**Food Microbiology and Food Safety**
Research and Development

Ynés R. Ortega
Charles R. Sterling *Editors*

# Foodborne Parasites

*Second Edition*

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Ynés R. Ortega • Charles R. Sterling
Editors

# Foodborne Parasites

Second Edition

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*Editors*
Ynés R. Ortega, PhD, MPH
Department of Food Science & Technology
and Center for Food Safety
University of Georgia
Griffin, GA, USA

Charles R. Sterling, PhD
School of Animal and Comparative
Biomedical Sciences
University of Arizona
Tucson, AZ, USA

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# Chapter 1
# Amoeba and Ciliates

**Ynés R. Ortega and Manuela Verastegui**

## 1.1 Preface

Amoeba and ciliates are two groups of protozoan parasites which have long been known to infect humans. Both are unicellular organisms. The amoeba has pseudopodia, which are cytoplasmic protrusions providing motility to the organism. Amoeba is commonly found in the environment and few are pathogenic to mammals.

The ciliates use the cilia (hair-like structures) on the surface of the organism for high motility. Ciliates such as *Paramecium* are commonly found in environmental waters. The only species pathogenic to humans is *Balantidium coli*, which is also found to infect pigs and nonhuman primates.

Amoeba and ciliates can be acquired either by ingestion of contaminated water or food and by contamination of products or surfaces by food handlers.

## 1.2 Amoeba

This group of parasites belongs to the phylum Sarcomastigophora, subphylum Sarcodina (Bruckner 1992). The cyst and trophozoite are the two distinct morphological stages of the amoeba. Some amoeba (commensal) can infect humans.

Nonpathogenic amoeba (commensal) that colonize the intestinal tract include *Entamoeba dispar*, *Entamoeba hartmanni*, *Entamoeba moshkovskii*, *Entamoeba*

Y.R. Ortega (✉)
Department of Food Science & Technology and Center for Food Safety,
University of Georgia, Griffin, GA, USA
e-mail: Ortega@uga.edu

M. Verastegui
Departamento de Microbiologia, Universidad Peruana Cayetano Heredia, Lima, Peru



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*polecki*, *Endolimax nana*, *Entamoeba chattoni*, *Entamoeba invadens*, *Iodamoeba butschlii*, and *Entamoeba coli*.

Amoeba can be identified by observing the morphology of trophozoites or cysts. Trophozoites can be observed only in fresh specimens from an infected individual. The arrangement, size, and pattern of the nuclear chromatin help in the identification of the various species. The size and position of the karyosome is also used in the speciation of the amoeba. The cytoplasm of the trophozoites may contain red blood cells (RBCs), bacteria, yeasts, and molds. The number and size of the nuclei in the cyst are taken into consideration when identifying the genera and species of amoeba, as well as chromatoidal bodies and vacuoles present in the cytoplasm. All of these characteristics are not easily noted in fresh preparations, requiring preparation of permanent stains of fecal smears and examined at 1000x magnification. Mixed infections are very common; therefore, observation of several parasitic structures is necessary for a conclusive diagnosis (Leber and Novak 2005).

The pathogenic amoeba for humans is *Entamoeba histolytica*. It was described by Fedor Losch in 1875 from a Russian patient with dysenteric stools (Lösch 1875). *E. histolytica* has been recovered worldwide and is more prevalent in the tropics and subtropics than in cooler climates. In areas of temperate and colder climates, it can be found in populations living in unsanitary conditions. Poor hygienic conditions, contaminated food and water, and malnourishment contribute to the high prevalence of amoebiasis in certain developing countries, particularly in children. In Bangladesh, a third of the children acquire amoebiasis in their first year of life (Morgado 2016). Amoebiasis is also frequently identified in the Indian subcontinent, Africa, and South America. In developed countries, amoebiasis is also frequently detected in travelers and immigrants.

The pathogenicity of *Entamoeba* has been controversial. In some instances, *E. histolytica* may cause invasive disease and extraintestinal amoebiasis, and in other instances, it may cause mild or asymptomatic infections. The host immune status, strain variability, environmental conditions, and the gastrointestinal microbiota are factors that may influence the clinical presentation of the disease. Axenic cultivation of the amoeba has facilitated the study of isoenzyme profiles including glucophosphate isomerase, phosphoglucomutase, malate dehydrogenase, and hexokinase in various isolates. Sargeaunt (1978) concluded that *Entamoeba* could be characterized based on their isoenzyme analysis and characterized in various zymodemes. Of the amoeba that infects humans, differences between *E. dispar* (nonpathogenic) and *E. histolytica* (pathogenic) are not only genotypic, but phenotypically distinct, although they are morphologically similar.

The life cycle of amoeba starts when the cyst, which is the infectious form, is acquired by ingestion of contaminated materials, such as food and water, or by direct fecal-oral transmission. Once in the intestinal tract, excystation occurs and trophozoites are released and propagate via asexual multiplication. Cyst formation occurs in the colon where conditions are unfavorable for the trophozoite. Cysts are excreted in the feces and can remain viable in the environment for up to several weeks if protected from environmental conditions (Garcia 1999).

*Entamoeba gingivalis*, an amoeba that infects the buccal cavity, is commonly associated with gingivitis and is localized in the soft tartar between the teeth and the oral mucosa. It does not have a cyst stage, and transmission is considered to be person to person or by contact with buccal secretions.

The free-living amoeba are frequently found in the environment, particularly in surface waters including ponds, lakes, and rivers. Three of these are of public health relevance: *Acanthamoeba*, *Naegleria*, and *Balamuthia*.

## *1.2.1* Entamoeba histolytica

*Entamoeba histolytica* has been described worldwide with an estimated 50 million cases annually. In areas of endemicity up to 50% of the population may be infected. It ranks second in worldwide causes of morbidity by parasitic infections (Laughlin and Temesvari 2005). Humans are the primary reservoirs of *E. histolytica*; however, it has been described as also infecting nonhuman primates. This transmission can occur via person-to-person or by ingestion of cysts present in contaminated food or water. The cysts excyst in the intestine and trophozoites are released and start dividing. Some will encyst and be excreted with the feces. In invasive amoebiasis, trophozoites may penetrate the bowel and disseminate to the liver, lungs, brain, pericardium, and other tissues. Invasive amoebiasis tends to affect men predominantly, but asymptomatic infection is equally distributed among both genders (Acuna-Soto et al. 2000). Immigrants from South and Central America and Southeast Asia are two groups with a high incidence of amoebiasis. Travelers are at high risk for acquiring the infection. In areas where *E. histolytica* and *E. dispar* are endemic, *E. histolytica* are more predominant in travelers and *E. dispar* are more predominant in residents. Amoebiasis in homosexual males is frequently transmitted by sexual behavior. Asymptomatic presentation is up to 30% (Walderich et al. 1997).

### 1.2.1.1 Morphology

Trophozoites are between 12 and 60 μm in diameter. The nucleus is characterized by evenly arranged chromatin on the nuclear membrane and a karyosome that is small, compact, and centrally located (Fig. 1.1). The cytoplasm is granular and has vacuoles containing bacteria or debris. In cases of dysentery, red blood cells may be present in the cytoplasm. Immature cysts are characterized by 1–2 nuclei, a glycogen mass, and chromatoidal bars with smooth round edges. Mature cysts have four nuclei, and the glycogen mass and the chromatoidal bars may disappear as the cyst matures. This process occurs while oocysts migrate in the intestine. The cyst measures 10–20 μm. Once the cyst is ingested by the new host, the gastric enzymes and neutral or alkaline pH in the intestines induce the trophozoites to become active, at which point they are liberated (Fig. 1.2).

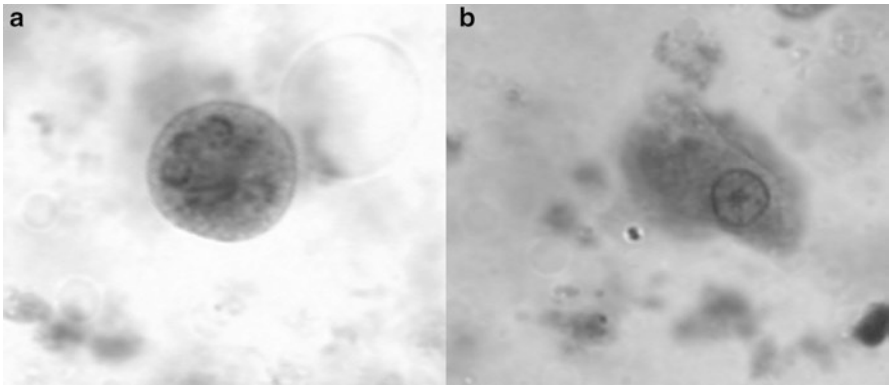


**Fig. 1.1** *Entamoeba histolytica* (**a**) cyst (**b**) trophozoite

#### 1.2.1.2 Clinical Significance

The World Health Organization estimates 50 million infections and 100,000 deaths per year (Anonymous 1997). The clinical presentation of *E. histolytica* can be asymptomatic, symptomatic without tissue invasion, and symptomatic with tissue invasion (Zaki and Clark 2001). Approximately 10% of infected individuals will have clinical symptoms such as dysentery, colitis, or, in few instances, amoebomas. Amoebomas are localized granulomatous tissues with tumor-like lesions resulting from chronic ulceration. They may be mistaken for malignancy. Amoebic dysentery is characterized by diarrhea with cramping, lower abdominal pain, low fever, and the presence of blood and mucus in feces. Ulcers start at the surface of the epithelium that deepens into a classic flask-shaped ulcer. Abdominal perforation and peritonitis are rare, but can be serious complications. Amoebic colitis is characterized by intermittent diarrhea over a long period of time and can be misdiagnosed as ulcerative colitis or irritable bowel syndrome (Leber and Novak 2005). In few cases amoebic infections can result in appendicitis. Entamoeba will invade the ileocecal appendix, produce inflammation, necrosis, and perforation. In these cases, symptoms are more severe including fever and pain in the lower right quadrant of the abdomen. Incubation period may vary from days to months.

*Entamoeba histolytica* can migrate to the liver causing liver abscesses. Symptoms include fever, nausea, weakness, and abdominal pain in the right upper quadrant. The biliary duct system can also be compromised resulting in jaundice. Other complications include hepatomegaly. Infection can also invade the lungs, pericardium, brain, etc. Symptoms may be acute or gradual and may include low-grade fever, pain in the right upper quadrant, and weight loss. In Mexico and Brasil, *E. dispar* has been reported in patients with invasive amoebiasis (Ximenez et al. 2010).

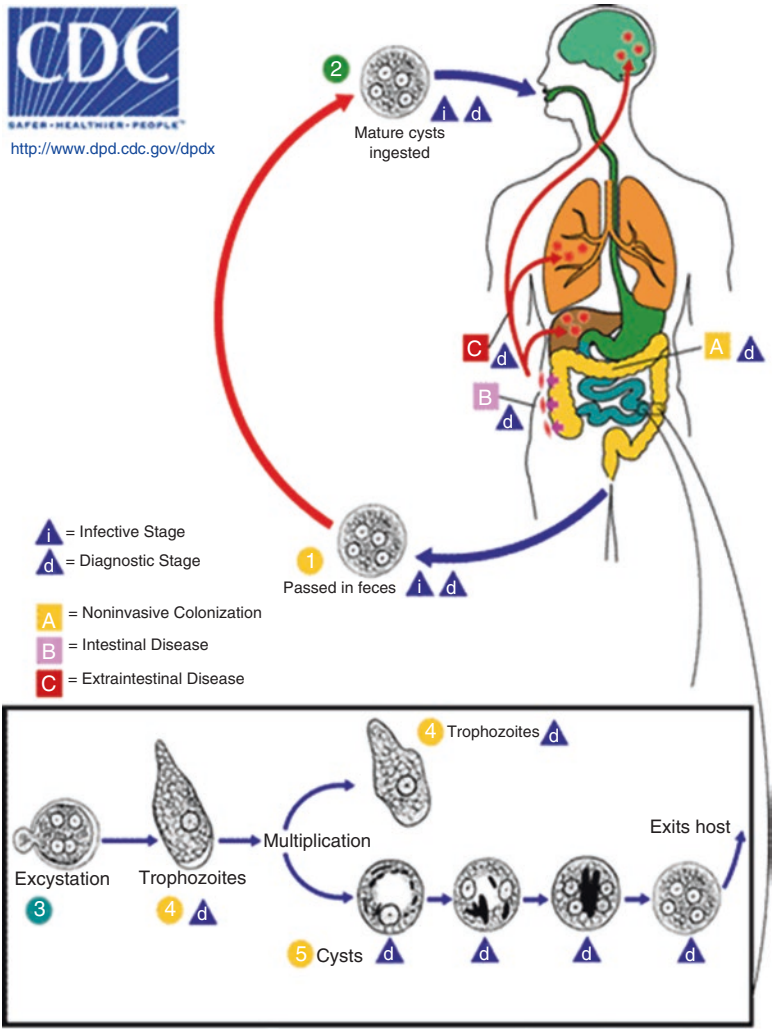


**Fig. 1.2** *Entamoeba histolytica* life cycle (Graph obtained from http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary)

### 1.2.1.3 Pathogenesis and Immunity

Adhesins, amoebapores, and proteases have been associated with lysis of the colonic mucosa in intestinal amoebiasis (Espinosa-Cantellano and Martinez-Palomo 2000). *Entamoeba* has a cell surface protein that has a sensory activity and contributes to the surface adhesion of the trophozoite. The Gal/GalNAc lectin recognizes galactose and N-acetylgalactosamine found on the human colonic mucin glycoproteins. Interaction between this lectin and the host glycoproteins is required for adherence and contact-dependent cytolysis (Petri et al. 1989). This lectin is unique in *E.*

*histolytica* and has been used to develop the ELISA diagnostic assay produced by TechLab. The trophozoite moves forming the pseudopod in front, the membrane moves to the uroid, which is a posterior foot. The amoeba collects surface antigens including host antibodies on the uroid. Membrane shedding is active at the uroid region eliminating the accumulated ligands including antigens, Gal/GalNAc lectins, and the 96 kDa surface protein with the host antibodies. This process may contribute to the evasion of the host immune defenses. Amoeba with a defective cytoskeleton cannot form a cap or form uroids and cannot cause cell cytolysis, suggesting that the cytoskeleton may play a role in contact-dependent cytolysis (Arhets et al. 1998).

The cysteine proteinases are a major virulence factor. These proteinases can degrade elements of the extracellular matrix including fibronectin, laminin, and type I collagen (Keene et al. 1986). These proteinases also interfere with the complement pathways and the humoral response of the human immune system. Gal/GalNAc lectin inhibits complement mediated lysis because it mimics the CD59, a membrane inhibitor of C5b-9 in human blood cells. The proteinases can degrade and inactivate C3 and C5 to circumvent the host immune response, as well as degrade secretory IgG and IgA; limiting the host humoral immune response (Kelsall and Ravdin 1993). The presence of IgA anti-lectin provides a marker of acquired immunity.

*E. histolytica* also secretes a pore-forming protein, the amoebapore containing three isoforms: A, B, and C. It works by inserting ion channels into artificial membranes and may be cytolytic to eukaryotic cells (Leippe et al. 1994; Rosenberg et al. 1989).

The mechanisms of defense of the host include production of mucin. The Gal/GalNac lectin binds to it. Whether it serves as defense or as an inducer for colonization needs to be determined (Petri et al. 1989).

The inflammatory response provides another mechanism of defense. In vitro and in vivo studies demonstrated that the presence of trophozoites cause the expression of a variety of cytokines, including IL-1b and IL-8. This production occurred in regions other than those in direct contact with the parasite (Zhang et al. 2000).

A subunit of the Gal/GalNAc lectin of 170 kDa induces production of IL-12 in human macrophages. The IL-12 promotes Th1 cytokine differentiation and, in turn, macrophage protection (Campbell et al. 2000).

Diagnosis of infection can be made by examination of fecal samples, material collected using a sigmoidoscope, tissue biopsy, and abscess aspirates. Conventional ova and parasite examination can be used to detect cysts and trophozoites based on morphological characteristics of the parasite. However, it will not discriminate *E. histolytica* from *E. dispar*. Trophozoites stained with trichrome stain can measure 10–60 μm and the nuclei are clearly noticeable.

Serological testing can be used. Several tests have been developed including ELISA, IHA, IFA, and latex agglutination. Serum antibodies have been identified in 85% of patients with proven amoebiasis (by histology) and in 99% of patients with extraintestinal amoebiasis. Persons with *E. dispar* do not develop detectable levels of antibodies (Leber and Novak 2005). Diagnosis is facilitated by the examination

of permanently stained slides. Diagnostic assays specific for *E. histolytica* in clinical specimens are available on the market (Tech Labs, Blacksburg, VA) (Garcia et al. 2000; Ong et al. 1996; Pillai et al. 1999). Zymodeme analysis has been used to differentiate between *E. histolytica* and *E. dispar*, which, although specific, is also expensive and time consuming.

Molecular assays such as PCR have been developed (Evangelopoulos et al. 2000; Rivera et al. 1996; Zindrou et al. 2001; Sanuki et al. 1997), and more sensitive and specific methods are emerging. Roy and collaborators compared a real-time PCR against the antigen detection tests and SS- rRNA and traditional PCR (72% sensitive and 99% specific). The real-time PCR was more sensitive (79% sensitive and 96% specific) than all the other assays, and the specificity was higher by PCR. Using the TechLab antigen detection kit only detected 49% of positive specimens (Roy et al. 2005). These tools have been very helpful for differentiating species as well as overcoming the need for skilled microscopists.

#### 1.2.1.4 Therapy

If treating asymptomatic infection with cyst excretion, a luminal amoebicide such as iodoquinol or paromomycin is recommended. If tissue invasion has occurred, tissue amoebicides such as metronidazole or tinidazole followed by iodoquinol or paromomycin are recommended (CDC 2017). Oral rehydration should be observed to maintain adequate fluid intake. Follow-up stool examination is important, since these treatments may lead to drug resistance. The multidrug resistance gene EhPgp1is constitutively expressed in drug resistant trophozoites (Ramirez et al. 2005).

## 1.3 *Dientamoeba fragilis*

*Dientamoeba*, originally considered an amoeba, is now considered nonflagellate trichomonad parasite and is closely related to *Histomonas* and *Trichomonas* spp.

