantly lower in secondary dengue infections and thus some anti-dengue IgM false-negative reactions are observed during secondary infections. According to the Pan American Health Organization (PAHO) guidelines, 80 % of all dengue cases have detectable IgM antibody by day 5 of illness, and 93–99 % of cases have detectable IgM by day 6–10 of illness, which may then remain detectable for over 90 days.

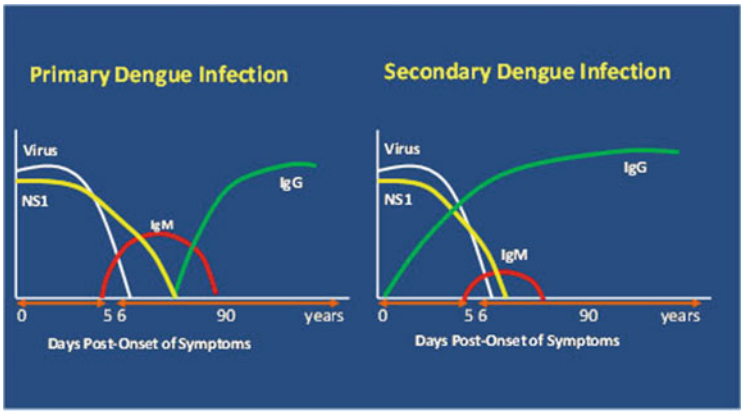

Fig. 15.2 Immune response to dengue infections

MAC-ELISA has become an important tool for routine dengue diagnosis, MAC-ELISA has a sensitivity and specificity of approximately 90 % and 98 %, respectively, but only when used 5 or more days after onset of fever (i.e., in convalescent phase). Different formats such as capture ELISA, capture ultramicroELISA, dot-ELISA, AuBioDOT IgM capture, and dipsticks have been developed. Serums, blood on filter paper, and saliva (but not urine) are useful for IgM detection if samples are taken in convalescent phase of illness. A variety of different commercial kits is available with variable sensitivity and specificity. Dengue diagnosis becomes even more challenging because dengue IgM antibodies also cross-react to some extent with other flaviviruses such as JEV, SLE, WNV, and YFV.

15.4.2 Testing Algorithms for Dengue

1. *PCR*: DENV can be detected in the blood (serum) from patients for approximately the first 5 days of symptoms. Currently, several PCR tests are employed to detect the viral genome in serum. In addition, virus can be isolated and sequenced for additional characterization. Real time RT–PCR assays have been developed and automated; but none of these tests are yet commercially available. Because antibodies are detected later, RT–PCR has become a primary tool to detect virus early in the course of illness. Current tests are between 80 and 90 % sensitive, and more that 95 % specific. A positive PCR result is a definite proof of current infection and it usually confirms the infecting serotype as well. However, a negative result is interpreted as "indeterminate." Patients receiving negative results before 5 days of illness are usually asked to submit a second serum sample for serological confirmation after the fifth day of illness (below).

2. *MAC ELISA*: IgM antibody capture ELISA (MAC-ELISA) format is the most commonly employed in diagnostic laboratories and commercial available diagnostic kits. The assay is based on capturing human IgM antibodies on a microtiter plate using anti-human-IgM antibody followed by the addition of dengue virus specific antigen (DENV1–4). The antigens used for this assay are derived from the envelope protein of the virus. One of the limitation of this testing is the cross reactivity between other circulating flaviviruses. This limitation must be considered when working in regions where multiple flaviviruses co-circulate. IgM detection is not useful for dengue serotype determination due to cross-reactivity of the antibody.
3. *IgG ELISA*: The IgG ELISA used for the detection of a past dengue infection utilizes the same viral antigens as the MAC ELISA. This assay correlates with the hemagglutination assay (HI) previously used. In general IgG ELISA lacks specificity within the flavivirus serocomplex groups. Primary versus secondary dengue infection can be determined using a simple algorithm. Samples with a negative IgG in the acute phase and a positive IgG in the convalescent phase of the infection are primary dengue infections. Samples with a positive IgG in the acute phase and a fourfold rise in IgG titer in the convalescent phase (with at least a 7 day interval between the two samples) is a secondary dengue infection.
4. *NS1 ELISA*: The nonstructural protein 1 (NS1) of the dengue viral genome has been shown to be useful as a tool for the diagnosis of acute dengue infections. Dengue NS1 antigen has been detected in the serum of DENV infected patients as early as 1 day post onset of symptoms (DPO), and up to 18 DPO. The NS1 ELISA-based antigen assay is commercially available for DENV and many investigators have evaluated this assay for sensitivity and specificity. The NS1 assay may also be useful for differential diagnostics between flaviviruses because of the specificity of the assay.
5. *PRNT*: Plaque Reduction and Neutralization Test (PRNT) and the microneutralization PRNT can be used when a serological-specific diagnostic is required, as this assay is the most specific serological tool for the determination of dengue antibodies. The PRNT test is used to determine the infecting serotype in convalescent sera. This assay measures the titer of the neutralizing antibodies in the serum of the infected individual and determines the level of protective antibodies this individual has toward the infecting virus. The assay is a biological assay based on the principle of interaction of virus and antibody resulting in inactivation of virus such that it is no longer able to infect and replicate in cell culture. Some of the variability of this assay is differences in interpretation of the results because of the cell lines and virus seeds used as well as the dilution of the sera.

15.5 Control

15.5.1 Chemotherapy

There is no specific treatment for dengue fever.