### *1.3.1 Morphology and Transmission*

The trophozoite measures 5–15 μm and pseudopodia are angular. No flagella are present. The cytoplasm is highly granular, and it is characterized as having 1–2 nuclei without peripheral chromatin and karyosome clusters of 4–8 granules. Cysts have not been identified in *Dientamoeba fragilis*. This parasite does not have a cyst form, and its transmission is less understood. However, transmission is suspected to be associated with helminth eggs such as *Ascaris* and *Enterobius*. Higher incidences have been reported in mental institutions, missionaries, and Indians in Arizona. It has been reported in pediatric populations (Garcia and Bruckner 1993). Symptoms

include fatigue, intermittent diarrhea, abdominal pain, anorexia, and nausea. It has been reported to cause noninvasive diarrheal illness. *Dientamoeba* colonizes the cecum and the proximal part of the colon. Reports of *Dientamoeba* are limited and this may be related to the difficulty in identifying the organisms. Asymptomatic cases of *D. fragilis* have been reported. This may be related to the description of two genetic variants using PCR-RFLP of the ribosomal genes (Johnson and Clark 2000).

### *1.3.2 Therapy*

Tetracycline or iodoquinol are recommended as the drug of choice for individuals with symptomatic infection. If coinfections include helminths such as *Enterobius*, mebendazole is usually included in the treatment (Butler 1996).

## 1.4 Nonpathogenic Amoeba

### *1.4.1* Entamoeba hartmanni

*Entamoeba hartmanni* is morphologically similar to *E. histolytica*/*E. dispar*. The trophozoite measures 5–12 μm and has one nucleus with a peripheral chromatin. The karyosome is small, compact, and centrally located. The cyst measures 5–10 μm. The mature cyst contains four nuclei. Chromatoidal bodies are like those of *E. histolytica*.

### *1.4.2* Entamoeba coli

It is commonly found in individuals in developing countries. It is characterized for having a cyst of 10–35 μm that may contain up to eight nuclei. Chromatoidal bars are splinter shaped and have rough pointed ends. The nuclei have distinctive characteristics, including the coarsely granular peripheral chromatin. The large karyosome is usually eccentric. The trophozoite measures between 15 and 50 μm and usually bacteria are present in the cytoplasm.

### *1.4.3* Endolimax nana

The trophozoite measures between 6 and12 μm and has a granulated and vacuolar cytoplasm. The cyst measures between 5 and 10 μm. It is usually oval and when mature may have up to four nuclei. The nuclei have nonvisible peripheral chromatin, and the karyosome is larger than the *Entamoeba.* Morphologically, it is very different than the *Entamoeba* species.

### *1.4.4* Iodamoeba butschlii

The trophozoite measures between 8 and 20 μm. The cytoplasm is granular and vacuolated. The cyst may be oval or round and measures between 5 and 20 μm. The mature cyst, contrary to the other amoeba, contains only one nuclei characterized by the absence of peripheral chromatin and a larger karyosome. It usually contains a large glycogen vacuole that stains brown when the sample is prepared using iodine. *Iodamoeba* can be easily differentiated from the other amoeba.

*Entamoeba coli*, *Endolimax nana*, and *Iodamoeba butschlii* can be easily differentiated from *E. histolytica* primarily by their size, followed by the nuclei characteristics and cytoplasmic inclusions.

## 1.5 Free-Living Amoeba

*Naegleria*, *Acanthamoeba*, and *Balamuthia* have been identified in the central nervous system of humans and other animals. *Acanthamoeba* can also cause keratitis, and both *Acanthamoeba* and *Balamuthia mandrillaris* may cause cutaneous infection in humans. *Naegleria fowleri* and *Acanthamoeba* spp. are commonly found in soil, water, sewage, and sludge. These amoebae feed on bacteria and multiply in the environment. They may harbor pathogenic bacteria to humans such as *Legionella*, *Mycobacterium avium*, *Listeria*, etc. Whether *Acanthamoeba* serves as a reservoir for human pathogens is unknown. Meningoencephalitis caused by *Naegleria* has been coined primary amoebic meningoencephalitis (PAM). It is an acute and fulminant disease that can occur in previously healthy children and young adults who have been in contact with fresh water about 7–10 days prior to development of clinical signs. It is characterized by severe headache, spiking fever, stiff neck, photophobia, and coma leading to death within 3–10 days after onset of symptoms. The amoeba finds their way through the nostrils, to the olfactory lobes and cerebral cortex.

*Acanthamoeba* and *Balamuthia* encephalitis is found primarily in immunosuppressed individuals with exposure to recreational fresh water. Chronic granulomatous amoebic encephalitis (GAE) has an insidious onset and is usually chronic. The invasion and penetration to the CNS may be through the respiratory tract or the skin. These amoebae have been predominantly associated with waterborne transmission in recreational waters. Whether these amoebae are associated with foodborne transmission has not been determined.

*Naegleria fowleri* is susceptible to amphotericin B alone or in combination with miconazole. Few patients infected with *Acanthamoeba* have survived when treated, but in most instances, patients with encephalitis have died. A successful recovery of a patient with GAE included surgery and treatment with sulfadiazine and fluconazole (Seijo et al. 2000). Skin infections have a good prognosis and usually require topical treatment with 2% ketoconazole cream.

## 1.6 Ciliates

Ciliates are highly motile protozoa. They are characterized by cilia present on the surface. Free-living ciliates can be found in environmental waters. The only species pathogenic to humans is *Balantidium coli*. It was initially identified in dysenteric stools of two patients and was later described by Leukhart in 1861 and Stein in 1862 (Diana 2003). *Balantidium* can exist in reservoirs such as pigs and nonhuman primates. *Balantidium coli* can be found in as many as 45% of pigs from intensive farming to 25% in wild boars (Weng et al. 2005; Solaymani-Mohammadi et al. 2004). In Denmark, 57% of suckling pigs had *Balantidium* (Hindsbo et al. 2000); however, balantidiasis has not been reported in humans. In some regions of Venezuela, balantidiasis was observed in 12% in humans and 33.3% in pigs. Nonhuman primates have also been reported carrying the infection in the tropics. Monkeys, chimpanzees, gibbons, macaques, and gorillas can harbor *Balantidium* (Nakauchi 1999).

Human infections can occur in warmer climates. Sporadic cases have been reported in cooler areas and in institutionalized groups with poor hygienic conditions. In the USA, it is rarely found in clinical specimens. Deficient environmental sanitation favors dissemination of the infection (Devera et al. 1999).

### *1.6.1 Life Cycle and Morphology*

The trophozoite and the cyst are the only two stages of *Balantidium coli*. The trophozoite is large and oval. It measures 50–100 μm long to 40–70 μm wide. The cyst measures 50–70 μm. The movement is rotary. The body is covered with longitudinal rows of cilia, and they are longer near the cytostome. The trophozoite is pear shaped with an anterior end pointed and the posterior end broadly rounded. The cytoplasm contains vacuoles with ingested bacteria and cell debri. The trophozoite and cyst contain two nuclei: one large bean-shaped nucleus and a round micronucleus (Fig. 1.3). The cyst form is the infective stage. It has a thick cyst wall. Trophozoites secrete hyaluronidase which aids in the invasion of the tissues. Cysts are formed as the trophozoite moves down the large intestine (Fig. 1.4).

### *1.6.2 Clinical Significance*

Frequently, *Balantidium* infections can be asymptomatic; however, severe dysentery similar to those with amoebiasis may be present. Symptoms include diarrhea or dysentery, tenesmus, nausea, vomiting, anorexia, and headache. Insomnia, muscular weakness, and weight loss have also been reported. Diarrhea may persist for

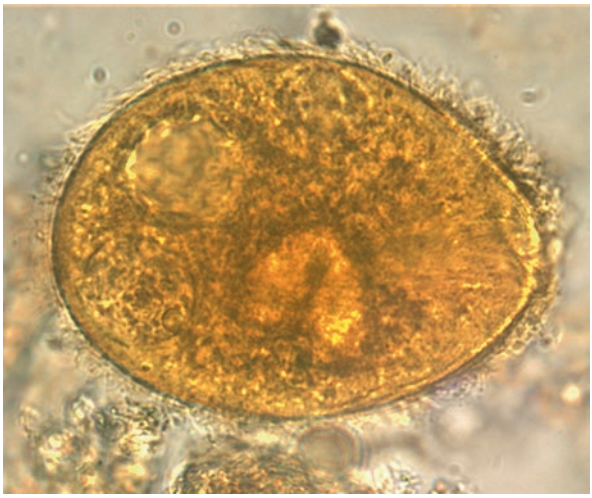

**Fig. 1.3** *Balantidium coli* trophozoite. *Arrow* points at the trophozoite cytostome (Picture obtained from http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary)

weeks or months prior to development of dysentery. Fluid loss is similar to that observed in cholera or cryptosporidiosis. Symptomatic infections can occur; resulting in bouts of dysentery similar to amoebiasis. Colitis caused by *Balantidium* is often indistinguishable from E. *histolytica* (Castro et al. 1983).

The organism can invade the submucosa of the large bowel, causing ulcerative abscess and hemorrhagic lesions. The shallow ulcers are prone to secondary infections by bacteria (Knight 1978). In few cases, extraintestinal disease in addition to the liver and the lung, other presentations such as peritonitis, urinary tract infection, and inflammatory vaginitis, have been reported (Karuna and Khadanga 2014). *Balantidium* has been described in the urinary bladder of an infected individual (Maleky 1998; Knight 1978; Ladas et al. 1989). Pulmonary lesions can occur in immunocompromised patients without obvious contact with pigs nor history of diarrhea prior to pulmonary infection (Anargyrou et al. 2003). *Balantidium* pneumonia has been described in a 71 old woman suffering from anal cancer (Vasilakopoulou et al. 2003). Chronic colitis and inflammatory polyposis of the rectum and sigmoid colon and an intrapulmonary mass have been described in a case with balantidiasis (Ladas et al. 1989).

After ingestion, the trophozoite excretes hyaluronidase, which aids in the invasion of the tissue. On contact with mucosa, mucosal invasion is accomplished by cellular infiltration in the area of the developing ulcer. The organism can invade the submucosa of the large bowel. Ulcerative abscesses and hemorrhagic lesions can occur. Some of the abscess formations may extend to the muscular layer. The shallow ulcers and submucosal lesions are prone to secondary bacterial infection. Ulcers may vary in shape and the ulcer bed may be full of mucus and necrotic debris.

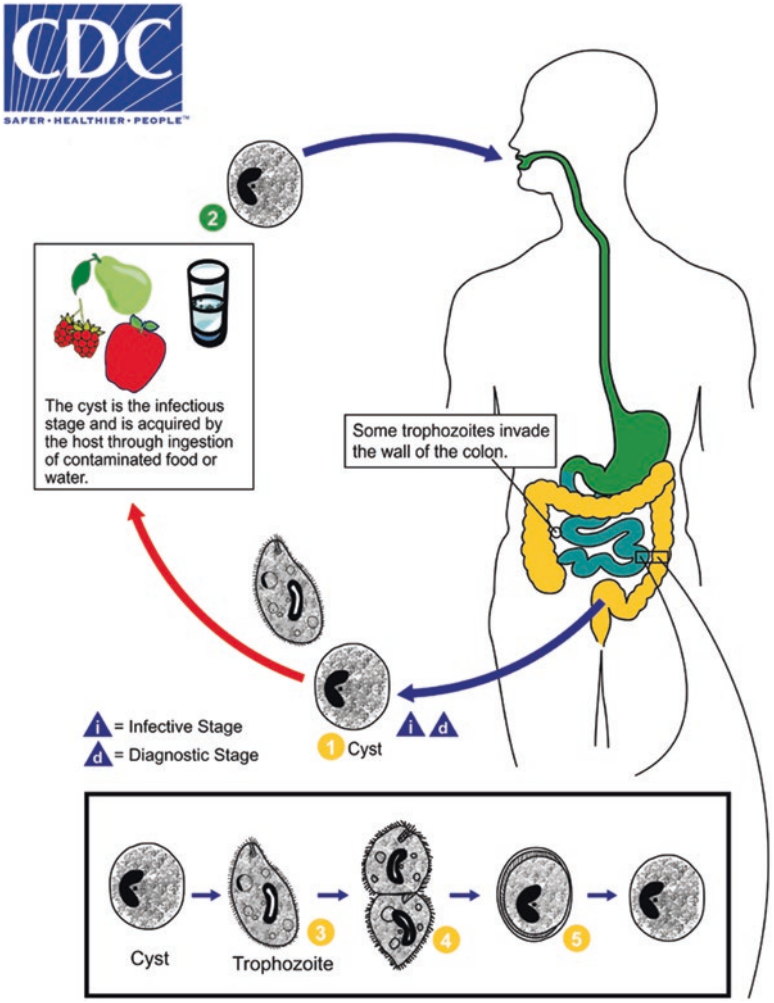


**Fig. 1.4** *Balantidium coli* life cycle diagram (Credit goes to CDC. Picture obtained from http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary)

### *1.6.3 Diagnosis and Treatment*

Wet preparation examinations of fresh and concentrated fecal material can determine the organism, as the shape and motility are characteristic of this ciliate. Tetracycline is the drug of choice, although it is considered an investigational drug for this infection. Iodoquinol or metronidazole may be used as alternative drugs.

### *1.6.4 Epidemiology and Prevention*

Several studies have demonstrated the presence of *B. coli* in developing countries. Balantidiasis has been reported in 8% of children of the Bolivian Altiplano (Basset et al. 1986).

Domestic hogs probably serve as the most important reservoir host for balantidiasis. In areas where pigs are the main domestic animal, the incidence of infection is high. Risk factors to acquire this infection include working at pig farms or slaughter houses. Infection can turn into an epidemic if conditions favor propagation in the community. This has been observed in mental hospitals in the USA, where poor sanitary conditions are common. Preventive measures include increased attention to personal hygiene and sanitation measures, since the mechanisms of transmission are via contaminated water or foods with *Balantidium* cysts.

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# Chapter 2
# Foodborne *Giardia duodenalis* and *Typanosoma cruzi*

**Charles R. Sterling**

## 2.1 *Giardia duodenalis*

### *2.1.1 Introduction*

Antonie van Leeuwenhoek (1632–1723), widely known for his work on the improvement of the microscope, and one of his disciples, yours truly, share something in common. We both have witnessed the flagellated "animalcule," also known as *Giardia*, from our own diarrheic feces under a microscope. While Van Leeuwenhoek made no connection of his observation to what might have been going on within him, that was certainly not the case with me. It is also certain that he had no idea as to how he might have acquired his infection, while I am most certain that my infection was foodborne.

My case history began circa May of 1990 while on a research trip to Peru to work with colleagues on another intestinal parasite, *Cryptosporidium*. Being a parasitologist, I had been careful on previous trips to Peru not to imbibe tap water or to eat leafy vegetables. On the last day of my trip in 1990, my colleagues and I ventured off to a restaurant well known to locals for some pisco sours and anticucho de corazon. I alone was hankering for a salad and assured by my friends that if there was one place in Lima where it was safe to eat one, it was here. A week later, back in the sanctuary of my office at the University of Arizona (UA), the symptoms of eructation, bloating, and diarrhea commenced. Putting two plus two together, I examined my own stool sample, only to witness the tumbling leaf motility of this flagellated demon. I immediately contacted an infectious disease specialist at the UA medical center who just also happened to be an expert on *Giardia* and obtained a prescription for Flagyl, the only drug for this infection available at that time in the United

C.R. Sterling, PhD (✉)
School of Animal & Comparative Biomedical Sciences, University of Arizona, Tucson, AZ 85721, USA
e-mail: csterlin@email.arizona.edu



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States. I continued with my duties at the UA, not knowing that my graduate students were recording my multiple daily voyages to the bathroom and delighting in the fact that I, a parasitologist, had defined myself as one who sits on one stool while looking at another! Within 5–6 days of treatment, I was more or less back to normal, vowing to avoid salads while traveling abroad. This case brings me to the point of thinking about how the salad components might themselves have come to bear this parasite: (1) fecal material was used to fertilize the fields in which the salad components were grown, (2) the field was contaminated by irrigation water bearing cysts, or (3) the food handler that prepared the salad was infected and did not practice good sanitation before passing his *Giardia* on to me. I don't even like to think about the latter possibility, but it certainly fits in with known *Giardia* transmission patterns. Enough about my very personal experience with *Giardia* and on to what we know about this organism and its foodborne transmission.

### *2.1.2 Overview of Importance, Life Cycle, and Biology*

Several recent excellent reviews have been written about the biology of *Giardia* and form the basis for what follows (Adam 2001; Carranza and Luhan 2010; Ankarklev et al. 2010). *Giardia duodenalis* (synonymous *Giardia lamblia* and *Giardia intestinalis*) is a ubiquitous enteric protozoan pathogen inhabiting the upper portions of the small intestines and affecting man and a variety of domestic and wild animals. It is one of the most commonly reported parasitic infections of humans in developed countries but extracts its greatest clinical impact in disadvantaged and developing countries, causing an estimated $3 \times 10^8$ cases per year (Lane and Lloyd 2002). Symptoms of intestinal discomfort and diarrhea can be persistent and pervasive, particularly in the young. Asymptomatic infections are more frequently encountered but may serve as a reservoir from which others can acquire infection, especially in endemic developing countries. Very few virulence factors have been identified for *Giardia*, and overall disease mechanisms are probably multifactorial.