For severe dengue, medical care by physicians and nurses experienced with the effects and progression of the disease can save lives—decreasing mortality rates from more than 20 % to less than 1 %. Maintenance of the patient's body fluid volume is critical to severe dengue care.

15.5.2 Prevention

At present, in the absence of an effective vaccine against DENV, the only method to control or prevent the transmission of dengue virus is to combat vector mosquitoes through:

- Preventing mosquitoes from accessing egg-laying habitats by environmental management and modification.
- Disposing of solid waste properly and removing artificial man-made habitats.
- Covering, emptying, and cleaning of domestic water storage containers on a weekly basis.
- Applying appropriate insecticides to water storage outdoor containers.
- Using of personal household protection such as window screens, long-sleeved clothes, insecticide treated materials, coils, and vaporizers.
- Improving community participation and mobilization for sustained vector control.
- Applying insecticides as space spraying during outbreaks as one of the emergency vector control measures.
- Active monitoring and surveillance of vectors should be carried out to determine effectiveness of control interventions.

In China, vector control includes programs in community participation and health education to reduce mosquito breeding in household water containers. At present, a vector surveillance system has only been established in Guangxi province, Guangdong province, Fujian province, Yunnan province, and Hainan province. Vector-based surveillance should be established nationwide to determine the distribution of competent vector species and to identify areas that may be at risk if mosquito distributions shift in response to climate change. In view of the current status of a large number of imported cases, it is crucial to provide health education targeted at the high risk groups including travelers from dengue epidemic countries in order to prevent importation of dengue fever. In addition, considering the increased travel between Southeast Asia and China, more surveillance is necessary to monitor DEN epidemiology in the region (Gao et al. 2010).

15.6 Research

For many years, much of the medical research community has been focused on the development of vaccines or drugs for mosquito-borne diseases. By contrast, there are few, if any, drugs available for treatment of the major arbovirus diseases (Botting and Kuhn 2012). Instead, greater progress has been made with the preventative, vaccine-based approach, from the yellow fever vaccine developed in the 1930s (Theiler and Smith 1937) through to the more recently developed vaccines for Japanese encephalitis (Halstead and Thomas 2010). Several vaccines are in development for dengue, the most advanced of which has just recently completed Phase IIb field trials in Thailand, with mixed results (Sabchareon et al. 2012).

Vaccine design for dengue has been far more challenging than for other arbovirus diseases owing to the existence of multiple serotypes, the complexity of the human immune response to dengue virus, and the propensity for sequential infections to result in more severe forms of the disease (Thomas and Endy 2011).

For all these diseases, some of the most effective interventions have targeted the mosquito instead of the pathogen through the use of insecticides (Ramirez et al. 2009; Raghavendra et al. 2011). Although insecticides have been shown to be effective in many contexts, the financial cost of their application can be prohibitively high, their widespread application logistically difficult in both very urban and remote areas, and their efficacy unstable owing to the evolution of resistance in their target insects. Despite the successes, the ongoing case burden demonstrates that insecticides, as they are currently being deployed, are not sufficient to bring these diseases under control.

During the past 15 years, researchers have been developing a range of alternative vector control strategies that do not rely on the use of insecticides or the creation of new vaccines or drugs. These approaches are typically focused on either reducing mosquito abundance or preventing the transmission of pathogens by the mosquito. Despite of environmental management and chemical treatment for vector control, there are now two major classes of interventions that have had demonstrated success. None of these methods is a panacea, and often a combination of approaches provides the best outcome (WHO 2004).

Biological control represents the first class of intervention and includes the use of natural predators or pathogens against mosquitoes. Recently, copepods have been successfully deployed to control *A. aegypti* larvae in water storage containers in small communities in Vietnam, leading to local elimination of adult mosquitoes and a reduction in dengue incidence (Sinh Nam et al. 2012; Kay and Vu 2005).

A different strategy uses *Wolbachia pipientis*, which is an obligate intracellular bacterium that lives inside insects and is transmitted vertically from mother to offspring. The infection affects insect sperm in a manner that prevents successful reproduction between infected males and uninfected females and between infected males and females that harbor different strains of *Wolbachia* (Yen and Barr 1971, 1973). This strategy was first deployed in 1967 in Burma as a measure against

filariasis vectors, when large numbers of *Wolbachia*-infected male *Culex quinquefasciatus* mosquitoes were released into wild populations, demonstrating the ability of these infected insects to eliminate local mosquito populations (Laven 1967).

Although present in many mosquito species, including *Culex pipiens* and *Aedes albopictus*, *Wolbachia* is not naturally present in any anopheline species that transmit malaria parasites or in *A. aegypti*, the primary vector of dengue viruses. In the past few years, three different *Wolbachia* strains have been successfully transinfected into *A. aegypti and Anopheles stephensi*, in which they have formed stable, inherited infections (Xi et al. 2005; McMeniman et al. 2009; Walker et al. 2011; Bian et al. 2013).

Wolbachia infections are currently being developed for a range of different control strategies ranging from population suppression approaches, in which *Wolbachia*-infected males effectively reduce the reproduction of wild females, to the use of *Wolbachia* to invade mosquito populations and reduce pathogen transmission by shortening the adult mosquito lifespan and/or preventing pathogen replication inside the mosquito (McGraw and O'Neill 2013).