*Giardia* possesses two morphologically distinct developmental stages in its life cycle: the motile flagellated trophozoite that inhabits the small intestine of its host and is capable of dividing by binary fission and the environmentally protected cyst stage that allows *Giardia* to survive outside the host's body once passed in feces (Fig. 2.1). In stained preparations, trophozoites have the appearance of a sting ray with two transcriptionally active nuclei and centrally positioned parabasal bodies that give it the appearance of a face staring back at you through the microscope. Trophozoites typically measure 12–15 μm in length by 5–9 μm in width. Their ventral surface is shaped as a concave disc which, with the aid of flagellar motion, assists trophozoites in adhering to the intestinal epithelium. Transit from the small intestines and under the right circumstances causes the trophozoites to form cysts. Trophozoites predominate in diarrheic stools and cannot survive for extended periods once passed from the host. Unique biological features of the trophozoite among eukaryotic organisms include the two already mentioned active nuclei, the lack of

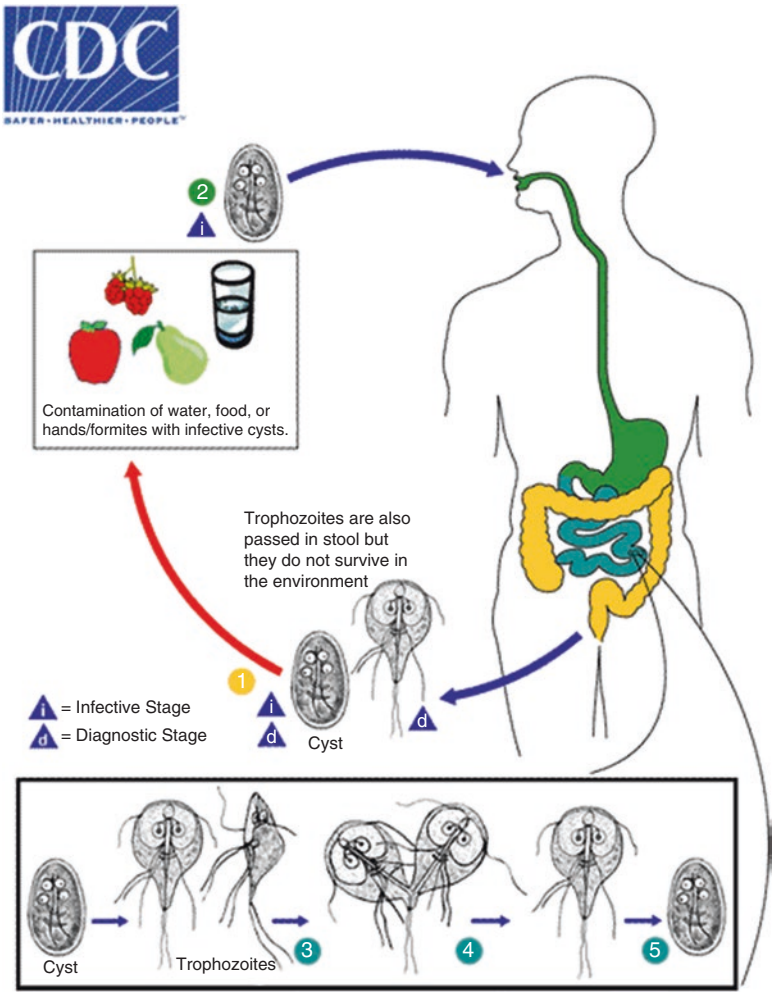


**Fig. 2.1** *Giardia duodenalis* life cycle. Image courtesy of DPDx (http://www.cdc.gov/dpdx/)

mitochondria, peroxisomes and Golgi, and the appearance of a "mitochondrial remnant" called the mitosome which probably has a function in anaerobic respiration. Another unique feature of the trophozoite is its ability to exhibit antigenic variation through the exchange of ~200 variant surface proteins (Prucca et al. 2011). In *Giardia*, the possibility of antigenic variation was first suggested by the marked difference in molecular sizes of the surface antigens (Nash and Keister 1985) among isolates that were quite similar genetically (Nash et al. 1985). These surface antigens are secreted into culture medium in large quantities and are the dominant molecules found when trophozoites are surface labeled. They were initially called excretory-secretory products (Nash et al. 1983; Nash and Keister 1985) and are now called variant-specific surface proteins (*VSP*) (Mowatt et al. 1991). This property of

*Giardia* probably plays a role in helping the organism evade host immune responses and also in expanding host diversity. In addition to the foregoing, the *Giardia* genome contains many genes required for meiosis (Ramesh et al. 2005), and recombination among isolates of one sub-assemblage, AII, genotype A2, has been reported from infected individuals of Peru (Cooper et al. 2007, 2010). Recombination also has been reported among assemblage B isolates of humans and also between assemblages previously considered independently evolving lineages (Lasek-Nesselquist et al. 2009; Siripattanapipong et al. 2011). Whether such reports of recombination represent true meiotic events remains to be established but is mentioned because *Giardia* has long been thought to be asexual.

Cyst formation is induced by host specific factors including high bile and low cholesterol levels and a basic pH (Lauwaet et al. 2007). Mature cysts possess a thick cyst wall composed of interlinking carbohydrates, sugars, and proteins and an enclosed zoite form with four nuclei. They typically measure 9 by 12 μm in size. Cysts allow the parasite to survive in the external environment in water and soil for months. Cysts are moderately susceptible to the action of commonly used disinfectants such as chlorine. Effective filtration methods remain the most effective barrier to preventing *Giardia* cysts from getting into potable water supplies (Plutzer et al. 2010). That said, many developing countries lack both conventional disinfection or filtration methodologies. Because cysts constitute the infective stage in the life cycle they play a major role in the foodborne transmission of giardiasis.

### *2.1.3 Speciation and Zoonotic Potential*

Currently, there are six recognized species of *Giardia.* These include two distinct species from birds, *G. ardeae* and *G. psittac*; two from rodents, *G. muris* and *G. microti*; one from amphibians, *G. agilis*; and a large taxonomic grouping termed *G. duodenalis* (Filice 1952) and meant to specify all *Giardia* from mammalian hosts other than mice (Thompson 2004). Numerous studies have clearly identified *G. duodenalis* as a species complex whose members have been divided into eight distinct genetic assemblages (Ryan and Cacciò 2013) (Table 2.1).

**Table 2.1** *Giardia doudenalis* genetic assemblages

| Assemblage | Host range |
|---|---|
| A | Humans, livestock, cats, dogs, beavers, guinea pigs, other primates |
| B | Humans, other primates, dogs, beavers, other wildlife |
| C/D | Dogs |
| E | Cattle, sheep, pigs, goats, alpaca |
| F | Cat |
| G | Rats |
| H | Marine mammals |

*Giardia duodenalis* isolates that commonly infect humans fall into two major genotypes or assemblages, each with a number of genetic subgroups (Adam 2001). One of the major controversies surrounding *Giardia* is related to its true zoonotic potential (Bauer 1994; Bemrick and Erlandsen 1988; Connaughton 1989; Faubert 1988; Monis and Thompson 2003; Thompson 1998). It should be pointed out that the finding of different genotypes in humans and animals indicates the lack of zoonotic transmission but that the finding of similar genotypes in different hosts, however, is not prima facie evidence that zoonotic transmission is occurring. (However, the finding of identical genotypes in two hosts would suggest that cross-species transmission had occurred in the recent past.) In other words, similarity of genotypes is a necessary but not sufficient condition to indicate epidemiologically significant transmission from one host to another. An important aspect of this controversy is not whether zoonotic transmission can ever occur but whether it is important in terms of biology and epidemiology. It is important to point out that human infectivity studies using isolates from animals infected with genotypes A and B have not been conducted. Such studies would likely put the zoonotic issue to rest as has been done for *Cryptosporidium* (DuPont et al. 1995).

In spite of the foregoing statements, investigators have isolated the same *G. duodenalis* assemblage from beavers and humans living within the same geographical area while investigating waterborne disease outbreaks, implying that zoonotic transmission was occurring (Isaac-Renton et al. 1993; Baruch et al. 1996; McIntyre et al. 2000). In one case, an infected beaver had an organism of the same genotype as that contaminating the water and causing human infection. When the beaver was removed, the water contamination was eliminated and the epidemic stopped (Isaac-Renton et al. 1993). More recent studies involving the use of molecular tools in endemic foci where the *Giardia* transmission frequency is high have also strongly implicated zoonotic transmission involving dogs, livestock, other primates, and humans (reviewed in Thompson and Ash 2015). If conclusively proven that zoonotic transmission of *Giardia* occurs with regular frequency, then this would importantly impact on the water and foodborne transmission of *Giardia* to humans.

### *2.1.4 Epidemiology and Foodborne Transmission*

There is little controversy over the fact that *Giardia* is transmitted via the fecal-oral route, either directly or indirectly. Transmission routes include direct person to person, animal to animal, potential zoonotic, potential zooanthroponotic, waterborne, and foodborne. The latter, which is the topic of this chapter, could include use of contaminated water in irrigation of food crops or in food preparation, as well as contamination due to poor sanitary habits used by food handlers. Most reports of the potential for foodborne transmission of *Giardia*, with perhaps the exception of my own experience, come from either reports of the presence of *Giardia* cysts on or in food, epidemiological studies of foodborne outbreaks, reports of poor personal hygiene practices used by food handlers, surveys of *Giardia* infection in food

handlers, and risk assessments for the risk of foodborne outbreaks due to the presence of *Giardia* in water used to irrigate fresh produce.

The finding of *Giardia* cysts on foods that humans consume is likely a good indication that the individual food product may, and I emphasize may, lead to *Giardia* infection and disease. It has already been demonstrated that a dose as low as 25 cysts may lead to infection (Rendtorff 1954). Foods from which cysts have been identified include dill, lettuce, bean sprouts, radish sprouts, and strawberries (Robertson and Gjerde 2001). Lettuce and leafy greens far and away lead the pack of vegetables shown to be positive (Cook et al. 2007; Dixon et al. 2013). The unanswered question is how did these foods become contaminated in the first place although many possibilities do exist. In addition to vegetables, shellfish, such as oysters and clams, through filter feeding, has been shown to harbor *Giardia* cysts (Graczyk et al. 1998, 1999a, b, 2003a, 2006; Gómez-Couso et al. 2005a, b; Hänninen et al. 2005; Shets et al. 2007). Theoretically, at least, consumption of raw shellfish could lead to infection and illness. Potential mechanical transmission of *Giardia* to food sources via synanthropic flies is also suggested by the finding of cysts on and within their bodies (Graczyk et al. 2003b).

Epidemiological investigations of possible foodborne disease outbreaks have provided some of the most compelling evidence for foodborne transmission of *Giardia*. Keep in mind that investigations of such outbreaks usually have involved multiple reported cases among individuals attending a single event where affected individuals who became sick all reported ingesting a common food. One of the first reported linkages to such an outbreak came from a nursing home in which evidence of foodborne transmission included a significant association ($P = 0.04$) deduced from sandwich consumption and illness (White et al. 1989). This type of association was not seen in individuals who ate cooked food. As it turned out, one of the food handlers had an infected child who attended a day care center and later became infected herself. She was the first, time-wise, of nine employees in the food service area who ultimately tested positive for *Giardia*. This same outbreak also provided compelling evidence for person-person transmission in this type of setting. Another outbreak occurred at a family party in which 9 of 25 people consumed fruit salad prepared by a mother who had a diapered child and pet rabbit that tested positive for *Giardia* (Porter et al. 1990). Yet another outbreak of *Giardia* came from a case-controlled investigation of employees in a corporate office setting who ate at a cafeteria whose food was prepared by a food handler who had giardiasis (Mintz et al. 1993). In this study, odds ratio and confidence interval data among 26 ill of 188 total individuals linked illness to the consumption of raw sliced vegetables prepared by this food handler. Additional overviews of such outbreaks are well summarized in the literature (Rose and Slifko 1999; Dawson 2005; Smith et al. 2007; Plutzer et al. 2010).

As pointed out in the above-noted outbreak situations, not only food but also food handlers were implicated in association with foodborne transmission. It's not surprising, therefore, that studies have been undertaken to screen food handlers for *Giardia* and other enteric parasites. One such screening report from Heathrow Airport, where a detailed medical history was mandated on all individuals prior to hiring of ground catering staff, showed that about 4% of applicants tested positive

and that many of these showed the presence of *Giardia* (McGirr 1969). Other such studies among food handlers have been reported from southern Brazil and western Iran (Takizawa et al. 2009; Colli et al. 2014; Kheirandish et al. 2014). The Brazilian studies, in particular, pointed to an association of infection with working conditions and improper hygiene. The take-home message, as in my own case, is traveler be safe rather than sorry.

*Giardia duodenalis* has frequently been described from numerous animal sources, and several studies have been conducted in both domestic and wild animals to assess whether or not feces from these animals possess a risk to human infection via contaminated food or water. In one study, conducted on dairy cattle in Ontario, Canada, *Giardia* was detected in 42% of cattle. Genotype assemblage B was isolated from 24.5% of cattle, while cattle-specific assemblage E was detected in 17.5% of cattle tested, suggesting that fecal material from cattle could pose a potential risk of zoonotic transmission, including through contaminated water or food (Coklin et al. 2007). The use of livestock waste bearing *Giardia* cysts, and its increasing environmental burden, likewise has called attention to such a source as contributing to human foodborne illness (Budu-Amoako et al. 2011). In a study conducted in California, *Giardia* genotypes were assessed from wild and domestic animals and linked to environmental loading that could impact on transmission of the parasite to humans. Interestingly, and in contrast to the study from Canada, most of the *Giardia* from cattle was of the host-specific E assemblage, while wild canids have a preponderance of *Giardia* from the A and B assemblages. The conclusion from this study was that cyst shedding from cattle may pose a lower risk to humans than that from other animals, such as wild canids (Oates et al. 2012). Another interesting study where genotyping studies were not conducted showed that 23.5% of rodents collected from produce fields of a major agricultural region of the Central California Coast harbored *Giardia*. Other potential human pathogens were also isolated from these rodents. The point of the study was to drive home the point that feces from these animals could contaminate preharvest crops (Kilonzo et al. 2013). Keep in mind, however, that rodent species of *Giardia* have not been implicated in causing human infections. In a longitudinal cohort study conducted in urban and rural settings of Vellore, India, an association was made of fly densities and infectious diarrhea. Fly densities were six times higher in the rural settings due largely to garbage disposal close to homes. Likewise, diarrheal episodes and duration were higher in individuals under the age of 5 included in the study. Pathogens isolated from the children included *Giardia*, and the point was made that mechanical transmission of such pathogens to food could likely occur in such a setting and be mediated by flies (Collinet-Adler et al. 2015).

A matched case-controlled study conducted in Southern England to investigate risk factors for sporadic giardiasis showed that consumption of lettuce had a positive and independent association with infection and disease (Stuart et al. 2003). This study was conducted using postal questionnaires. Another study in an agricultural area of Mexico identified the presence of *Giardia* cysts (range < 17–1633 per 100 liters) in irrigation water heavily impacted by humans and animals. Therefore,

ample opportunity existed for contaminated water to find its way onto produce crops (Mota et al. 2009). This study went one step further, and a risk assessment study was performed to assess the potential of infection from produce items of this area. The risk assessment for *Giardia* from lettuce was determined to be $2 \times 10^{-1}$ at the highest detectable concentration. Much of the produce, including lettuce, from this area is imported into the United States on an annual basis. The authors were quick to point out the need for mitigation strategies that would help minimize the risk of human infection.

### *2.1.5 Detection*

Standardized methods for detecting *Giardia* cysts in water, based on entrapment by filtration, elution, concentration by immunomagnetic separation (IMS), and identification by specific morphometric criteria and immunofluorescence, have been developed and standardized (US-EPA Method 1623 and 1623.1 1999, 2012). Use of these methods can be enhanced by following with PCR-RFLP or sequencing to determine specific parasite species or genotype (reviewed in Koehler et al. 2014). Such standardized methodologies, however, have not been established for *Giardia* isolation or detection from foods. As already noted, however, *Giardia* has been detected on food products. The incorporation of IMS, following more conventional concentration and separation methods used for foods, led to recovery of 46% ± 19% *Giardia* cysts on artificially contaminated leafy green products (Cook et al. 2007). Another study involved cyst recovery involving a washing procedure by drum rotation and sonication followed by centrifugation and IMS led to an average recovery of 67% (Robertson and Gjerde 2001). Lately, a technique employing inertial microfluidic separation, which does not use IMS and is reported to be less costly and time-consuming to techniques that use IMS, had an average recovery of 68% for *Giardia* cysts from spiked lettuce samples. This technique utilized initial concentration methods previously refined and developed (Dixon et al. 2013) and depends on the presence of a microfluidic *G. duodenalis* inertial separation chip that can channel eluates containing cysts into recovery and waste reservoirs followed by epifluorescent detection of cysts following staining with specific FITC-labeled monoclonal antibodies (Fig. 2.2). Alternatively, PCR can be used to detect *Giardia* cysts from vegetables (Dixon et al. 2013), and a recent study has shown this method to be ten times more sensitive than light microscopy (Ramirez-Martinez et al. 2015). Bear in mind that the abovementioned methods for *Giardia* cyst detection in produce have largely been made on artificially seeded samples. With a minimum detection of $10^3$ cysts by PCR in 50 g of seeded lettuce (Ramirez-Martinez et al. 2015), one can only wonder what the minimum detection level will be in field harvested or store bought produce. Also bear in mind that cyst numbers on such produce are likely to be low, but that human infection can be produced by low cyst numbers.

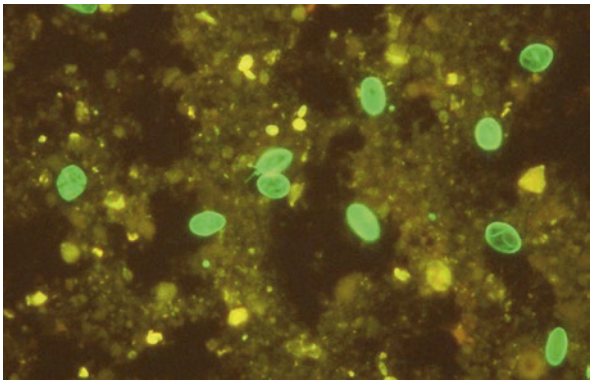

**Fig. 2.2** FITC-labeled *Giardia* cysts

### *2.1.6 Treatment*

Giardiasis, or disease caused by *Giardia* infection, is a worldwide issue. The number of drugs available to treat this condition has not varied widely over the past several decades. Metronidazole (MTZ), commonly known as Flagyl, has been a mainstay in treating this disease. I have known this from a personal perspective. Another nitroimidazole compound, tinidazole (TNZ), is now more commonly used since it can be administered as a single dose with fewer side effects. However, increasing treatment failures are being reported (reviewed in Escobedo et al. 2016). Because of this and because albendazole (ABZ) has also been reported to affect this parasite in vitro, a meta-analysis of the efficacy of this drug compared to TNZ was conducted for the treatment of *Giardia* infections in children (Escobedo et al. 2016). Meta-analysis can be used analytically to summarize results from different but comparable studies. This study showed that TNZ had a higher efficacy than ABZ on the treatment of *Giardia* infection from children of developing countries, but that there was a dire need for well-designed multicentric trials. In addition, it pointed to the need for newer drug development against this infection.

### *2.1.7 Control*

In order to consider methods of controlling the spread of *Giardia* via food products, one has to have an appreciation and understand of all the ways in which food can become contaminated in the first place. These sources of possible food contamination have been beautifully summarized (Smith et al. 2007; Budu-Amoako et al. 2011) and include the following: poor hygiene by food handlers, use of contaminated feces, manure or slurry for crop fertilization, pasturing infected livestock near crops, direct defecation of infected wild hosts onto crops, direct contamination of foods by synanthropic hosts (birds and insects), aerosols used for spraying that may be contaminated, use of contaminated wastewater for irrigation, washing foods in

contaminated water and then consuming raw, use of contaminated water for making frozen/chilled foods, and use of contaminated water for making products that receive minimum heat or preservative treatment. From the foregoing, therefore, it is obvious that any measures that could be used to prevent foodborne disease would need to consider the above and adopt appropriate strategies that could possibly mitigate all these potential sources of contamination. Accomplishing all of this is easier said than done, especially in developing countries that lack resources to carry out the appropriate required measures. With growth of the global market economy, let's not forget about the exportation and importation of food products. In most instances, they are not tested, so the ever present risk of infection remains. We can and must do more to prevent not only foodborne giardiasis but foodborne illness in general.

## 2.2 *Trypanosoma cruzi*

### *2.2.1 Introduction*

*Giardia duodenalis* and *Trypanosoma cruzi* do share something in common. They are both members of the phylum Sarcomastigophora and subphylum Mastigophora. Otherwise, they are quite different parasites. Fortunately for me, I have had no personal experience of infection with *T. cruzi. Trypanosoma cruzi* is the causative agent of Chagas disease, so named in honor of the individual who first described it, Carlos Chagas. According to one published report in honor of the 100th anniversary of his birth, Chagas first found this trypanosome in the hindgut of the "barber" bug, also known as kissing bug. He next found the parasite in the blood of a cat and then a girl. Finally, he succeeded in fulfilling Koch's postulates by reproducing the infection in small animals and recovering and identifying the parasite from these animals (Lewinsohn 1981). The early accounts of his discover are not without controversy however (Löwy 2005). It has long been speculated that Charles Darwin may have suffered from Chagas disease. Recent reports, however, have come to the conclusion that he either had Crohn's disease (Orrego and Quintana 2007) or conditions rooted in mitochondrial dysfunction (Hayman 2013).

### *2.2.2 Overview of Importance, Life Cycle, and Biology*

Chagas disease, caused by *T. cruzi*, is variably estimated to infect between 5 and 18 million people, largely of Latin America (Stanaway and Roth 2015; Bern 2015). The disparity in these figures could reflect prevalence data reported in 1991 (18 million) and in 2010 (5.7 million) (Bern 2015). There are numerous challenges in estimating prevalence including biases centered on community-based studies where the disease is known to be endemic (Stanaway and Roth (2015). Because of this, the

true burden of disease will likely depend on new data collection using newer methods, including risk assessment modeling.

Chagas disease can be divided into three clinical phases: acute, indeterminate, and chronic (Pereira and Navarro 2013; Stanaway and Roth 2015). Following a variable incubation period, lasting 3–112 days depending on the mode of transmission (Shikanai-Yasuda and Carvalho 2012), an acute phase of infection ensues lasting 4–8 weeks. Symptoms may range from asymptomatic to febrile with lymphadenopathy. During this phase there is detectable parasitemia (Bern 2015). Death during the acute phase generally involves children (Rassi and Marin-Neto 2010). The acute phase is followed by the indeterminate phase in which as many as 50% of infected people show chronic asymptomatic infection (Stanaway and Roth 2015). There may be no long-term sequelae to this, or 20–30% may progress to chronic Chagas cardiomyopathy which may take years to decades to develop (Bern 2015) or to manifestation of gastrointestinal disease involving megaesophagus and megacolon. These later manifestations of Chagas disease are observed in about one-third of chronic chagasic patients (Pereira and Navarro 2013).

The life cycle of *T. cruzi* as typically presented in text books involves development of the parasite in an invertebrate, transmission of the parasite via the feces of the invertebrate (vector) to a susceptible vertebrate host, development in a vertebrate host, and transmission back to the invertebrate host when it feeds on the blood of the infected vertebrate host (Fig. 2.3). Many species of triatomine bugs, also

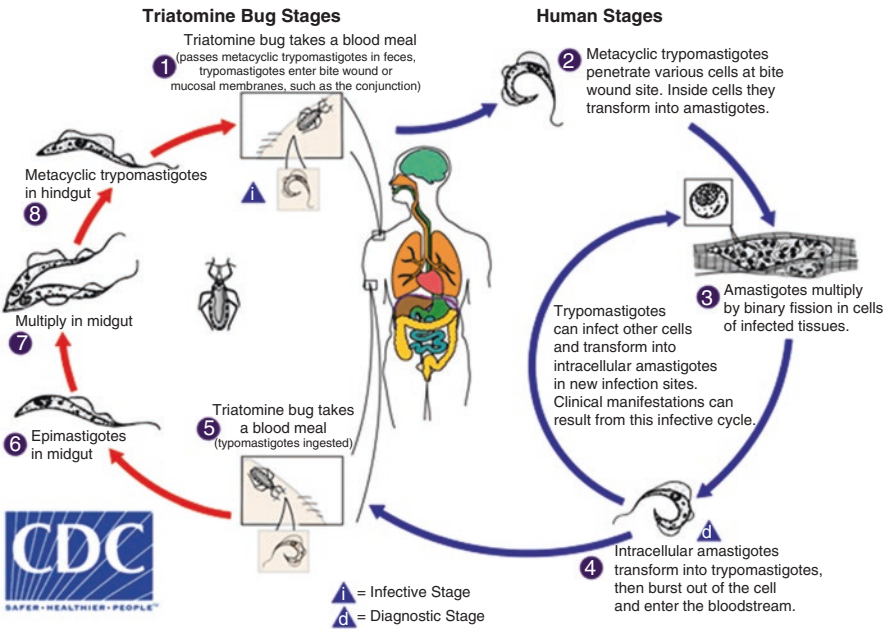


**Fig. 2.3** *Trypanosoma cruzi* life cycle

known as kissing bugs, serve as intermediate hosts and many species of vertebrates as final definitive hosts. There are many likely routes of transmission involving these hosts that will be subject of discussion later.

The vectors of *T. cruzi* are rather robust in size, and, therefore, one would assume that feeding activity by such an insect would be readily detected. Most infected individuals, however, rarely report being aware they were bitten (Shields and Walsh 1956). These bugs are exquisitely sensitive to $CO_2$ (Guerenstein and Lazzari 2009) and once they find their host inoculate salivary substances that serve to anesthetize the skin area around the bite and therefore may go undetected (Dan et al. 1999). Successful vectors of this infection to humans further take on the habit of defecating while they feed. This leaves infective metacyclic trypomastigotes in the bug's feces on the skin near where the bug has bitten, only to be rubbed into the wound near the bug's bite. The bug's saliva also serves to induce cell chemotaxis, bringing inflammatory cells to site that can become infected (Mesquita et al. 2008). Infection of cells is dependent on specific receptor ligand interactions leading to internalization of the parasite into a parasitophorous vacuole (Barrias et al. 2013). This is also the case in terms of oral infection (Yoshida 2009). Furthermore, a stage-specific glycoprotein, gp82, has been shown to resist proteolytic digestion by pepsin at acidic pH and binds to both gastric mucin and to target epithelial cells, such that parasites recovered from the stomach of mice 1 h after oral inoculation remain infective (Cortez et al. 2006). Another glycoprotein, gp90, plays a role in inhibition of cell invasion, and those *T. cruzi* strains that have a gp90 isoform susceptible to peptic digestion are more likely to be infective via the oral route (Yoshida 2009). Once inside cells, trypomastigotes are converted to amastigotes and replicate, undergoing a morphological transformation that results in more trypomastigotes being derived. Figures 2.4, 2.5, 2.6 and 2.7 depict the transformation of trypomastigotes to amastigotes and eventually back to trypomastigotes by electron microscopy. Some of the trypomastigotes get into the blood circulation, while many continue with the cycle of replication in tissues. Parasites in the blood stream become available to the kissing bug when it feed on an infected host. Inhibition of host responses induced by the parasite is responsible for maintaining persistent infection which may lead to chronic disease over time (Nardy et al. 2015). Innate and adaptive host responses probably lead to declining parasitemias over time but insure the presence of enough parasites in the peripheral circulation so that infection of the vector can occur. Once the vector has taken in an infective meal, trypomastigotes transform into epimastigotes in the bug's midgut, multiply, migrate to the hindgut, and transform into infective metacyclic trypomastigotes. It is these metacyclic forms that are also responsible for foodborne transmission of the disease should the bug leave its calling card on food that may be consumed by humans. This will be discussed in more detail later on. One of the remarkable thinks about this life cycle is all of the environmental challenges *T. cruzi* is faced with in transitioning from an insect to vertebrate host (Jimenez 2014). Issues the parasite must face include thermal shifting, nutrient availability, osmotic stress, and oxidative stress, not to mention all the stressors of host immune responsiveness.

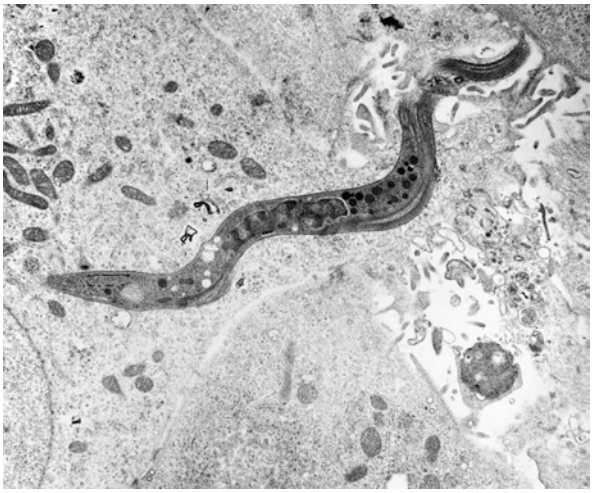

**Fig. 2.4** *T. cruzi* trypomastigote entering cell

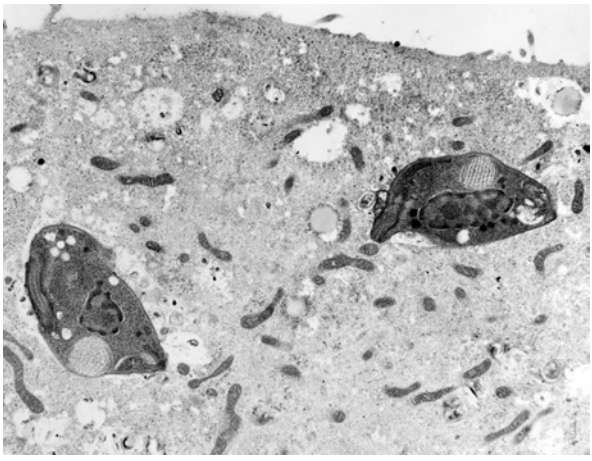

**Fig. 2.5** *T. cruzi* transforming into amastigotes

### *2.2.3 Epidemiology and Foodborne Transmission*

From an epidemiological perspective, the transmission of *T. cruzi* basically involves three cycles: enzootic, zoonotic, and anthroponotic. The enzootic cycle, which involves transmission between wild animals and involves vectors, defines what occurs in the United States and other sylvatic regions of the Americas. *Trypanosoma cruzi* is quite common in such animals as raccoons and wood rats. In a survey conducted in Texas, 90% of raccoons and 37% of woodrats tested serologically positive for *T. cruzi* (Charles et al. 2013). These same authors point out that *T. cruzi* has been identified from over 20 different wildlife species in the United States. While some of these wildlife species are synanthropic, none is more so than man's best friend, the dog, which may also harbor *T. cruzi* (Barr 2009; Esch and Petersen 2013).

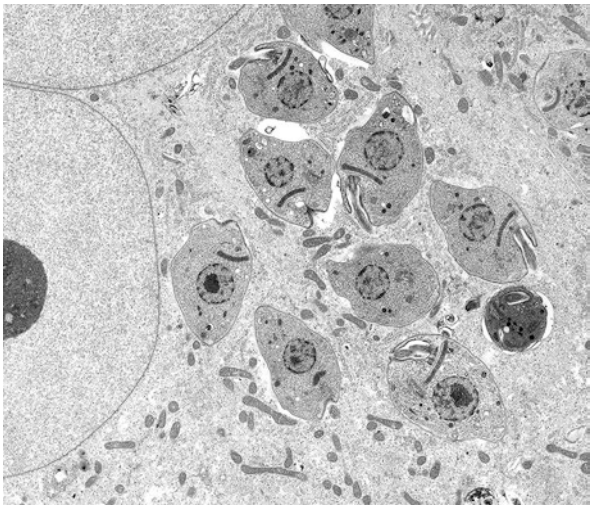

**Fig. 2.6** *T. cruzi* epimastigotes and one amastigote

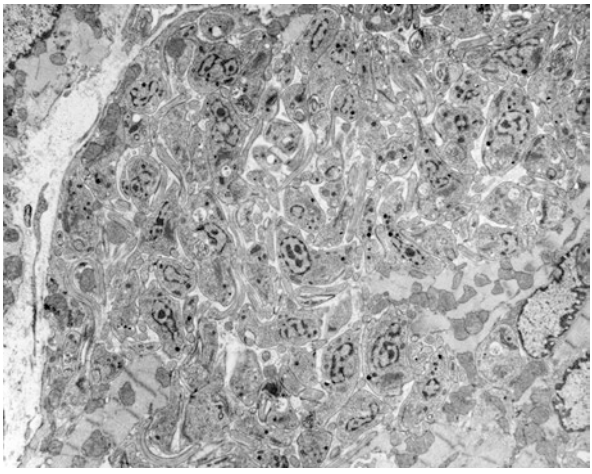

**Fig. 2.7** *T. cruzi* intracardiac trypomastigotes

In spite of this, only a handful of autochthonous cases have been described from the United States (Garcia et al. 2015). Reasons for this are likely linked to vector defecating habits and the fact that our 11 species of triatomines don't like to inhabit human dwellings that are well constructed (Klotz et al. 2014). It is estimated, however, that there are as many as 300,000 immigrants residing in the United States who have Chagas disease (Bern 2015).

Zoonotic transmission involves animal to human transmission involving vectors. As previously pointed out, a synanthropic type of relationship most likely exists in order to facilitate transmission. In this instance, transmission could occur in either rural or urban settings depending on the type of animal harboring infection and living close to man. An excellent example of a very rural type setting is seen in the

Brazilian Amazon where 33 species of wild animal and 27 species of triatomines, most of which are infected with *T. cruzi* exist and either live near man or man enters their domain (Coura 2013).

Anthroponotic transmission involves person-to-person or perhaps person-to-animal transmission either involving vectors or not. If vectors are involved, the implication is that they inhabit man's poorly constructed domicile that favors vector infestation and feed almost exclusively on human blood. Thirteen triatomines are recognized as being domestic species (Coura 2013). Direct routes of transmission from person-to-person and not involving vectors may involve blood transfusion, organ transplantation, and vertical or congenital transmission (Bern et al. 2011). Collectively, all of these transmission routes have to be considered in highly endemic setting and many of the direct routes in settings where immigrants have come from countries endemic for the disease.

Recently, oral/foodborne transmission of Chagas disease has gained attention and has only been mentioned in passing, if at all, in many textbooks of Parasitology. An excellent overview behind oral transmission and the thinking behind foodborne transmission of *T. cruzi* has been published (Pereira et al. 2010). Oral routes have been known for some time in susceptible omnivorous and insectivorous animals. Oral transmission was reinforced in 1931 by putting infective feces of triatomines on the oral mucosa of animals, thus infecting them. Numerous studies followed demonstrating that oral ingestion of infected triatomes led to infection in opossums, armadillos, and other animals. *Trypanosoma cruzi* infected mice were also used to orally infect cats as were blood samples from the infected mice (Pereira et al. 2010).

The association of infection with foods that humans might consume and oral transmission was reported in 1961 (cited by Jörg 1992) when mice, cats, and dogs drank milk containing the feces of infected triatomines and became infected. Later, a variety of foods were contaminated with *T. cruzi* suspensions and fed to mice, all of which became infected (Lainson et al. 1980). Since then, numerous such experiments have been repeated in mice (Pereira et al. 2010). An interesting study, which dealt with the survival of *T. cruzi* in various fruits and vegetables, demonstrated that the parasite could survive for a period between 6 and 72 h with the largest survival rate found between 6 and 18 h post contamination (Añez and Crisante 2008 cited in Pereira et al. 2010). Even though the parasites used in this study were derived in cultures, the foregoing is important because unlike what one may logically think, it is apparent that the parasite can survive outside its vector host for extended periods of time increasing the likelihood of foodborne transmission should that food become contaminated with vector feces.

The first published report of an outbreak of Chagas disease possibly associated with foodborne transmission was made in Brazil in 1965 (Silva et al. 1968). Seventeen individuals of an Agricultural School, who ate together, became ill, and the link with Chagas disease was made when infected people presented with clinical symptoms of acute myocarditis. All fell sick practically on the same day and ultimately six died. A hypothesis was put forth that food may have become contaminated with the urine of an infected marsupial. Another outbreak in Brazil in 1986 was epidemiologically linked to the possible contamination of food by opossum

feces that brought illness to 26 people consuming various foods at a farm meeting (Shikanai-Yasuda 1987; Shikanai-Yasuda et al. 1991). All 11 opossums collected from the area tested positive for *T. cruzi*, while none of the other farm animals sampled were infected.

A large outbreak of Chagas disease in 2005, and again in Brazil, was linked to the consumption of sugarcane juice sold at a stand along a Brazilian motorway. There were 25 confirmed cases and 3 deaths (Secretaria de Vigilância em Saúde of Brazil 2005). In this instance, it was hypothesized that triatomines were ground up in the preparation of the juice from sugarcane or that the sugarcane was contaminated with the feces of wild infected animals. Infected vectors were found near and in the stand selling the juice. Many additional cases, 437, 3/4 of which were linked to outbreaks also occurred in the Brazilian Amazon region between 1968 and 2005 and were suspected to have been caused by the consumption of açai juice prepared from a reddish/purple fruit from the açai palm tree (Pereira et al. 2010). In one localized outbreak occurring in Brazil in 1996 and involving 17 individuals, a definite link with triatomine feces was made since the açai juice was prepared at night and vectors were attracted to the light and fell into the juice being prepared and were ground up (Valente et al. 1999, 2009). Many more such cases have been reported from Brazil, and the vast majority has been linked to contamination of açai or sugarcane juice by triatomines and triatomine feces which ended up in the juice because of the way in which the juice is prepared (Pereira et al. 2009).