Recent approaches have focused on population suppression for *Aedes polynesiensis* on South Pacific islands as a means of filariasis control (O'Connor et al. 2012; Chambers et al. 2011). The strategy is based on bidirectional incompatibility, a complexity of the *Wolbachia*-induced reproductive phenomenon, which results in unsuccessful mating between mosquitoes carrying genetically distinct *Wolbachia* strains. Field experiments have commenced in Australia to test the ability of artificially introduced *Wolbachia* infections to invade and establish in wild *A. aegypti* populations. To date, *Wolbachia w*Mel has been successfully introduced into Australian mosquito populations (Walker et al. 2011; Hoffmann et al. 2011) and has remained at fixation for more than 18 months.

The second class of intervention strategy involves genetic modification of the vectors. There are four main approaches for genetic modification of the vector. The sterile insect technique (SIT) is the oldest and most tested example of such a strategy. In a SIT approach, male insects are exposed to either γ-irradiation or sterilizing chemicals, causing large-scale random damage to the insect chromosomes or dominant-lethal mutations in the sperm (Alphey et al. 2010). These males are then released in far larger numbers than occur in the wild male population, and when they mate with wild females, viable offspring are rarely produced. With ongoing releases of these males, the population reduces to low levels or is completely eliminated.

SIT has a mixed history of success for mosquitoes, as some trials have demonstrated reductions in target populations, whereas other trials have not (Alphey et al. 2010; Benedict and Robinson 2003). The most successful initiatives include the eradication of *C. quinquefasciatus*, a local vector of West Nile virus, on an island of Florida, USA (Patterson et al. 1970), and the elimination of *Anopheles albimanus*, a local malaria vector, in El Salvador (Lofgren et al. 1974), both of

which were achieved by the release of chemosterilized males. The development of SIT approaches is underway for other mosquito vectors, with the aim of controlling malaria (Helinski et al. 2008; El Sayed et al. 2009), Chikungunya disease, and dengue (Oliva et al. 2012).

The second approach known as release of insects carrying a dominant lethal (RIDL) operates similarly to traditional SIT but offers several improvements, most notably with a focus on female-killing effects. Instead of random mutations, males carry and deliver female-acting transgenes into the population. One approach uses a construct that reduces the expression of a gene which is active in the flight muscle in female pupae. The result is that daughters of the released males are unable to fly to find mates or human hosts (Fu et al. 2010). The second approach is based on transgenes that induce mortality later in life, either in pupae (Phuc et al. 2007) or in adults (Bargielowski et al. 2011). Of the genetic modification-based approaches, RIDL is the most advanced with respect to implementation, as the technology is currently being trialed by *Oxitec* in Brazil and Malaysia (Lacroix et al. 2012).

A third genetic modification strategy, one that is still in the early stages of development, is aimed at improving the natural defense system of the mosquito based on RNAi. RNAi is an insect immune response that recognizes and degrades invading viral RNA. In one approach, a genetic construct was developed that expresses copies of an inverted repeat from a dengue virus 2 (DENV-2) genomic RNA. The resulting double-stranded RNA that forms then triggers the RNAi response and protects the mosquito from colonization of its tissues by the dengue virus encountered in blood meals (Franz et al. 2006). In another approach, insect densoviruses were engineered to deliver RNA copies of genes required for vector competence in the mosquito (Gu et al. 2011).

A fourth genetic modification approach makes use of HEGS, which are selfish genetic elements that were discovered in bacteria but have since been experimentally engineered and introduced into mosquitoes for future use in disease control. HEGs encode endonucleases that recognize and cut specific DNA sequences (of ~30 bp). As HEGs insert into these specific recognition sequences, they are protected from their own activity. In an organism that is heterozygous for the HEG, the endonuclease will cut the intact copy of the recognition sequence in the chromosome that does not contain the HEG. Because HEGs can be engineered to recognize specific sequences, they can be developed to target mosquito genes required for vector competence (Windbichler et al. 2011). Alternatively, HEGs can be used as a form of population suppression by targeting genes to induce sterility and reductions in survival or sex ratio distortions (Burt 2003; Deredec et al. 2011). Thus far, HEGs have been successfully introduced into *A. aegypti* (Traver et al. 2009) and *Anopheles gambiae* (Windbichler et al. 2011). In simple simulation modeling, HEGs have been predicted to be able to eliminate populations of *A. gambiae* in as little as a few years after their introduction (Deredec et al. 2011).

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Chapter 16
Tsutsugamushi Disease in China

Xiaoying Zheng

Abstract Tsutsugamushi disease (Scrub typhus) is caused by the obligate intracellular bacterium *Orientia tsutsugamushi* that is transmitted by trombiculid mites. Scrub typhus is an infectious disease first discovered in China, as early as 1,600 years ago. There are six species of trombiculid mites that act as scrub typhus vector in different areas of China; another ten species of trombiculid mites have proved to be naturally infected with *O. tsutsugamushi*. The clinical manifestations of Scrub typhus range from subclinical disease to organ failure to fatal disease. Diagnosis of scrub typhus depends on a history of chigger biting and clinical manifestations with laboratory examination. Doxycycline is a key antibiotic used in scrub typhus patients' treatment. Prevention and control strategy includes vector and reservoir host control and personal protection and health education.

Keywords Tsutsugamushi disease • Scrub typhus • *Orientia tsutsugamushi* • Trombiculid mite • China

16.1 Introduction

16.1.1 Finding History of Tsutsugamushi Disease in China

Tsutsugamushi disease (Scrub typhus), an acute febrile illness, is caused by the obligate intracellular bacterium *Orientia tsutsugamushi* that is transmitted by trombiculid mites. Scrub typhus is an infectious disease first discovered in China, as early as 1,600 years ago; there are records about epidemiology, clinical symptoms, and prevention of the scrub typhus in China's ancient medical treatise.