The oral/foodborne transmission of Chagas disease is not confined to Brazil alone. In December of 2007, *T. cruzi* was detected in a 9-year-old student of a school in Caracas, Venezuela. Subsequently, 20 persons of the same school were hospitalized with acute symptoms, and parasites were detected. Ultimately a preliminary clinical interview, including serologic screening, was performed on 1000 individuals from the school within a week of the outbreak. From this total, there were 119 confirmed cases and 16 undefined cases. One child died. In this study, a positive correlation was found between ingestion of guava juice and risk of infection (Alarcón de Noya et al. 2010b). Electrocardiogram changes have been followed and reported from many of these individuals (Marques et al. 2013). In addition, a follow-up on this outbreak and others occurring in Venezuela using haplotyping techniques showed multiclonal and common infections in patients, vectors, and reservoirs (Muñoz-Calderón et al. 2013). Other reports within this time frame on the oral transmission of Chagas disease came in from Colombia and Bolivia (Alarcón de Noya et al. 2010a; Rueda et al. 2014).

In total, more than 1000 cases of infection with *T. cruzi* has been linked with oral infection in different regions of Brazil, Colombia, Bolivia, Guayana Francesa, Argentina, and Ecuador (Reuda et al. 2014). In the Amazon Basin in Brazil, orally transmitted Chagas disease has emerged as the principal mode of transmission, even though intra- and peridomestic control measures were in effect (Shikanai-Yasuda and Barbosa Carvalho 2012).

Although the vertical transmission of Chagas disease via breast milk has been implicated from very early studies, the risk of infection through this route has not been established. In spite of this it is recommended that a mother with Chagas

disease only discontinue breast feeding if she is experiencing the acute phase of the disease, has reactivation due to immunosuppression, or bleeding nipples (Norman and López-Vélez 2013).

### 2.2.4 Detection

The detection of *T. cruzi* parasites in food implicated in or epidemiologically linked to foodborne infection or outbreaks is rarely, if ever, accomplished. There are several likely reasons why this hasn't happened: (1) Unlike *Giardia*, *T. cruzi* does not have an environmentally resistant cyst stage it its life cycle. (2) Trypomastigotes, or metacyclic trypomastigotes, cannot survive for an extended period of time in foods and most likely in juices. (3) By the time an infection or outbreak is detected, that source of infection is no longer available to sample. This latter point is because it can take weeks for acute symptoms of disease to appear and make medical personnel aware that such an infection or outbreak has occurred. In spite of the foregoing, excellent recommendations recently have been made regarding epidemiological, clinical, and diagnostic approaches that can be utilized in conjunction with oral/foodborne outbreaks of *T. cruzi* infections (Alarcón de Noya et al. 2015). The following summarizes these recommendations.

The first thing likely to happen is that a patient or patients present with acute symptoms, and a confirmed diagnosis of what is causing the symptoms must be made. In the case of *T. cruzi*, this can most readily be accomplished through blood smears, ELISA to detect antibodies, and/or combinations of these tests along with PCR of the positive blood sample. Other laboratory tests involving blood chemistry should also be performed. At the same time, it is advised to interview the patient(s) toward food habits and assess hygienic habits along with living conditions. It is also pertinent to ask if insects and other animals are present in or around the home. Epidemiological questionnaires are most useful. Clinical follow-ups including appropriate therapy, blood tests, and heart monitoring using ECG should then follow for extended time periods following infection. Lastly, and if it can be done, insect vectors and/or animals should be collected from the suspected area in which the infection or outbreak likely occurred and appropriate PCR testing performed to identify likely genotype linkages with PCR results obtained from the patient(s).

### 2.2.5 Treatment

Benznidazole is considered to be the first-line treatment, with minimal side effects, for the treatment of acute phase Chagas disease (Bern 2015) and has been shown to be efficacious in maintaining sustained clearance of the parasite even 1 year later (Chatelain 2015). Nifurtimox and benznidazole are the only drugs with proven efficacy against *T. cruzi*, although gastrointestinal and neurotoxic side effects have been

noted with nifurtimox. Both drugs reduce the severity of symptoms, shorten the clinical course of illness, and reduce the burden of parasitemia (Bern 2015). In the United States, these drugs can only be obtained from the CDC and used under investigational protocols.

Two new drugs, posaconazole and ravuconazole, have received clinical testing in comparison with benznidazole, but came up short even though able to clear parasites due to lack of sustained activity (Chatelain 2015). A Chagas Drug Discovery Consortium was created in 2010 following funding from the NIH and included several laboratories and new investigators, mainly from the United States. It is hoped that this consortium will lead the way to new drug development that will have sustained activity, much like many antimalarials, in targeting all stages of the parasite occurring in humans. Currently, there is no treatment for chronic Chagas disease, and there is still debate about the persistence of parasites during this phase of infection (Chatelain 2015). Clearly, new drugs are needed to help control this infection in all of its clinical phases.

### *2.2.6 Control*

Considerable inroads have been made in reducing the burden of Chagas disease in many Latin American countries. These control measures have largely, but not exclusively, been aimed at controlling populations of anthropocentrically oriented triatomine vectors. Most authors acknowledge that total eradication of *T. cruzi* is virtually impossible owing to enzootic cycles of transmission involving more than 150 potential vectors (Coura 2013; Alarcón de Noya et al. 2015.

Much of the success of vector elimination in areas of Latin America is because of spraying campaigns. The reason for their success or failure can directly be linked to the life cycle of the bug and efforts to sustain spraying long term. The only stage of a triatomine life cycle where these insects can readily disperse is the adult. Only adults have wings. Nymphal instars are wingless and are more likely to stay near a constant and steady source of blood (Schofield 1985). Of concern, however, is the evolution of insecticide resistance among certain common vectors (Mougabure-Cueto and Picollo 2015). The Pan American Health Organization and the World Health Organization have assisted in the coordination of four large-scale control initiatives for controlling this disease: the Southern Cone Initiative (1991), the Andean Initiative (1997), the Central American and Mexico Initiative (1998), and the Amazon Initiative (2004) (Stanaway and Roth 2015). Collectively, these initiatives have included educating the public, making suggestions for housing improvements, insecticide spraying, and more rigorous screening of blood donors. In some areas, a drastic reduction in disease prevalence has been observed, while worldwide a reduction of as much as 50% has been seen within the past 25 years (Bonney 2014). It has been pointed out that there needs to be a sustained political effort aimed at controlling this disease to prevent lapses and regression in success areas (Bonney 2014). As part of a team that has studied disease transmission and control near Arequipa, Peru,

I can vouch for this need. Despite a control effort via spraying that took place in 1995, the vectors and parasite rapidly reemerged (Delgado et al. 2011).

The control of foodborne transmission will likely depend on governments, such as in Brazil, implementing guidelines of good manufacturing practices and mandatory pasteurization of beverages and foods that may be related to the oral transmission of T. cruzi (Bern and Montgomery 2009; Pereira and Navarro 2013). In addition, surveillance must aim at food safety, particularly at institutional levels (schools, nursing homes, universities, canteens, restaurants, and food vendors) (Alarcón de Noya et al. 2015). Improved health and medical education are also an important part of this control formula.

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# Chapter 3
# *Cyclospora cayetanensis*

**Vitaliano A. Cama and Ynés R. Ortega**

## 3.1 Preface

*Cyclospora cayetanensis* (*Cyclospora*) is a coccidian parasite that infects the enteric tract of humans (Ortega et al. 1993) and may cause disease when infectious oocysts are ingested by humans. The routes of transmission include the consumption of contaminated food (Connor and Shlim 1995) or water (Wright and Collins 1997). When excreted, the oocysts of *Cyclospora* are immature and not sporulated, therefore noninfectious, and require 7–15 days with ideal environmental conditions to fully mature and sporulate to become infectious (Fig. 3.1).

*Cyclospora* was properly described as a coccidian parasite in 1993, and shortly after there were several foodborne outbreaks of cyclosporiasis, which were epidemiologically associated with the consumption of imported berries. The drastic reduction of importation of berries from specific regions led to the absence of significant outbreaks of cyclosporiasis in the USA. However, between 2013 and 2016, there were large multistate outbreaks of cyclosporiasis in the USA, this time associated with the consumption of imported cilantro (FDA 2016; CDC 2014, 2015, 2016; CDC 2016).

V.A. Cama, DVM, PhD (✉)
Parasitic Diseases Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, 1600 Clifton Road, MS D-65, Bldg 23, Rm. 10-164, Atlanta, GA 30329-4018, USA
e-mail: Vec5@cdc.gov

Y.R. Ortega
Department of Food Science & Technology and Center for Food Safety, University of Georgia, Griffin, GA, USA



Y.R. Ortega, C.R. Sterling (eds.), *Foodborne Parasites*, Food Microbiology and Food Safety, DOI 10.1007/978-3-319-67664-7_3

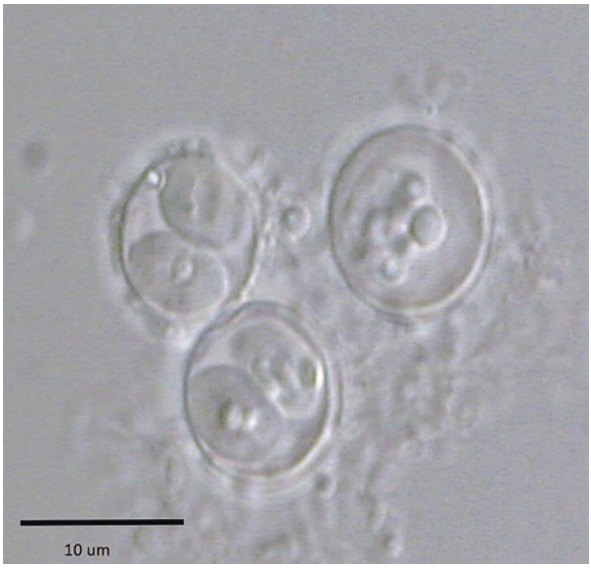


**Fig. 3.1** *Cyclospora cayetanensis* oocysts. Unsporulated oocysts present a morula-like body and when sporulated they differentiate to form two sporozoites

## 3.2 Background/History

The epidemiology of *Cyclospora* is unique: it is endemic in several regions of the world, almost exclusively in nonindustrialized countries (Markus and Frean 1993; Ortega et al. 1993), whereas it is epidemic in the industrialized world. Some of those outbreaks have had important impact in food safety practices and regulations (Charatan 1996; Herwaldt 2000; Herwaldt and Beach 1999). Cyclosporiasis has also been reported in travelers returning from endemic areas (Gascon et al. 1995; Soave et al. 1998).

*Cyclospora cayetanensis* was described as a parasite in 1992–1993 (Ortega et al. 1993) and gained significant public health awareness a few years later when it was identified as the causative agent of several foodborne outbreaks in the USA. The taxonomic classification and description of human infectious *Cyclospora* date back to 1992. There are previous reports in the literature that describe organisms quite similar to *Cyclospora cayetanensis*. One of the first reports was published in 1979 (Ashford 1979), describing an organism with microscopic characteristics compatible with oocyst of *C. cayetanensis*. This report concluded that the described organism was most likely a new species of *Cystoisospora* infecting humans (Ashford 1979).

In the late 1980s, other investigators reported similar organisms which autofluoresced when observed under UV light (Long et al. 1991). Based on this observation, the investigators concluded that such organism was either a blue-green algae or a cyanobacterium. Thus, it was proposed that the yet to be identified organism was a cyanobacterium-like body or CLB, a term that was used in some publications. Other terms used in the literature to describe organisms that could have been *Cyclospora* were coccidian-like body (also used the acronym CLBs) (Hoge et al. 1993) or big *Cryptosporidium* (Naranjo et al. 1989). The latter was based on microscopy observation of acid-fast stained organisms and transmission electron micrographs that showed sections of organisms resembling encysted flagellates.

Shortly thereafter, studies led by Ortega demonstrated that organisms that had been described as "CLBs" or "big *Cryptosporidium*" were indeed coccidian parasites. The conclusive evidence was based on the successful maturation of the organisms, where the authors were able to clearly demonstrate that the previously unclassified organism had an oocyst containing two sporocysts, and each sporocyst contained two sporozoites. These morphologic characteristics demonstrated by light microscopy and novel freeze-fracture substitution electron microscopy conclusively classify the organism as protozoan parasite of the genus *Cyclospora* (Ortega et al. 1993). Following the guidelines of the International Code of Zoological Nomenclature, this parasite was named *Cyclospora cayetanensis* (Ortega et al. 1994).

*Cyclospora* has been associated with the multiple foodborne outbreaks, where the primary risk factor was either the consumption of fresh berries or leafy greens, such as basil and cilantro. Thus, efforts continue to be directed to the development of diagnostic tools, not only for clinical settings but also for the detection of this parasite in environmental and food samples, as well as for epidemiological and trace-back investigations.

## 3.3 Biology

The oocysts of *Cyclospora cayetanensis* are spherical, with 8–10 μm in diameter (Fig. 3.1). Despite that its life cycle has not been fully demonstrated, evidence so far supports the notion that this is a parasite that only infects humans (Ashford 1979; Ortega et al. 1993, 1994). *Cyclospora* has been described in the feces of various animal species, including ducks (Zerpa et al. 1995), chickens (Garcia-Lopez et al. 1996), dogs (Carollo et al. 2001; Yai et al. 1997), and monkeys (Chu et al. 2004); however, no evidence of tissue infections with *Cyclospora* were described in those studies. Oocysts of *Cyclospora* spp. have been observed in nonhuman primates. Interestingly, genetic analysis of the small subunit RNA gene has shown that each species of *Cyclospora* infected only a specific primate host: *Cyclospora papionis* was only found in baboons, green monkeys were only infected with *C. cercopitheci*, and only colobus monkeys had infections with *C. colobi* (Eberhard et al. 2001). *Cyclospora macacae*, a recently described species, was only reported in rhesus monkeys (Li et al. 2015).

There have been multiple attempts to develop an animal model for *C. cayetanensis*. Animal infectivity studies using mice (nine strains of mice, including adult and neonatal immunocompetent and immune-deficient inbred and outbred strains), rats, sand rats, chickens, ducks, rabbits, gerbils, hamsters, ferrets, pigs, dogs, owl monkeys, rhesus monkeys, and cynomolgus monkeys were all unsuccessful. These findings suggested that *C. cayetanensis* could be a monoxenous or species-specific parasite that only infects humans (Eberhard et al. 2000).

Studies to fully elucidate the life cycle of *C. cayetanensis* in humans have not been possible. Nevertheless, infectious stages of *Cyclospora* have been identified in biopsies of human small intestine, which has been the basis for a proposed (and yet to be fully demonstrated) life cycle.

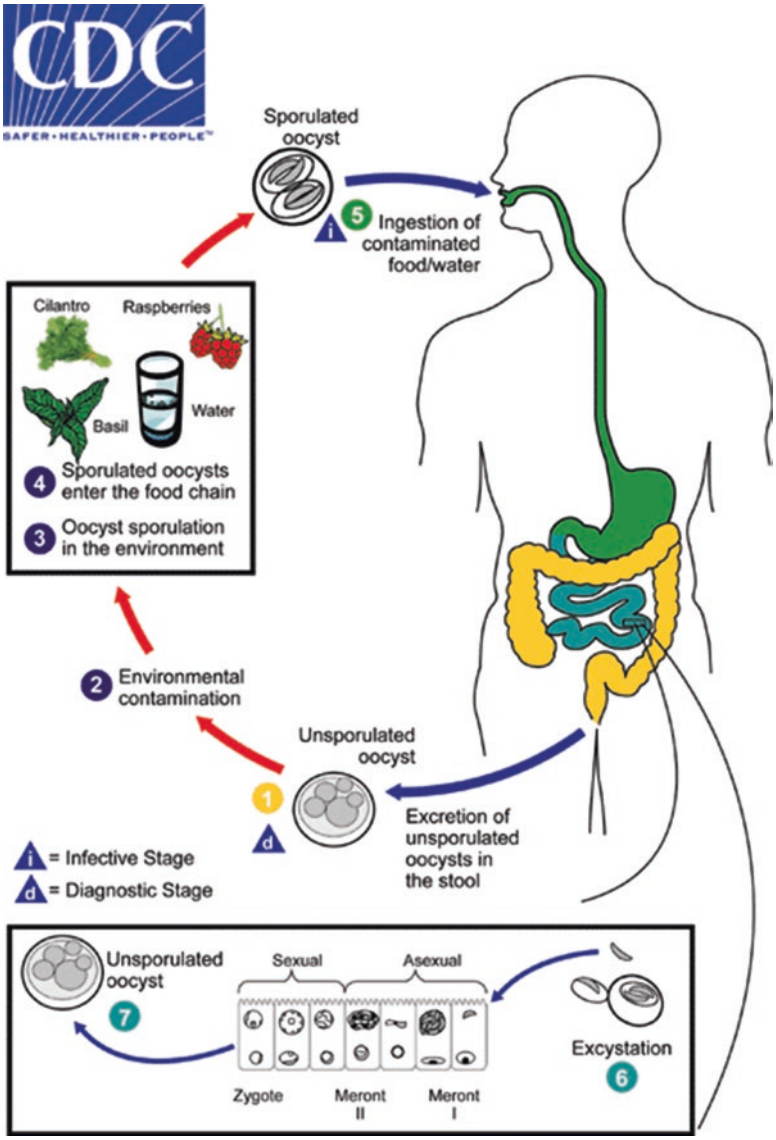


**Fig. 3.2** *Cyclospora cayetanensis* life cycle (Graph obtained from http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary).

It is proposed that cyclosporiasis starts after a susceptible host ingests sporulated oocysts, which are the infectious stages. After ingestion, the oocysts are ruptured in the upper gastrointestinal tract and their two sporocysts are liberated. Shortly thereafter, two sporozoites are released from each sporocyst and proceed to infect epithelial cells of the intestine (Fig. 3.2). The extent of infection in the human gastrointestinal tract has not been clearly determined. Ortega and collaborators

(Ortega et al. 1997a) have described intracellular stages from histological sections of upper gastrointestinal biopsies from 17 Peruvian patients. Meronts and macro- and microgametocytes were identified in the cytoplasm of jejunal enterocytes, demonstrating that oocysts could be formed in this section of the enteric lumen. However, the extent of intestinal infection or preferential site of infection has yet to be reported. This apparent lack of information is due in part to the absence of an animal model to study human cyclosporiasis (Sadaka and Zoheir 2001).

There are anecdotic reports of *Cyclospora* in ducks (Zerpa et al. 1995), dogs (Yai et al. 1997), chickens, and monkeys (Chu et al. 2004), where these hosts were presumed to be naturally infected with *Cyclospora cayetanensis*. The report in ducks is from the microscopic exam of the droppings of a duck owned by a *Cyclospora*-infected patient, while fecal droppings of ducks of non-infected people were *Cyclospora* negative (Zerpa et al. 1995). Two dogs in Sao Paulo, Brazil, were reported positive for *Cyclospora*; however, a follow-up study of 140 dogs from the same city did not find any infected dogs (Carollo et al. 2001). In another study, dogs, chickens, and monkeys from Nepal were found microscopy positive for *Cyclospora*, and these samples were further confirmed by PCR. The authors concluded that infections in these species needed further confirmation, including the histological demonstration of infectious stages (Chu et al. 2004).