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Ge Hong (AD 284–364 or 343) was the first scientist who described Scrub typhus. There are details of Scrub typhus, including epidemiology, clinical symptoms, prevention, and treatment of tsutsugamushi disease in his book "Bao Pu Zi" and "Zhou Hou Fang" (Li 1997; Yu 2000). Chao Yuanfang (AD 610) also described the epidemiology and treatment of the diseases. There are more records of scrub typhus in Tang Dynasty (618–907); for example, Sun Si Miao had recorded scrub typhus caused through the bite of sand mite and its pathogenesis and treatment in his book "Qian Jin Fang."

Taiwan had scrub typhus cases reported since 1908; there were total of 250 cases with 27 deaths reported from 1912 to 1914. In 1931, the first scrub typhus case was reported in Penghu Island, Fujian Province, and 252 cases with 16 deaths were reported in 1938 (Yu 2000).

Peng and Xie (1949) report the first scrub typhus case in Guangzhou in 1946–1948. Peng and Xie inoculated the patient's blood into the abdominal cavity of mice, and weeks later they demonstrated *O. tsutsugamushi* from peritoneal exudate, liver, and spleen swabs seen inside and outside the cells.

Liang (1952) found the *Leptotrombidium deliense* in Guangzhou, and he also reported an easy method of raising trombiculid mite. Zhao (1953) isolated *O. tsutsugamushi* from patients' blood, rodents, and chigger mites in Guangzhou and also studied the biological characteristics of *O. tsutsugamushi*.

In 1951, Scrub typhus outbreaks occurred in garrison and residents in Fujian Province. For example, in Pingtan County, Fujian Province, the incidence was as high as 800/100,000, with the case fatality rate as high as 13 %. Yu and Wu (1959) isolated *O. tsutsugamushi* from patients, rodents, and chigger mites in Fujian.

Scrub typhus outbreaks in 1952 in Guilin, Guangxi was reported (Lv anf Liang (2000)).

In 1954, scrub typhus outbreak occurred in Toumen Island, Linhai County, Zhejiang Province, and scrub typhus cases were reported in Yongjia County, Zhejiang Province in 1956. Further investigation showed that scrub typhus foci exist in Qingtian, Lishui, the mountain area of southern Zhejiang Province. *Rattus confucianus* are the main animal host, and *L. gaohuense* act as the main vector for *O. tsutsugamushi* in these areas.

Forty scrub typhus cases were reported during June–July 1956 in Yun Rong, Yunnan Province. And *O. tsutsugamushi* was isolated from patients, *R. flavipectus*, and *L. deliense*; more than 40 cities and counties proved the etiology of the existence of scrub typhus in Yunnan Province.

Tsutsugamushi disease cases were reported in Xichang, Sichuan Province, in 1961, and *O. tsutsugamushi* was separated from *L. deliense*.

In 1967–1968, scrub typhus cases were reported in Guzhang County, west part of Hunan Province. In 1981, scrub typhus cases were confirmed by using serological methods in Guzhang for the first time, and the following year *O. tsutsugamushi* was isolated from patients, the *Apodemus* mouse (*Apodemus agrarius*), and *L. deliense*. Also cases of scrub typhus were reported and *O. tsutsugamushi* isolated from Jishou County, Hunan Province, in 1984 was found to be isolated from *L. jishoum*, which is the main vector of trombiculid mite in these areas.

In 1973, Tsutsugamushi disease cases were reported in Moto County, Tibet. *O. tsutsugamushi* was separated from *L. deliense*.

In July 1982, scrub typhus cases were reported in southern Xiuning County, Anhui Province, and scrub typhus cases were confirmed by serum immunofluorescence test.

Lin (1985) reported scrub typhus cases in Wuzhishan, Hannan Island; Lin (1986) isolated *O. tsutsugamushi* from patients.

In Shandong Province, tsutsugamushi cases were reported in the mountain area of Yimeng and Wulian County in 1986, and a scrub typhus outbreak occurred in the suburb area of Jinan city in 1988.

In Jiangsu Province, scrub typhus cases were reported in Jiang Pu County in 1986 and in Dongtai County in 1987. After that tsutsugamushi cases were reported in another six counties. Also *O. tsutsugamushi* was isolated from patients, rodents, and chigger mites. *L. scutellare* was proved as the main vector mite in the region of these areas.

The 1989, scrub typhus outbreaks occurred in Baodi County, Ji County of Tianjin City. Scrub typhus foci were proved to be distributed in Heilongjiang, Liaoning, and Jilin provinces during 1981–1991.

Human populations showed *O. tsutsugamushi* infection in Xinjiang and Gansu provinces by seroepidemiological survey in 1994. Scrub typhus epidemic occurred in Yicheng and Jiang counties, Shanxi Province, from August to November 1995; scrub typhus outbreaks also occurred in Taihang Mountain area of Baoding city, Hebei Province, from September to October 1997 (Chen et al. (2000)). In Jiangxi Province, scrub typhus outbreak occurred in Shang gao County in 1998.

Epidemiological investigations on scrub typhus conducted in China continued, so the infection route, treatment method, and the vector mites were cleared. The death cases of scrub typhus decreased. In the early 1990s, scrub typhus did not classify as a notifiable infectious disease. But tsutsugamushi disease cases were still found in classic endemic areas; even new endemic areas emerged. In 2006, scrub typhus cases were reported through the national disease report management information system in China. The number of reported cases has a rising trend, and the endemic area in China is expanding. In 2006, 1,244 cases of scrub typhus with incidence of 0.09/100,000 were reported. In 2007, 1,332 cases with one death and 0.1/100,000 incidence were reported; In 2008, 2,592 cases with three deaths and 0.2/100,000 incidence were reported (Wu 2010).

For example, in Guangzhou, a classic endemic area of tsutsugamushi disease, a total of 1,648 cases were reported from 2006 to 2010 and it increased year by year. Cases were found in all age groups, 77.67 % were above 35 years old, and farmers accounted for 50.37 % of total cases. The scrub typhus peak time was from May to October, which accounted for 82.34 % of total cases. The suburbs of Panyu, Conghua, Zengcheng, Huadu, and Baiyu were the high incidence places, which accounted for 74.77 % of total cases (Hu et al. 2012).

16.1.2 Biology of the Pathogen of the Disease

O. tsutsugamushi is the causative organism of tsutsugamushi disease in China. The organism is an obligate intracellular pathogen, which needs to infect eukaryotic cells in order to multiply. The envelope is similar to that of Gram-negative bacteria. Normally *O. tsutsugamushi* grows in the host cell cytoplasm, but sometimes it can also be seen in the nucleus. The serotypes of *O. tsutsugamushi* in China are mainly Karp, Gilliam, Kato, Kawazaki, TA763, TA716, TA686 (Wang et al. 1996), and many more serotypes continue to be reported. Karp strain mainly exists in the south of Yangtzi River; Gilliam strain exists in Shandong, Jiangsu, Jilin provinces, and Tianjing City. There also exists Kawazaki in Jiangsu Province. Three strains, Karp, Gilliam, and Kato, were proved to exist in Herongjiang Province.

The toxicity of *O. tsutsugamushi* to experimental animals depends on different strains. In China, the virulence of *O. tsutsugamushi* is usually more strong in areas south of the Yangtze River and Heilongjiang Province than in temperate regions of Jiangsu, Shandong, Tianjin, Liaoning, and Jilin Province.

16.2 Epidemiology

16.2.1 Geographical Distribution

Scrub typhus was endemic in the area of south of 31° north latitude only before 1985 in China (Li 1997). It extends from Taiwan at east to Yunnan, Sichuan, and Tibet at west; from Hainan at south to Zhejiang and Hunan provinces at north. Endemic areas were Guangdong, Hainan, Guangxi, Fujian, Tibet, Taiwan, and Anhui provinces in southern China. Scrub typhus epidemic areas seem to expand after 1985 (Su et al. 1989). Scrub typhus outbreaks occurred in Shandong, Jiangsu, and Tianjin provinces since 1986 (Su et al. 1989; Wang 1992). During 1992–1994, scrub typhus foci were proved to be distributed in Heilongjiang, Liaoning, and Jilin provinces, northeast part of China (Xu et al. 1999; Liu et al. 1991). In 1995, Scrub typhus patients or infected people were reported in Shanxi, Henan, and Hebei provinces (Zheng et al. 1997; Xia et al. 2007). So far more than 22 provinces or areas have reported scrub typhus cases or infected population. According to the forecast of the animals' geographic information, the foci of scrub typhus not only limited to the above-mentioned areas, the endemic areas should be where both the *Rattus* in genus *Rattus* and trombiculid mites in subgenus *Leptotrombidium* exist.

16.2.2 Population Distribution

In China, tsutsugamushi cases were regularly reported since 1952. Annually reported scrub typhus cases were around 1,000 cases before 1984, but there were more than 2,000 cases a year since 1987 (see Table 16.1). The increase in the

Table 16.1 Numbers of Tsutsugamushi disease cases occurred in China from 1952 to1989

Year	Country	Guangdong Province	Fujian Province	Yunnan Province	Zhejiang Province	Jiangsu Province	Shandong Province
1952	400	400	–	–	–	–	–
1953	1,102	197	905	–	–		
1954	1,844	138	1,706	–	–	–	–
1955	1,526	194	1,332	–	–	–	–
1956	760	95	648	31	–	–	–
1957	929	227	558	26	118	–	–
1958	785	476	234	19	56	–	–
1959	812	200	313	41	258	–	–
1960	717	223	364	38	90	–	–
1961	573	195	191	56	131	–	–
1962	1,179	181	511	319	168	–	–
1963	1,313	480	245	488	97	–	–
1964	1,534	492	358	498	110	–	–
1965	1,129	408	189	385	122	–	–
1966	924	364	120	333	106	–	–
1967	894	314	45	448	59	–	–
1968	396	38	100	226	32	–	–
1969	315	161	14	126	14	–	–
1970	749	308	65	273	103	–	–
1971	548	210	59	210	50	–	–
1972	558	225	90	160	74	–	–
1973	1,103	409	196	397	101	–	–
1974	963	307	245	339	64	–	–
1975	961	358	207	319	44	–	–
1976	779	296	281	145	33	–	–
1977	559	290	75	150	31	–	–
1978	1,003	569	50	329	42	–	–
1979	661	401	44	168	11	–	–
1980	704	78	18	218	60	–	–
1981	856	420	25	358	24	–	–
1982	868	396	111	335	43	–	–
1983	970	244	227	356	33	–	–
1984	1,193	219	149	187	76	–	–
1985	1,605	252	142	526	44	–	–
1986	1,519	348	93	659	62	–	–
1987	2,239	44	108	900	70	278	240
1988	2,590	15	463	1,034	48	207	310
1989	2,542	39	169	962	111	150	284

number of scrub typhus cases was mainly due to the expansion of endemic areas and improvement in diagnostic techniques. Scrub typhus did not classify as a notifiable infectious disease since 1990. But scrub typhus still exists in China, for example, Guangzhou, a classic scrub typhus epidemic area, still has about 500 cases a year. In 2012, the local scrub typhus epidemic occurred in Guangzhou. Scrub typhus cases were reported through the national disease report management information system since 2006 in China.