People who become infected with *C. cayetanensis* may present gastrointestinal symptoms that may include diarrhea, flatulence, weight loss, abdominal discomfort, and nausea (Mansfield and Gajadhar 2004). Although the exact pathogenesis for these symptoms is not yet defined, jejunal biopsies have shown an altered mucosal architecture with shortening and widening of the intestinal villi due to diffuse edema and infiltration by a mixed inflammatory cell infiltrate. There was also reactive hyperemia with vascular dilatation and congestion of villous capillaries, which are compatible with inflammation of the upper intestinal tract. The histological examination of infected jejunal cells has shown parasitophorous vacuoles containing sexual and asexual forms. Type I and II meronts, with 8–12 and 4 fully differentiated merozoites, respectively, were found at the luminal end of jejunal epithelial cells. These findings demonstrated of the developmental cycle of a coccidian and that infected tissues had pathological changes that could be linked to the symptoms observed among those infected with *Cyclospora cayetanensis* (Ortega et al. 1997a).

## 3.4 Clinical Significance

Diarrhea, malaise, and lack of energy and appetite are symptoms associated with several gastrointestinal pathogens and are present in patients with cyclosporiasis in developed countries, where the disease occurs sporadically. In endemic areas, however, infections with *C. cayetanensis* affect specific age groups and are usually detected in children 4–10 years of age. About 50% of these children will experience diarrhea and other gastrointestinal discomforts, including malaise, bloating, and anorexia. *Cyclospora* has also been identified in 7.5% patients with chronic renal

failure in Egypt (Ali et al. 2000). In endemic settings, infections may resolve spontaneously, suggesting that immunity may play a role in clearance of infections.

## 3.5 Transmission and Epidemiology

Foodborne outbreaks in the USA have increased in absolute numbers since 1973. Data analysis from the Foodborne Outbreak Surveillance System revealed 190 outbreaks between 1973 and 1997. During this period, *Cyclospora* and *E. coli* O157:H7 were newly recognized as causes of foodborne illness (Sivapalasingam et al. 2004). However, there has been an increased in the number of multistate outbreaks, from 2.5/year between 1973 and 1980 to 13.5 in the period 2001–2010. Although most of the outbreaks were caused by *Salmonella*, leafy greens (13%) and fresh fruits (13%) were the second and third most frequent food products implicated in those outbreaks (Nguyen et al. 2015). The increment in multistate outbreaks can be due to better reporting and surveillance systems; however, the consumption of imported fresh vegetables and produce has also increased since 1973.

Epidemiological studies have demonstrated that the transmission of *C. cayetanensis* occurs primarily through the foodborne route. Waterborne transmission has also been documented, with some reports directly implicating drinking water as the source of infection, while in others water was suspected to be responsible for contaminating and disseminating the oocysts in fresh produce.

The first report of waterborne transmission described that consumption of contaminated chlorinated water was the source of *Cyclospora* infections between expatriates in Nepal (Rabold et al. 1994). In Illinois, a retrospective epidemiological investigation of a *C. cayetanensis* outbreak identified drinking water as the source of infection (Huang et al. 1995). A prospective epidemiological study in Haiti (Lopez et al. 2003) reported variations in the percentage of cases of *Cyclospora* infections, from 12% to 1.1% between February and April 2001. One artesian well used for drinking water was found positive and led to this study; however, none of the wells was positive thereafter. Therefore, no epidemiological associations were established between infections and water. Using molecular methods, two studies in California reported the detection of *C. cayetanensis* DNA in surface waters (Dowd et al. 2003; Shields and Olson 2003), suggesting that *Cyclospora* might have been present in water and that the waterborne transmission potential needed to be further studied.

Currently, *Cyclospora* is primarily considered a foodborne parasite, although early on it was considered originally a diarrheal disease affecting returning travelers. This concept changed in the spring of 1996, when a large foodborne outbreak of cyclosporiasis occurred in North America, affecting 1465 people from 20 states, the District of Columbia, and two provinces (Herwaldt and Ackers 1997). Epidemiological investigations confirmed that the vast majority of cases were associated with 55 events where raspberries were served. These investigations later demonstrated a significant association between cyclosporiasis and consumption of

imported raspberries (Herwaldt and Ackers 1997). Detailed epidemiological descriptions of specific events included a wedding reception in Massachusetts (Fleming et al. 1998) and a luncheon in Charleston, SC, where 38 of 64 attendees met the case definition of cyclosporiasis (Caceres et al. 1998). Cluster investigations in Florida showed that raspberries were the only food common to this outbreak and confirmed them to be imported as well (Katz et al. 1999).

In the spring of 1997, another large outbreak of cyclosporiasis affected the USA and Canada. Epidemiological and trace-back investigations identified 41 infection clusters that comprised 762 cases of acute cyclosporiasis and 250 sporadic cases of cyclosporiasis. Similar to the 1996 outbreak, there were significant associations between *Cyclospora* infections and consumption of imported raspberries. As a consequence of this second outbreak, exports of raspberries were voluntarily suspended in May 1997 by producers in Guatemala, the primary producers in those years (Herwaldt and Beach 1999).

In addition to raspberries, other fresh produce has been implicated in the transmission of *Cyclospora*. Basil was directly implicated in a 1999 outbreak in Missouri, where 62 cases were identified. All of these people had previously eaten either pasta chicken salad at one event or tomato basil salad at another event (Lopez et al. 2001). European countries also have documented cases of foodborne cyclosporiasis. Although a specific vegetable was not identified, the food associated with disease risk was lettuce imported from southern Europe that was spiced with fresh green herbs (Doller et al. 2002).

Snow peas have also been implicated as sources of transmission of cyclosporiasis. An outbreak found that 50 potential cases of cyclosporiasis were linked to the consumption of snow peas imported from Guatemala (Anonymous 2004).

In 2013, 631 cases of cyclosporiasis were reported in the USA multistate outbreak, with cases confirmed from 25 states. Another outbreak on the same year occurred in Texas, with additional cases reported in other states. These outbreaks occurred in the summer season and were linked to either imported cut lettuce or cilantro from Mexico (CDC 2013). In 2014, another summer outbreak of cyclosporiasis was reported, affecting 304 individuals from 18 states and New York City. Although epidemiological evidence linked all cases to the consumption of imported cilantro, the parasite was not isolated in any of the samples of produce or water tested (CDC 2014). In the summer 2015, 546 cases of cyclosporiasis were reported in the USA, spanning 31 states. The epidemiological investigation associated this outbreak with the consumption of cilantro from Mexico. As a result of these outbreaks, the FDA issued an import alert taking effect every year from April 1 to August 31, which allowed the import into the USA of cilantro only from producers in the Green List. To be included in the Green List, the producer or exporter had to demonstrate that their products were farmed and harvested following good production practices as defined by the US-FDA. Producers that were not on the Green List have to provide documentation to FDA to demonstrate that their produce met the requirements from the Green List, so that FDA will be confident that those products will be in compliance with the required food safety practices. Farms wishing to be in the Green List have to be inspected and certified to be in compliance with the

System for Reduction of Risk from Contamination (SRRC) program. Processing facilities wishing to be in the Green List will have to be inspected and certified to be in compliance with required Good Production Practices. Any farms not participating in the SRRC program can request FDA on-site inspections (FDA 2015). In 2016, after the implementation of the Green List, no outbreak was reported in the USA. Overall, there were 134 cases reported from 25 different states between May 1 and September 15 (CDC 2016). In 2016, there was an outbreak in Canada, reporting 87 cases of cyclosporiasis; however, the source of the outbreak was not identified. In 2017, more than 1060 cases of cyclosporiasis were reported in the USA affecting 40 states. No food specific food sources have been implicated in these cases (CDC 2017).

*Cyclospora* infections had been previously reported in travelers returning from developing countries. Although infections have been reported in areas of Southeast Asia, Papua New Guinea, Indonesia, India, Pakistan, Nepal, the Middle East, North Africa, the United Kingdom, the Caribbean, the USA, Central America, and South America, the true distribution and prevalence of this parasite in any population are frequently unknown. Infections were reported in travelers returning from South America (Drenaggi et al. 1998). In 1997, *Cyclospora* was reported in 5 of 469 returning travelers with diarrhea (Jelinek et al. 1997). In 2010, an outbreak of cyclosporiasis occurred in two succesive voyages of a cruise ship that departed from Fremantle, Australia. It was estimated that at least 266 passengers and 48 crew members had cyclosporiasis. The fresh produce taken onboard from Southeast Asian ports may have been the likely source of infection (Gibbs et al. 2013). Among expatriates living in endemic areas, a study in Nepal reported a higher risk of diarrhea among foreigners during the first 2 years of residence (Shlim et al. 1999).

Endemic cyclosporiasis has been reported in several areas of the world, mainly in developing countries. Although there are socioeconomic and geographical similarities with other related pathogens including *Cryptosporidium*, *Giardia*, and bacterial and viral disease, there are also marked differences. In general, *Cyclospora* has been reported affecting children in areas where access to clean water or sanitation is marginal or suboptimal. A prospective study in Peru found that children between the ages of 3 and 6 years were more frequently affected (Bern et al. 2002), but as children got older, the infections were rare after 10 years of age. These findings suggest that humans living in endemic areas, where exposure to the parasite is likely frequent, can develop protective immunity.

These observations, however, may only apply for specific settings where the parasite is endemic. For example, a study on upper middle class Peruvians, living less than 10 miles away from a shanty town where *Cyclospora* was endemic, showed that this population was affected by sporadic cases, with an epidemiological pattern that closely resembles cyclosporiasis in developed countries (Ortega et al. 1997a). Although the study sites were in the same city, the socioeconomic and sanitary infrastructure was markedly different, emphasizing the role of sanitation as an important factor for cyclosporiasis.

Endemic cyclosporiasis has been reported in multiple sites around the world. The first reports suggested that *Cyclospora* infections were more commonly found in

children (Ortega et al. 1993) especially those under 5 years of age (Hoge et al. 1995). In Latin America, *Cyclospora* infections have been reported in children with diarrhea in Brazil (2/315) (Ribes et al. 2004) and 6.1% of people living in impoverished areas in Venezuela (Chacin-Bonilla et al. 2003). A study conducted among 36 case and 37 control Egyptian children reported *Cyclospora* infections in 5.6% of malnourished children compared to 2.8% of the controls (Rizk and Soliman 2001). It should be noted that *Cyclospora* is not considered an HIV opportunistic agent, with similar incidence rates among immunocompromised and immunocompetent people. Nonetheless, a recent study in Egypt reported 6% *Cyclospora* infections among Hodgkin's lymphoma patients receiving chemotherapy (Rizk and Soliman 2001). Additionally, *Cyclospora* infections were reported in 7 of 71 Venezuelan HIV-infected patients and 7 of 132 otherwise normal children ages 1–12, while the highest frequency was observed in children 2–5 years of age (Chacin-Bonilla et al. 2001). In another study, *Cyclospora* was found in 3.3% of samples from HIV + patients in Northern India. Diarrhea was present in 75% of the cases with cyclosporiasis (Mohandas et al. 2002).

An epidemiological study among Guatemalan children detected *Cyclospora* in 117 (2.1%) of 5520 specimens, and most cases were in children <5 years of age. In that study, *Cyclospora* infections were more strongly associated with diarrhea than *Cryptosporidium* infections (Bern et al. 2000). Another study reported the prevalence of *Cyclospora* in Guatemala at 1.5%; however, none of the infected cases were raspberry farm workers (Pratdesaba et al. 2001).

The epidemiology in endemic settings also showed a seasonal pattern. Findings from a 3-year longitudinal study (1995–1998) in children living in an impoverished area of Peru showed an incidence rate of 0.20 cases per child per year, which was constant among children 1–9 years of age. In this study, the infections were more frequent during the warmer months, December to May, showing a seasonal pattern (Bern et al. 2002). A longitudinal study in several areas of Nepal from April 1995 to November 2000 found marked seasonality, with highest infection rates occurring during the summer and rainy season of the year (Sherchand and Cross 2001).

Additional studies have reported endemic cyclosporiasis on all continents, primarily affecting children. *Cyclospora cayetanensis* oocysts were reported in 1.1% of 5836 Peruvian children studied over 2 years (Madico et al. 1997). A study in Lagos, Nigeria, reported an overall prevalence of 0.9% (Alakpa et al. 2002, 2003). In Yunnan, China, 5.29% of pediatric diarrhea cases had *Cyclospora* (Zhang et al. 2002). In 2002, Uga found *Cyclospora* in the Bekasi District in West Java, Indonesia (Uga et al. 2002). *Cyclospora* infections have been reported in pediatric and adult patients with diarrhea in Tanzania (Cegielski et al. 1999) and the sub-Saharan region (Markus and Frean 1993).

Although *Cyclospora* is considered to be primarily transmitted through food products, very few studies on produce have been conducted outside outbreak investigations. Surveys of fresh produce were conducted in an endemic area of Peru, detecting *Cyclospora* in 1.8% of the samples (Ortega et al. 1997b). Other reports identified *Cyclospora* oocysts in lettuce, water spinach, herbs, salad greens, precut salad, and leafy greens in as high as 21% of the tested samples (El-Said 2012). A

study in Canada found *Cyclospora* in 1.7% of precut and packaged salad greens, although in this case most of these products were reportedly produced in the USA (Dixon et al. 2013). Another study in Italy in the Apulia region identified *Cyclospora* in 12.2% of the tested vegetables (Giangaspero et al. 2015).

## 3.6 Diagnosis

Diagnosis of these parasites is done by the identification of the oocysts or sporocysts in the fecal samples of the infected individuals. Oocysts can be observed using phase contrast or bright field or DIC microscopy (Fig. 3.1). *Cyclospora* oocysts autofluoresce; therefore, screening of a suspected sample should be done using an epifluorescence microscope with an excitation filter of 360/40 nm, a long pass dichroic mirror of 400 nm, and a 420 nm emission filter (Fig. 3.3) (Lindquist et al. 2003). A Kinyoun, Ziehl-Neelsen, or carbolfuchsin-modified acid-fast stain can be used to facilitate diagnosis; however, it should be taken into consideration that *Cyclospora* oocysts take the stain variably; therefore, if a sample contains a low number of oocysts, they could be missed. A safranin stain has also been reported to facilitate the detection of *Cyclospora* (Fig. 3.3) and may work better than acid-fast stains because oocysts of the bright pink color of the stain and most oocysts take the safranin stain (Visvesvara et al. 1997).

These procedures are more frequently used in human clinical specimens; however, they may not be efficient when examining environmental samples as oocysts are usually found in very small numbers and there are many other environmental structures that may look alike. Therefore, it is recommended to rely on DNA-based detection tools to identify *Cyclospora* in food or environmental samples. In 1996, a PCR assay for *Cyclospora* was developed using clinical samples (Relman et al.

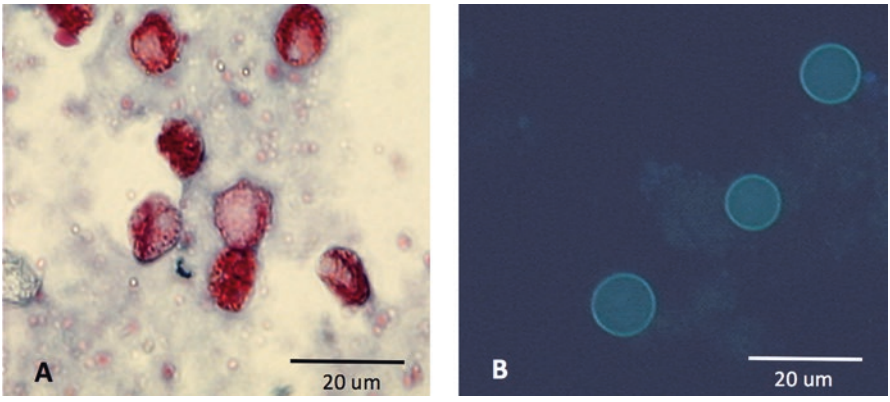


**Fig. 3.3** *Cyclospora cayetanensis* oocysts. (**A**) Modified acid fast stain. (**B**) Autofluorescence of oocysts when exposed to UV light

1996). However, it was later determined that this PCR, although sensitive, and useful for human clinical samples, the DNA locus targeted for amplification was also present in parasites that do not infect humans. Therefore, when working with environmental samples, the DNA of coccidian parasites noninfectious to humans could also be amplified, with an amplicon of the same size as *Cyclospora*. A PCR-RFLP which could differentiate among these coccidian parasites was developed (Jinneman et al. 1998). This was followed by new strategies to develop more sensitive and specific assays. These included the oligonucleotide ligation assay (OLA) (Jinneman et al. 1999) and real-time PCR (Varma et al. 2003). The sensitivity of these assays could be improved by optimizing the DNA extraction procedures and eliminating PCR inhibitors. Some of these strategies included the use of FTA filters (Orlandi and Lampel 2000) and inclusion of resin matrix treatment during oocyst disruption (Jinneman et al. 1998). Other tests have been developed where samples are no longer archived for additional studies. This includes the FilmArray Gastrointestinal Panel that simultaneously detects 22 different enteric pathogens directly from stool specimens (Buss et al. 2015). Studies on the *Cyclospora* mitochondrial genome will be allowing a better understanding of the biology of the parasite as well as the development of diagnostic tools (Cinar et al. 2015). Other tools including multilocus sequence typing tools have also been described with the objective to potentially be useful in trace-back studies (Guo et al. 2016).

## 3.7 Treatment and Control

The treatment of choice for cyclosporiasis is trimethoprim or sulfamethoxazole (TMP/SMX) (Madico et al. 1993; Pape et al. 1994). In a randomized control trial, ciprofloxacin was suggested as an alternative treatment for patients with cyclosporiasis who are allergic to sulfa drugs (Verdier et al. 2000). However, patients allergic or intolerant to TMP/SMX may take ciprofloxacin as an alternative treatment (Verdier et al. 2000). Patients with AIDS have a high rate of adverse reactions to this therapy.

Because the mechanisms of transmission of *Cyclospora* are primarily associated with the consumption of contaminated water or foods, prevention of infections can be achieved by avoiding fresh produce in areas of endemicity and drinking purified water (by filtration or boiling). Since *Cyclospora* is highly resistant to water chlorination, this procedure should not be considered as an alternative water treatment.

Prevention is the best strategy to avoid these infections. Because removal of these coccidian parasites is poorly achieved from fresh foods, and because they are resistant to sanitizers and disinfectants, it is preferred to implement good agricultural practices at farms to prevent the introduction of the pathogens to fresh produce and fruits.

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# Chapter 4
# *Cystoisospora belli* and *Sarcocystis* spp.

**Ynés R. Ortega and Vitaliano A. Cama**

*Cystoisospora belli* formerly known as *Isospora belli* and the *Sarcocystis* species primarily *S. hominis* and *S. suihominis* are parasites that infect the enteric tract of humans (Beck et al. 1955; Frenkel et al. 1979). These parasites cause disease when infectious oocysts (*Cystoisospora*) and sporocysts or tissue cysts (*Sarcocystis*) are ingested by humans. However, their routes of transmission are different: *Cystoisospora* can be acquired through the ingestion of human-shed sporulated oocysts in food or water, while *Sarcocystis* could be acquired through the ingestion either of viable sporocysts in muscles of intermediate hosts, or human-shed oocysts (Fayer et al. 1979 and Wright and Collins 1997). Both parasites are coccidia, thus the infectious stages have morphological similarities. Both have been associated with foodborne outbreaks and cause infections of the intestinal tract of humans. *Cystoisospora* is an opportunistic parasite and more comonly identified in immunocompromised individuals, particularly in AIDS patients. *Sarcocystis* have worldwide distribution, however infections are more frequently reported in travelers returning from endemic regions. *Cystoisospora* is frequently identified in immunocompromised individuals, particularly in AIDS patients.

Y.R. Ortega (✉)
Department of Food Science & Technology and Center for Food Safety, University of Georgia, Griffin, GA, USA
e-mail: Ortega@uga.edu

V.A. Cama
Center for Disease Control and Prevention, Atlanta, GA, USA



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## 4.1 Background/History

*Cystoisospora belli* is an infrequent parasite of humans, with most cases reported from tropical areas. In the immunocompetent population, *Cystoisospora* infections are usually asymptomatic (Teschareon et al. 1983). Immunocompromised patients, however, can present infections associated with severe clinical disease (Soave 1988). *Cystoisospora* is well recognized as a cause of chronic diarrhea in patients with AIDS (Pape et al. 1994). Infection can be acquired by ingestion of water or foods containing oocysts. The clinical presentation can be severe in the immunocompromised. *Sarcocystis* infections have been identified worldwide being most frequent in areas where raw or undercooked meats are consumed. If *S. hominis* or *S. suishominis* oocysts are ingested then they associated with gastrointestinal illness. However, if the infection is acquired by ingestion of contaminated meats, food, or water containing sporocyst of *Sarcocystis* spp, the infection is primarily localized in muscle. The likely agent is *S. nesbitti* and infection will result in muscular sarcocystosis (Fayer et al. 2015).

## 4.2 *Cystoisospora belli*

Formerly *Isospora belli*, this coccidian parasite may cause self-limiting or asymptomatic infections in immunocompetent people. However, it can cause protracted severe gastrointestinal illness among HIV-infected patients with low CD4+ cell counts. Infections can be rarely detected in other organs such as mesenteric or respiratory lymph nodes, liver, spleen, or gall bladder.

### *4.2.1 Biology*

*Cystoisospora* oocysts are elliptical measuring 20–36 μm by 10–20 μm. These oocysts are shed unsporulated in the feces of infected individuals and after 1–4 days the oocysts are fully sporulated. In environmental samples, however, the sporulated forms are likely to be found. Within the oocysts' wall, two spherical structures (spheroblasts) can be observed. Spheroblasts mature and differentiate after 24–28 h into sporocysts when favorable conditions of temperature and humidity are present. Each sporocyst is spherical in shape, measuring 10–12 μm in diameter, and each contains four sporozoites which are the stages that eventually invade ad infect enterocytes.

### *4.2.2 Clinical Significance*

Human cystoisosporiasis can present with severe enteric illness in AIDS patients or people with other forms of immunosuppression. The most common manifestation is watery chronic and persistent diarrhea, low-grade fever, malaise, steatorrhea, and

weight loss. Profuse diarrhea in immunocompromised patients can result in severe dehydration and malabsorption (DeHovitz et al. 1986; Ma et al. 1983; Pape et al. 1989; Shein and Gelb 1984). If not treated, this infection can result in death.

Reactive arthritis and acalculous cholecystitis have also been reported as sequelae. (Benator et al. 1994; Gonzalez-Dominguez et al. 1994).

### *4.2.3 Transmission and Epidemiology*

*Cystoisospora belli* infections are infrequently reported in immunocompetent individuals; however, patients with impaired immune systems may develop chronic life-threatening diarrhea. Within this population, there has been a sharp drop in the incidence of cystoisosporiasis due to the prophylactic use of trimethoprim sulfamethoxazole for *Pneumocystis carinii*, and later the wide spread availability of effective anti-retroviral therapies. The clinical and epidemiological features of human cystoisosporiasis are usually found in the literature as case reports.

In the USA, a review of human stools received in Kentucky clinics from March to September 2003 did not detect *Cystoisospora*-positive specimens (Ribes et al. 2004). Similar findings were reported from Japan, where a 7-year study of 4,273 specimens collected from patients with infectious enteritis and admitted to hospitals found only three positive samples (Obana et al. 2002). In developing countries, however, *C. belli* has been reported in children with diarrhea (Tavarez et al. 1991).

In patients with very low CD4+ counts or AIDS-associated infections, *C. belli* can lead to chronic diarrhea and wasting syndrome, primarily among patients not receiving or with sub-therapeutic plasma levels of antiretroviral medications (Figueroa et al. 1985; Maiga et al. 2002). In developing countries, the prevalence of *C. belli* infections in HIV-infected patients varies from 1.5% to 17% (Brandonisio et al. 1999; Cimerman et al. 1999; Cranendonk et al. 2003; Ferreira 2000; Gassama et al. 2001; Joshi et al. 2002; Lainson and da Silva 1999; Lebbad et al. 2001; Mohandas et al. 2002; Wiwanitkit 2001).

Severe *Cystoisospora* infections have also been reported in immunosupressed patients with neoplastic diseases (Makni et al. 2000; Resiere et al. 2003) and chronic renal failure (Ali et al. 2000).

### *4.2.4 Diagnosis*

The detection of *Cystoisospora* intracellular stages is through histological examination of intestinal biopsies of infected individuals, particularly in AIDS patients. Infection in the biliary tract, lymph nodes, spleen, and liver of HIV patients has also been reported (Bernard et al. 1997). Diagnosis of these coccidia is usually done by the identification of the oocysts in the fecal samples of the infected individuals. Oocyst shedding can be intermittent and at low numbers; thus, repeated ova and

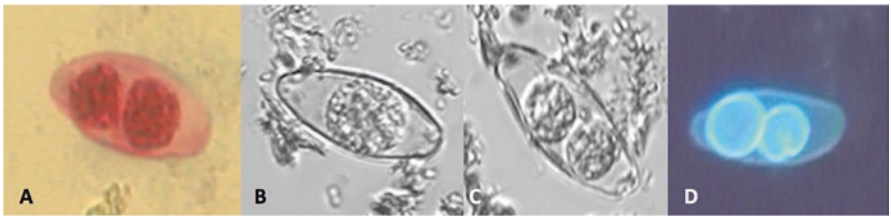


**Fig. 4.1** *Cystoisospora belli* oocysts. (**A**) Modified acid-fast stain, (**B**, **C**) unsporulated and sporulated oocysts, (**D**) autofluorescence of sporulated oocyst

parasite testing may be needed for an accurate diagnosis. Oocysts can be observed using phase-contrast, bright-field, or DIC microscopy (Fig. 4.1). Oocysts and sporocysts autofluoresce when observed using an epifluorescence microscope set with an excitation filter of 360/40 nm, a long pass dichroic mirror of 400 nm, and a 420 nm emission filter (Lindquist et al. 2003). Oocysts can also be observed in duodenal aspirates. A Kinyoun, Ziehl-Neelsen, or carbolfuchsin modified acid-fast stain can be used to stain oocysts (Ng et al. 1984). If a sample contains low number of oocysts, they could be missed on direct examination; thus, concentration methods are recommended. Polymerase chain reaction has also been described for detection of *Cystoisospora* (Muller et al. 2000).

### *4.2.5 Treatment and Control*

Trimethoprim sulfamethoxazole (TMP/SMX) is the drug of choice for treatment of cystoisosporiasis. TMP-SMX has also been used as chemoprophylaxis in persons with HIV infection. The incidence of cystoisosporiasis decreased in immunocompromised patients with low CD4+ counts when receiving antiretroviral therapy combined with TMP-SMX. Ciprofloxacin is an alternative treatment for patients allergic or intolerant to TMP/SMX (Verdier et al. 2000). Two patients with AIDS and cystoisosporiasis that were sulfonamide allergic were treated successfully with pyrimethamine alone, 75 mg/d, and recurrence was prevented with daily pyrimethamine therapy, 25 mg/d (Weiss et al. 1988). Doxycycline and nifuroxazide have also been studied in AIDS patients. Relapsed *Cystoisospora* infections were long-term treated with doxycycline (Meyohas et al. 1990). Since *Cystoisospora* can be acquired by the consumption of contaminated water or foods infection can be prevented by avoiding consumption of raw fresh produce in areas of endemicity and treating drinking water (by filtration or boiling). Coccidia are highly resistant to sanitizers and disinfectants; therefore, good agricultural practices that include the use of safe irrigation water will reduce the potential introduction of oocysts in products that are consumed raw.

## 4.3 *Sarcocystis* spp.

Sarcocystosis was considered an infrequent parasite infecting humans. Infection with this parasite can have two clinical presentations, intestinal and muscular sarcocystosis (Bunyaratvej et al. 1982; Fayer 2004). *Sarcocystis hominis* and *S. suihominis* infections can result in intestinal sarcocystosis, whereas muscular sarcocystosis can result from infections with multiple *Sarcocystis* species, particularly *S. nesbitii* (Fayer et al. 2015). More cases of muscular sarcocystosis have been reported among returning travelers and has gained the attention of the medical community.

### *4.3.1 Biology*

*Sarcocystis* is a parasite that has a definite and intermediary host to complete its life cycle. This coccidian parasite infects a variety of animals including those of commerce significance affecting poultry and livestock as well as other mammals, birds, reptiles, and fish (Fayer et al. 2015).

Various animal species can serve as definite and intermediate hosts. Humans are definite hosts for *S. hominis* and *S. suihominis*. Oocysts of these parasites are indistinguishable by microscopy; they are elliptical in shape, measuring 16 by 10 μm. The life cycle of these two species starts when the definite host (human) eats undercooked meat of an infected intermediate host (cattle or swine) containing tissue cysts (sarcocysts). When the sarcocysts are digested, bradizoites are released, and these invade the cells of the lamina propria of the intestinal epithelium where they differentiate into gametocytes. Sexual multiplication occurs resulting in the formation of unsporulated oocysts. The oocysts are then excreted in the feces of the infected individual. It is not unusual to find sporocysts in the feces along with oocysts.

Intermediate hosts (cattle and swine) get infected when they ingest the oocysts or sporocysts. Oocysts excystation occurs in the gastrointestinal tract of the animal, and sporozoites are released. They invade the endothelial cells of the blood vessels where multiple asexual multiplication cycles (schizogony) occur. Merozoites are eventually formed and will infect smooth, cardiac, and neural tissues. Sarcocysts containing bradizoites are formed (Dubey 1993). However, it is not known if bradizoites, the dormant stage of the parasite, can cause reinfections in neighboring tissues. Humans can serve as intermediate hosts for a various *Sarcocystis* species. *S. nesbitti* has been identified in a large outbreak involving travelers returning from the Tioman Island, Malaysia. Studies using the 18S rDNA from sarcocysts of rodents and oocysts in snake feces suggest that cobras may be the definite host of *S. nesbitti* (Lau et al. 2014).

*Sarcocystis* infections are frequently observed in travelers particularly those in Southeast Asia. Within the Malaysian population, the prevalence of intramuscular infections with *Sarcocystis* was less than 21%. Another outbreak of *Sarcocystis nesbitti* was described in Pangkor Island, Malaysia, in 2012. Eighty-nine of 92 college

students and teachers reported having fever, myalgia, and myositis. Identification of the *Sarcocystis* 18S rRNA gene and sarcocysts were confirmed in two students (Abubakar et al. 2013).

A large outbreak of muscular sarcocystosis in 2011–2012 in individuals traveling to the Tioman Island, Malaysia, was reported. Sixty-two probable and six confirmed cases were tourists. Myalgia (100%), fatigue (91%), fever (82%), headache (59%), and arthralgia (29%) were the most common symptoms. Blood eosinophilia and elevated serum creatine phosphokinase (CPK) levels were observed during the fifth week after departure from the island. *Sarcocystis nesbitti* DNA was recovered from one muscle biopsy. The source of infection was not determined (Esposito et al. 2014).

Occasionally, local infections may present complications due to large localized inflammatory reactions. Eight out of eleven presented with infections of the tongue and nasopharynx (Pathmanathan and Kan 1992). Potential association with vasculitis has also been proposed (McLeod et al. 1980).

### *4.3.2 Clinical Significance*

Clinical disease associated with human sarcocystosis is usually limited (Dissanaike 1994; Pozio 1991), and most human infection studies demonstrated spontaneous resolution within a month post-infection (Chen et al. 1999). Infection studies in human volunteers (Lian et al. 1990) reported clinical symptoms such as anemia, abdominal pain, diarrhea, fatigue, and dizziness on day 3 post-infection. Sporocysts and oocysts were found in the feces on day 8. The patent period of sporocyst excretion was more than 42 days. In addition to digestive malaise, another symptom associated with *Sarcocystis* infections may be muscle aches (Pamphlett and O'Donoghue 1990).

Experimental infections in human volunteers have been reported in the literature. Eight medical students ate raw meat from a pig experimentally infected with *Sarcocystis suihominis*. At 6–24 h after the meal, all persons suffered from acute clinical symptoms, particularly diarrhea and vomiting, chills, and sweating which decreased by the second day (Piekarski et al. 1978). In another study at the Institute of Medical Parasitology, University of Bonn, eleven medical students and six members of the institute participated in a meal with raw pork of an experimentally *Sarcocystis suihominis*-infected pig. Only individuals who ingested excessively high quantities of infected meat suffered severe symptoms (Kimmig et al. 1979).

No serological result association was found between heart conditions and *Sarcocystis* infections in Egyptian patients (Azab and el-Shennawy 1992), but serological evidence associates this disease with chronic fatigue syndrome (Pamphlett and O'Donoghue 1992).

Twenty-two intestinal specimens surgically resected due to segmental enterocolitis were classified into three groups: (1) acute inflammation with hemorrhage and necrosis, (2) constrictive lesion, and (3) false diverticulum with perforation. The predominant finding was unisegmental involvement, distributed in the jejunum, ileum, and ileocolon. Microscopically, small parasitic structures, interpreted to be an unconventional excystation stage of *Sarcocystis hominis*, were present on the

luminal border and within the crypt-lining epithelial cells (Dubey 1976). At the ulcerated area, tissue invasion by Gram-positive bacteria was consistently seen and considered as second pathogen (Bunyaratvej and Unpunyo 1992).

Companion animals can also get infected with *Sarcocystis* spp., as well as agricultural animals such as cattle, sheep, and horses which are of economic importance. Infected animals can have poor growth, poor feed use, reduced milk production, wool and hair loss, and abortion (Fayer et al. 2015).

### *4.3.3 Transmission and Epidemiology*

Despite its biological similarities with *Toxoplasma* and *Cystoisospora*, *Sarcocystis* is not considered an AIDS opportunistic infection (Dionisio et al. 1992). Most reports of cases of *Sarcocystis* in animals and humans are from Asia.

#### 4.3.3.1 *Sarcocystis* in Animals

In Czechoslovakia, *Sarcocystis cruzi*, *S. hirsuta*, and *S. hominis* were detected in 87% of 200 cattle and *S. ovicanis* and *S. tenella* in 92% of 100 sheep examined. All of 200 pigs examined were negative (Gut 1982).

In New Zealand, muscle tissue from the esophagus and diaphragm of 500 beef cattle was examined. All cattle were infected with *Sarcocystis*: 98% had *Sarcocystis cruzi* and 79.8% had *Sarcocystis hirsuta/Sarcocystis hominis* (Bottner et al. 1987).

Muscle tissue from the esophagus, diaphragm, and heart of 100 cattle from Belgium was examined for *Sarcocystis* infection. Of these, 97% were positive. Thick-walled cysts were recovered from 56% of animals, but these could not be identified as *S. hirsuta* and/or *S. hominis* on morphological grounds (Vercruysse et al. 1989).

In India, muscle samples from 890 slaughtered pigs were examined for the presence of sarcocysts. The prevalence rate was 67.98%, of which 43.14% was *Sarcocystis miescheriana* and 47.11% was *Sarcocystis suihominis* (Saleque and Bhatia 1991).

The prevalence of *Sarcocystis* in the muscle of 36 caribou examined in Newfoundland, Canada, was 53%. Infected animals were more frequently found in the central part of the island (Khan and Fong 1991).

Various animals in Ethiopia were tested and found positive for *Sarcocystis* infection: 93% of sheep, 82% of cattle, 81% of goats, 16.6% of donkeys, and 6.6% of chickens, from a total of 671 animals. However, none of the 40 heart muscles from bovine, ovine, caprine, and donkey fetuses examined harbored *Sarcocystis* (Woldemeskel and Gebreab 1996).

In Fars Province of Iran, 786 (57.7%) of 1,362 animals examined were positive for *Sarcocystis*. The prevalence was significantly higher ($p < 0.05$) in animals owned by nomadic Assyrians (67.95%) than in those owned by local people (41.86%). Animals older than 2 years of age (69.98%) were more infected compared to younger animals (30.02%). *Sarcocystis gigantea* was predominantly identified in

the esophagus; *S. medusiformis* mainly in the diaphragm; *S. tenella* in the esophagus, diaphragm, tongue, and heart; and *S. arieticanis* in the esophagus, in the tongue, and occasionally in the diaphragm (Oryan et al. 1996).

In Japan, *Sarcocystis suihominis* was detected for the first time in the heart and diaphragm of 5 out of 600 older culled breeding pigs slaughtered in the Saitama Prefecture (Saito et al. 1998).