The number of scrub typhus cases had an increasing trend. For example, total of 1,648 cases were reported in Guangzhou from 2006 to 2010 and it increased year by year. Scrub cases were found in all age groups, from 4-month baby to 94-year-old person. 77.67 % of scrub cases were above 35 years old, and farmers accounted for 50.37 % of total cases. The scrub typhus peak time was from May to October, which accounted for 82.34 % of total cases (Wu 2010).

The population distribution features of the scrub typhus patients in Shangdong Province also showed that farmers are the major part of scrub typhus patients. The onset age of the 1,291 patients ranged between 1 and 92 years old. 639 out of 1,291 patients were over 55 years old, accounting for 49.5 %. Patients were farmers, workers, students, and preschool children. However, most of the cases were farmers, up to 84.8 % (1,095/1,291). Generally, no gender difference is observed in scrub patients. Out of 1,291 scrub typhus patients in Shangdong Province, 640 patients were male and the other 651 patients were female, occupying 49.6 % and 50.4 % respectively. The gender ratio was 1:1.02 (Ding et al. 2012).

16.2.3 Route of Infection

Scrub typhus is transmitted by some species of trombiculid mites. Chiggers are the larvae of the trombiculid mites. In the life cycle of trombiculid mites, only larvae feed on hosts, including humans and all classes of terrestrial vertebrates.

16.2.3.1 Vector Mites

There are more than 500 species of trombiculid mites in China; they are distributed in family Trombiculidae (including in subfamily Trombiculinae and subfamily Gahrliepiinae) and family Leeuwenhoekidae (Subfamily Leeuwenhoekiinae) (Li 1997).

Now known vectors of tsutsugamushi vectors in China are species belonging to Genus *Leptotrombidum*. A chigger mite identified as the vector should have following four basic conditions (1) It should be locally dominant mite species, it's seasonal distribution, geographical distribution associated with the number of scrub cases; (2) the mite naturally infected with *O. tsutsugamushi*; (3) the mite should have the ability to bite on hosts and transmit the pathogen; (4) the mite could transmit *O. tsutsugamushi* transovarially to the offspring. Six species of trombiculid species had been identified as the vectors of tsutsugamushi disease. They are *L. deliense* (Li et al. 2002; Zheng et al. 1995), the main vector of scrub typhus in the south of Yangtze River (Guangdong, Fujian, Hainan, Guangxi, Zhejiang, Taiwan, Yunnan, Sichuan, and Tibet); *L. scutellare* (Wu et al. 2004; Wu 2005), the vector of scrub typhus in and Fujian, is the main vector for autumn scrub typhus in Shandong and Jiangsu provinces and the vector for winter scrub typhus in Fujian Province; *L. kaohuense* (Wu et al. 2012), the vector for scrub typhus in Southern

mountain areas of Zhejiang Province; *L. rubellum* (Wang et Liao 1984; Wang 1988), the vector for scrub typhus in coastal areas of Fujian Province; *L. insulare* (Wei et al. 1988), the vector for scrub typhus in East Rocky islands of Zhenjiang Province; and *L. jishoum*, the vector for scrub typhus in the west part of Hunan Province (Li et al. 1988).

Ten species of chiggers were found naturally infected with *O. tsutsugamushi* but still were not identified as the vector for scrub typhus. They are *L. yui* (Chen et Hsu 1955), *L. orientale*, *L. apodemi*, *L. palpalis*, *L. linhuaikongense*, *L. imphalum*, *Ascoschoengastia indica*, *Odontacarus majesticus* (Chen et Hsu 1955), *Walchia pacifica*, and *W. chinensis* (Chen et Hsu 1955; Li et al. 2002).

16.2.3.2 Animal Hosts

Scrub typhus is a zoonosis. The pathogen is *O. tsutsugamushi*. It is transmitted from animal hosts to humans through the biting of vector chigger mites.

There 21 species of animal including in Order Rodentia and Order Insectivora were found naturally infected with *O. tsutsugamushi in* China (see Table 16.2), some of them are main hosts of chigger mites.

Small mammals were commonly found to be infested by chigger mites and most host species harbored several species of mite. The species diversity of chigger mites in Yunnan was much higher than diversities reported previously in other provinces of China and in other countries. A single species of rodent, *Eothenomys miletus* (Rodentia: Cricetidae), carried 111 species of chigger mite, thus demonstrating the highest species diversity and heaviest mite infestation of all recorded hosts (Zhan et al. 2002). Two species acting as major vectors for scrub typhus (tsutsugamushi disease), *Leptotrombidium scutellare* and *L. deliense*, were identified as the dominant mite species in this sample. In addition to these two major vectors, 12 potential or suspected vector species were found. Most species of chigger mite had a wide range of hosts and low host specificity. For example, *L. scutellare* parasitized 30 species of host. The low host specificity of chigger mites may increase their probability of encountering humans, as well as their transmission of scrub typhus among different hosts. Hierarchical clustering analysis showed that similarities between different chigger mite communities on the 18 main species of small mammal host did not accord with the taxonomic affinity of the hosts. This suggests that the distribution of chigger mites may be strongly influenced by the environment in which hosts live (Zhan et al. 2002).

The host animals of winter scrub typhus in Shandong Province, *A. agrarius*, were predominant in the field and the seasonal fluctuation was correlated significantly to that of scrub typhus ($r = 0.810$, $p < 0.005$) and *R. norvegicus* was predominant indoors (Liu et al. 2003).

16.2.3.3 Transovarian Transmission of *O. tsutsugamsuhi*

Because the chigger mites bite on host only once during their life cycle, so transovarial infection is the only conceivable way by which this can be accomplished. The

Table 16.2 List of animal hosts of *O. tsutsugamushi* in China

Animal	Geographic distribution (Provinces)
Order Rodentia	
Family Muride	
Genus Rattus	
R. Losea	Fujian, Guangdong, Hainan, Guangxi, Zhejiang, Taiwan
R. novegicus	Fujian, Guangdong, Hainan, Guangxi, Zhejiang, Jiangsu, Sichuan, Taiwan
R. flavipectus	Fujian, Guangdong, Hainan, Guangxi, Zhejiang, Yunnan
R. rattus	Guangdong, Taiwan
R. rufescens	Taiwan
R. slandeni	Guangdong, Guangxi, Yunnan
R. confucianus	Fujian, Zhejiang, Jiangsu, Yunnan
R. fulvesens	Fujian, Zhejiang
R. nitidus	Fujian, Yunnan, Tibet
R. bowersii	Fujian
Genus Mus	
M. musculus	Fujian, Guangdong, Zhejiang, Taiwan
M. bactrianus	Fujian, Taiwan
Genus Apodemus	
A. agrarius	Fujian, Zhejiang, Jiangsu, Shandong, Hunan, Jilin, Heilongjiang
A. speciosus	Liaoning, Jilin, Heilongjiang
Genus Bandicota	
B. indica	Fujian, Guangdong, Zhejiang, Yunnan, Taiwan
Family Cricetidae	
Genus Cricetidae	
C. triton	Shandong, Shanxi, Liaoning
Genus Microtus	
M. fortis	Fujian, Hunan
Genus Eothenomys	
E. melanogaster	Fujian
*E. miletus**	Yunan
Order Insectivora	
Genus Suncus	
S. murinus	Fujian, Guangdong, Zhejiang, Guangxi
Genus Anourosorex	
A. squamipes	Yunnan
Genus Croidura	
C. lasiura	Jiangsu
Domestic animal	
Rabbit	
Pig	
Cat	
Chicken	

*No *O. tsutsugamushi* naturally infection reported.

trombiculid larvae feed on its host animal infected with *O. tsutsugamushi* and pick up the pathogen organisms from it. After detaching from the host the larvae develop to the free-living adult mite; then the adult mites lay eggs which hatch to produce chiggers that carry *O. tsutsugamushi* and can transmit them to new host animals or humans.

O. tsutsugamushi could maintain in *L. deliense* at least 360 days and it could transmit transovarially to the offspring for four generations in inoculating infection mites and 270 days and two generations in mites that infected through biting (Zheng et al. 1995). *O. tsutsugamushi* could disappear in *L. deliense* after a period of time. It was necessary for chigger mites to bite the infected host animal to keep the cycle of *O. tsutsugamushi* in trombiculid.

16.2.4 Foci Type of Scrub Typhus

Before 1986, scrub typhus was only found endemic in southern China. Because human infections typically occur in the summer, it is called "summer type." During the autumn–winter period of 1986, a new type of scrub typhus was identified in Shandong and northern Jiangsu provinces of northern China. This newly recognized scrub typhus was subsequently reported in many areas of northern China and was then called "autumn–winter type."

The autumn–winter-type scrub typhus in northern China occurred exclusively from September to December with a peak occurrence in October, which was different from the summer type in southern China.

16.3 Clinical Aspects

Scrub typhus is an acute febrile illness which generally causes nonspecific symptoms and signs. The clinical manifestations of Scrub typhus range from subclinical disease to organ failure to fatal disease. Scrub typhus usually presents with fever, rash, eschars, lymph node enlargement, and complications involving respiratory, liver, kidney, cardiac, or central nervous system (Wang 2009; Liu et al. 2010). Multiple organ injuries are more common in children (Zhang and Long 2011). In the elderly patients (Feng et al. 2008), fever, eschar, and swelling of lymph node were the major clinical features of tsutsugamushi disease and skin rash was rare. The disease development was also associated with a low platelet count and an increase in the peripheral white blood cell count. Onset of the disease was usually insidious. Clinical manifestations were complex and multiple organ damage was frequently observed. Deaths are attributable to late presentation, delayed diagnosis, and drug resistance.

Clinical manifestations seem to be severe in summer-type scrub typhus endemic areas. Clinical data of scrub typhus patients diagnosed in Guangzhou Eight People's hospital during 5 years showed that fever, eschar, ulcer, the swelling of

lymph nodes, and rash were common signs and symptoms. The disease was often complicated by liver injury, heart injury, kidney injury, secondary infection, hydrocephalus, shock, and disseminated intravascular coagulation (DIC) (Li et al. 2009).

In comparison with the summer type, complications associated with autumn–winter-type scrub typhus were less severe, and abnormalities of routine hematological parameters were less obvious. According to data of scrub typhus cases from 2006 to 2011 in Shandong Province, the most common manifestations were fever (100 %), eschar or skin ulcer (86.3 %), fatigue (71.6 %), anorexia (71.6 %), and rash (68.6 %). Predominant complications included bronchopneumonia, toxic hepatitis, and acute cholecystitis in 21.6 %, 3.9 %, and 2.9 % of the cases, respectively. Severe complications include toxic myocarditis, heart failure, pneumonedema, pleural effusion, and emphysema (Zhang et al. 2012).

16.4 Diagnosis

16.4.1 Clinical Diagnosis

Diagnosis of scrub typhus depends on a history of contact with chigger mites and clinical manifestations include fever, headache, skin rash, and eschar in patients.

Eschar is an important finding for the diagnosis of scrub typhus. The presence or absence of eschar was thoroughly examined. Among the 176 scrub typhus cases confirmed by IFA, 162 (92.0 %) cases had eschar; 128 patients (79.5 %) had eschars on the front of the body. Eschars were primarily detected in males within 30 cm below the umbilicus (19 patients, 35.8 %). Distributions on the lower extremities and the front chest above the umbilicus were 22.6 % (12 patients) and 20.8 % (11 patients), respectively. A different pattern was seen in females. The most prevalent area was the front chest above the umbilicus, which accounted for 40.7 % (44 patients) of all the detected eschars. To verify the value of eschars for the diagnosis of scrub typhus and to characterize genotypes of *O. tsutsugamushi* in patients, Liu et al. (2006) examined eschars and blood specimens of seven patients from Shandong Province. All seven eschars and acute-phase blood samples were positive, while no specific DNA amplicons were obtained from the seven convalescent-phase blood samples collected after antimicrobial drug therapy.

16.4.2 Laboratory Diagnosis

Presumptive diagnosis may be confirmed by laboratory tests, in particular serology. Weil felix test followed by ELISA-based test for *O. tsutsugamushi* can make proper diagnosis (Yu et al. 2009), although indirect immunoflourescence assay (IFA) or indirect immuno-peroxidase test (IIP) and polymerase chain reaction (PCR)- and Q-PCR-based tests are considered gold standard in the confirmation of tsutsugamushi diseases (Zhan et al. 2010).

Appropriate history and finding of eschar are often pathognomonic but can be missed by inexperienced observers. Lack of knowledge among physicians can lead to underdiagnosis and improper treatment. The diagnosis is often missed because of similarities with other tropical febrile infections. Many unusual manifestations are present.

16.5 Control

16.5.1 Chemotherapy

Scrub typhus is an important febrile disease in China, and antibiotics have been used to treat this disease.

Doxycycline is a key antibiotic used in scrub typhus patients' treatment (Zhou 2013). Doxycycline treatment (200 mg/day in adults, 2.2 mg/kg in children >8 years old) either as a unique dose or as short treatments (3–7 days) may save adults and children. Every suspect patient should be treated. Children who are under 8 years should be prohibited to use doxycycline.

Macrolides, quinolones, and chloramphenicol were also used to treat scrub typhus patients. Doxycycline was found to act more quickly, but more adverse drug events occur when using this regimen compared to azithromycin and chloramphenicol.

Both antibiotics and traditional Chinese medicine have been used to treat scrub typhus (Yao and Wang 2012).

16.5.2 Prevention

In China, the main reservoir hosts of tsutsugamushi disease are rodents. Chigger mite is the only vector. No vaccine for tsutsugamushi disease is yet available. Prevention and control strategy includes vector and reservoir host control and personal protection and health education.

16.5.2.1 Vector and Reservoir Hosts Control

In endemic area, the density of chigger mite and incidence of tsutsugamushi disease are positively correlated; hence, surveillance mite density has important significance in the forecast of epidemic situation. Environmental management and disinfection are effective ways to control the mites and rodents (Jiang et al. 2013). Study on chemical control of *L. deliense* showed that Pyridaben was effective against the mites (Liu et al. 2000). Studies on prevention strategies emphasize that personal

hygiene, methods of personal protection, and keeping the living environment clean will provide strategies to eliminate rats and mites. As a result of adopting these strategies, no case of scrub typhus occurred in Pingtan Island in 2003–2004 (Guo et al. 2006).

16.5.2.2 Personal Protection

Personal protection is an effective measure to prevent scrub typhus. Chiggers mainly inhabit in the bushes or shrubs. People should avoid sitting or drying clothes in such environment.

Before entering these areas where patients have already been discovered, people should fasten cuffs, leg openings, and shirt tie into the waistband to avoid chigger bites. People can use insect repellent to protect themselves from the chigger bites.

16.5.2.3 Health Education

Health education or community engagements are helpful in scrub typhus prevention. The health education included attending lectures, videotapes, and prevention handbooks on the clinical characteristic, transmission, and prevention of tsutsugamushi disease.

Health education of the army and residents in Nanao and Nanpenglie islands where the incidence of the disease has increased recently showed: after health education, the rate of understanding of the related knowledge on tsutsugamushi disease was significantly better than before. Before the epidemic season in 1999, the health education and other measures were extensively used and there was no case that year (Huang et al. (2002); Wang et al. (2001)). The health education should be based on local conditions, with programs and methods in which people were interested.

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