A survey was carried out to investigate the occurrence of *Sarcocystis* infection in the loin of 482 Japanese and imported beef. The prevalence of *Sarcocystis* was lower in Japanese beef (total 6.31%: 0% in Holstein castrated, 12.96% in Holstein milk cow, 3.33% in Japanese shorthorn, and 11.58% in Japanese black cattle) than in beef imported from America (36.78%) or Australia (29.49%). All detected cysts except one were identified as *Sarcocystis cruzi.* One thick-walled cyst was found in Australian beef, but it could not be distinguished between *Sarcocystis hirsuta* and *Sarcocystis hominis* (Ono and Ohsumi 1999).

In the Upper East Region (UER) of Ghana, a cross-sectional study was carried out to estimate the prevalence of parasitic infections in local crossbred pigs. Ten out of sixty villages with a population of 200–1,000 inhabitants were randomly selected for the study. The number of pigs varied from 50 to 200 pigs per village. *Sarcocystis* spp. were observed in 28.3% animals (Permin et al. 1999).

A survey of *Sarcocystis* infection was conducted in Mongolia between June 1998 and July 1999. The prevalence of infection was: cattle 90.0% (27/30), yak 93.3% (28/30), hainag 100% (30/30), sheep 96.9% (753/777), horses 75% (3/4), and camels 100% (5/5). The heart was most commonly infected in cattle (100%), yak (86.7%), and hainag (100%); the tongue was most commonly infected in sheep (100%) and horses (100%) (Fukuyo et al. 2002).

Domestic animals have also been reported harboring *Sarcocystis*. In Rio de Janeiro, Brazil, 0.8% of fecal samples had *Sarcocystis* sp. in a survey of 131 domesticated and stray cats (Serra et al. 2003).

#### 4.3.3.2 Human Infections

Reports of human infections with *Sarcocystis* are limited. Most of them are from Asia. In Thailand, six patients aged 3–70 years presented with acute enteritis associated with *Sarcocystis* infection (Bunyaratvej et al. 1982).

In Tibet, fecal specimens of 926 persons from Duilongdeqing, Milin, and Linzhi counties were examined. The prevalences of *Sarcocystis hominis* in the three counties were 20.5%, 22.5%, and 22.9%, respectively, with an average of 21.8%. *Sarcocystis suihominis* prevalence was 0, 0.6%, and 7.0%, respectively. No significant difference in infection rate was found between different age or sex groups. *Sarcocystis* was detected in 42.9% of beef specimens from markets. The infected cases were generally asymptomatic (Yu 1991).

In Malaysia, *Sarcocystis* cysts have been reported from domestic and wild animals, including domestic and field rats, moonrats, bandicoots, slow lorises, buffalo, and monkeys. The overall seroprevalence in humans was 19.8% among the main racial groups in Malaysia (Kan and Pathmanathan 1991).

*Sarcocystis* spp. were identified in 14 of 1228 Vietnamese individuals (1.1%) who came to Central Slovakia in the course of 18 months in 1987–1989. The subjects were from the northeastern part of the country from Hanoi-Haiphong areas (Straka et al. 1991).

Three cases of muscular sarcocystosis from West Malaysia were reported. Eight of eleven cases were associated with malignancies, especially of the tongue and nasopharynx (Pathmanathan and Kan 1992).

In earlier studies the prevalence of muscle sarcocystosis in Malaysia was determined by examination of tongue tissues from autopsies of subjects aged 12 years or more. Of 100 tongues examined, 21% were found to contain *Sarcocystis*. The number of cysts per case varied from 1 to 13. The age range of positive cases was from 16 to 57 years (mean 37.7 years). The prevalence did not differ with regard to race, sex, or occupation (Wong and Pathmanathan 1992).

*Sarcocystis* sp. was identified in 23.2% of 362 Thai laborers who were going abroad for work. *Sarcocystis* was highly prevalent in male laborers (83.3%) ($p < 0.01$). The laborers from northeastern Thailand ($n = 278$) had a higher prevalence (26.6%) of *Sarcocystis* infection ($p < 0.01$) (Wilairatana et al. 1996).

Fifty samples of raw kibbe from 25 Arabian restaurants in the city of Sao Paulo, Brazil, were examined for the presence of *Sarcocystis*. Sarcocysts were found in all 50 samples. Based on cyst wall structure, *S. hominis* (94%), *S. hirsuta* (70%), and *S. cruzi* (92%) were identified. Most were found as mixed infections (Pena et al. 2001).

Infrequent tissue location has been reported. A patient from West Malaysia presented with *Sarcocystis* in the larynx (Kutty and Dissanaike 1975). *Sarcocystis* was identified in biopsy specimens from two adults in Singapore, one in Bombay, one in Uganda, and in the heart of a child in Costa Rica. Among the sarcocysts seen in 40 cases (35 old, 5 new), seven morphological types were recognized, each representing one to several different species, all of which are zoonotic. Of this group, 13 probably were acquired from Southeast Asia, 8 from India, 5 from Central or South America, 4 each from Africa and Europe, 3 from the USA, 1 from China, and 2 from unknown localities (Beaver et al. 1979; Gut 1982). Symptoms associated with these infections included muscle soreness or weakness, subcutaneous swellings, eosinophilia, and periarteritis or polyarteritis nodosa.

Nonetheless, there is no conclusive evidence of pathogenicity of the mature sarcocysts (Beaver et al. 1979). Molecular tools may help discriminate the species affecting humans and their distribution worldwide.

Occasionally, local infections may present complications due to large localized inflammatory reactions. Eight out of eleven presented with infections of the tongue and nasopharynx (Pathmanathan and Kan 1992). Potential association with vasculitis has also been proposed (McLeod et al. 1980).

The prevalence of intestinal sarcocystosis has been reported in various countries. Those with highest prevalence include Poland with 10.4% (125 fecal samples), Tibet (20–22.9% of 926 fecal samples), and Thailand (23.2% of 362 fecal samples). Muscular sarcocystosis is determined by serology or histology. Countries with highest prevalence include 19.7% (of 243 blood donors by serology) and 21% (by histology of 100 tongue tissues) in Malaysia, 44.4% in Yugoslavia (*Sarcocystis* positive by IFAT of 341 suspected to be *Toxoplasma* positive), and 34% (of 50 by ELISA) in Australia (Poulsen and Stensvold 2014).

In 2014, a study in northern Cambodia determined a 10% prevalence of muscular sarcocystosis (of 1081 individuals). Many *Sarcocystis* cases were associated with a wedding celebration and Chinese New Year festivities, where they had consumed raw or insufficiently cooked beef (Khieu et al. 2017). The largest and international outbreak of muscular sarcocystosis originated in Tioman Island, Malaysia, during 2011–2012. With the use of surveillance networks (GeoSentinel and TropNet), 62 probable and 6 confirmed cases of muscular sarcocystosis were identified. Most of the patients who were from Europe who had returned from Tioman Island complained of myalgia, fatigue, fever, and headache. In only one case, *Sarcocystis nesbitti* DNA was isolated from a muscle biopsy (Esposito et al. 2014).

Fecal samples from immunodeficient patients in Shiraz, Iran were examined for the presence of enteric protozoan oocysts. Sarcocystis-like sporocysts were detected in the stools of an AIDS patient and confirmed to be *Sarcocystis cruzi* using molecular tools (Agholi et al. 2016).

### *4.3.4 Diagnosis*

Diagnosis of intestinal sarcocystosis is done by identifying oocysts or sporocysts in the feces of infected individuals who can present with enteritis. Sporocysts measure 10x15 μm and can be observed by phase-contrast microscopy. It can also be observed using an epifluorescence microscope as oocysts and sporocysts autofluoresce (Fig. 4.2). Low number of sporocysts can be present in the stool samples; thus, flotation or sedimentation methods can concentrate and facilitate detection of the parasites. Diagnosis of extraintestinal sarcocystosis is by examination of biopsy tissues. This method can give a definite diagnosis for sarcocystosis; however, parasites are diffusely distributed and may not be present in the samples collected (Fig. 4.3). Myositis can be indicative of infection but is not specific for sarcocystosis. Mehrotra performed microscopic examination of excised tissues of cases that presented with lumps, pain in the limbs, or a discharging sinus and showed characteristic cysts of *Sarcocystis* (Mehrotra et al. 1996). Patients can also present with dyspnea on excertion and chest pain suggesting the involvement of *Sarcocystis* in the heart (Harris et al. 2015).

*Sarcocystis hirsuta* isolates from cattle, *Sarcocystis hominis*-like isolates, and a *Sarcocystis cruzi* isolate can be detected by PCR amplification of the 18S rDNA. These species could be fast and easily differentiated by sequencing of the amplified products and PCR-RFLP (Fischer and Odening 1998; Yang et al. 2002).

An IFA test (indirect fluorescent antibody test) for the diagnosis of sarcocystosis in the intermediate host was identified in mice inoculated experimentally with *Sarcocystis dispersa*; however, cross-reaction was observed with *Frenkelia* (Cerna and Kolarova 1978). Habeeb studied the humoral response in patients with idiopathic cardiac diseases and rheumatic diseases. The enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody technique (IFAT) using *Sarcocystis fusiformis* antigen could specifically identify cases with *Sarcocystis* and idiopathic cardiac diseases (Cerna and Kolarova 1978; Habeeb et al. 1996).

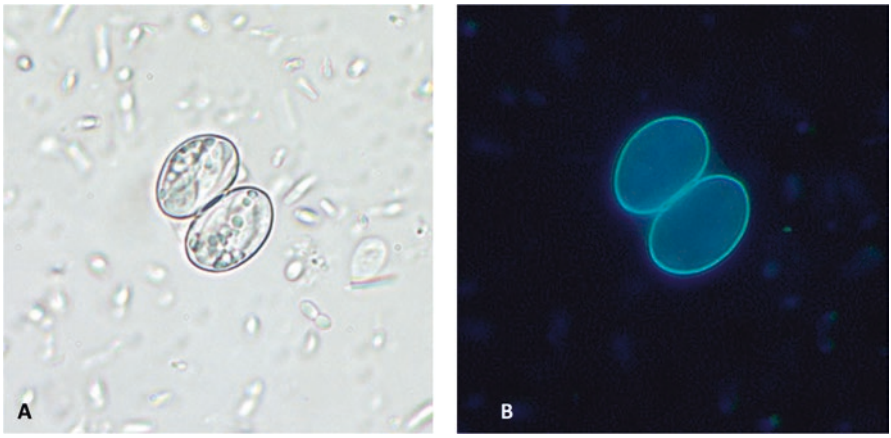


**Fig. 4.2** *Sarcocystis* spp. (**A**) DIC of sporulated oocyst, (**B**) autofluorescence of sporulated oocyst. Image courtesy of CDC-DPDx (https://www.cdc.gov/dpdx/sarcocystosis/index.html)

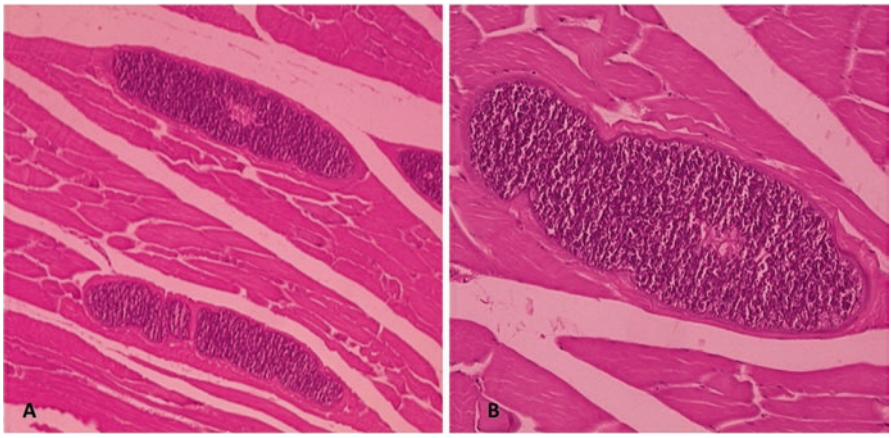


**Fig. 4.3** *Sarcocystis* spp. in muscle tissue. (**A**) Section of muscle containing two sporocysts, (**B**) sporocyst containing multiple bradizoites

### *4.3.5 Treatment and Control*

No therapeutic treatment is available for intestinal sarcocystosis in animals or humans. *Sarcocystis* infections in humans treated with acetylspiramycin for 15 days stopped excreting oocyst after 30 days post-treatment (Fayer et al. 2015). Al benda-zole and trimetoprim sulfamethoxazole are considered alternative therapies. Corticosteriods and non steroidal anti-inflamatory medication may aleviate symp-toms (Harris et al. 2015).

An outbreak of muscular sarcocystosis was reported in a USA military team in rural Malaysia. Seven of fifteen members developed acute illness consisting of myalgia, bronchospasm, fleeting pruritic rashes, transient lymphadenopathy, and subcutaneous nodules associated with eosinophilia, elevated erythrocyte sedimentation rate, and elevated levels of muscle creatine kinase. Diagnosis was confirmed by biopsy. Health in one patient improved with albendazole treatment, but symptoms lasted for more than 5 years. The other soldiers had a self-limiting illness, and one was asymptomatic (Arness et al. 1999).

*Sarcocystis* infections can be prevented by eating well-cooked meats, particularly game meats and pork (Yu 1991) and drinking and preparing foods with filtered water.

## 4.4 Conclusion

Consumption of contaminated fresh produce or drinking contaminated water can result in infections with *Cystoisospora* and *Sarcocystis* infection. Additionally, the consumption of raw or undercooked infected meats can also result in sarcocystosis. Safe eating practices, like boiling water and cooking or freezing meats can reduce the chance of infection. Much is to be learned on *Sarcocystis* and the host-parasite interactions. Very few of the cases of sarcocystosis have been confirmed by finding the parasite in muscle biopsies. Molecular testing cannot always detect infected patients as parasites may not be distributed homogeneously in muscle tissues. Therefore, serological assays are used to confirm exposure. Better tools need to develop to isolate, detect, and characterize the *Sarcocystis* infecting humans. Thanks to new molecular tools available to differentiate *Sarcocystis* species in the coming years, we will be able to have a better appreciation of the parasite distribution worldwide.

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# Chapter 5
# *Cryptosporidium* and Cryptosporidiosis

**Lihua Xiao and Vitaliano A. Cama**

## 5.1 Introduction

*Cryptosporidium* spp. are apicomplexan parasites that inhabit the brush borders of the gastrointestinal and respiratory epithelium. Initially thought to be only a pathogen of young animals such as calves and lambs, cryptosporidiosis is now known as an important cause of diarrhea, enterocolitis, and cholangiopathy in humans (Checkley et al. 2015). Healthy children and adults and young animals with cryptosporidiosis usually have a short-term illness accompanied by watery diarrhea, malabsorption, and weight loss. In humans and animals with immunodeficiencies and snakes, however, the infection can be protracted and life-threatening (Chalmers and Davies 2010).

*Cryptosporidium* oocysts are environmentally resistant; retain their infectious potential for considerable time in moist environments, such as water, soil, and fresh seafood; and produce (Reinoso et al. 2008) and survive some common drinking water and wastewater disinfection treatments (Castro-Hermida et al. 2008; Keegan et al. 2007; Kitajima et al. 2014; Nasser 2016). Two important fecal-oral transmission routes include direct contact with infected persons (person-to-person or anthroponotic transmission) or animals (zoonotic transmission) and consumption of contaminated water (waterborne transmission) or food (foodborne transmission) (Xiao 2010). Thus, *Cryptosporidium* spp. are well-recognized waterborne, foodborne, and zoonotic pathogens, having caused many cryptosporidiosis outbreaks in the United States and other industrialized countries (Aberg et al. 2015; Cope et al. 2015; De et al. 2016; Gertler et al. 2015; Goni et al. 2015; Hlavsa et al. 2015; McKerr et al. 2015; Ng-Hublin et al. 2015; Rosenthal et al. 2015; Stensvold et al. 2015b; Utsi et al. 2015; Webb et al. 2014; Widerstrom et al. 2014). Water and

L. Xiao (✉) • V.A. Cama
Division of Foodborne, Waterborne and Environmental Diseases, and Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA 30329, USA
e-mail: lax0@cdc.gov



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food probably also play an important role in the transmission of cryptosporidiosis in endemic areas, even though the disease burden attributable to them is not fully clear (Sarkar et al. 2013).

## 5.2 Taxonomy

*Cryptosporidium* spp. belong to the family Cryptosporidiidae, which is a member of the phylum Apicomplexa. Cryptosporidiidae is a basal member of Apicomplexa and is considered to be mostly related to gregarines (Clode et al. 2015). Extracellular gregarine-like reproductive stages have been described in *C. andersoni* and *C. parvum* (Ryan and Hijjawi 2015). Thus, *Cryptosporidium* spp. are no longer considered coccidian parasites.

*Cryptosporidium* spp. were first recognized by Tyzzer in 1907, who described *Cryptosporidium muris* in the stomach of laboratory mice (Tyzzer 1907, 1910). Shortly after, Tyzzer described a second species in laboratory mice, *C. parvum* (Tyzzer 1912). This new species differed from *C. muris* not only by infecting the small intestine instead of the stomach but also by having smaller oocysts, the environmentally robust stage of the parasite. Several *Cryptosporidium* species have been named over the next 80 years, but uncertainties have been associated with some of them because of morphologic and biologic similarities among species. It was not until the mid-1990s that the status of some of the described species has been validated by genetic characterization of isolates, and numerous new species have been described since (Ryan et al. 2014; Xiao et al. 2004a).

Currently, there are 30 established *Cryptosporidium* species, including *C. hominis* and *C. viatorum* in humans; *C. parvum* in ruminants and humans; *C. andersoni*, *C. bovis*, and *C. ryanae* in cattle and other bovine animals; *C. xiaoi* in sheep; *C. suis* and *C. scrofarum* in pigs; *C. ubiquitum* in ruminants, rodents, and primates; *C. canis* in dogs; *C. felis* in cats; *C. erinacei* in horses and hedgehogs; *C. cuniculus* in rabbits; *C. muris*, *C. tyzzeri*, and *C. proliferans* in rodents; *C. wrairi* in guinea pigs; *C. rubeyi* in squirrels; *C. fayeri* and *C. macropodum* in marsupials; *C. meleagridis*, *C. baileyi*, *C. galli*, and *C. avium* in birds; *C. varanii* and *C. serpentis* in reptiles; *C. fragile* in amphibians; and *C. molnari* and *C. huwi* in fish (Table 5.1). Most of these species have some degree of host specificity. There are also >50 host-adapted *Cryptosporidium* genotypes that do not yet have species names, such as horse, hamster, ferret, skunk, mink, squirrel, bear, deer, fox, mongoose, panda, wildebeest, seal, duck, woodcock, muskrat I and II, opossum I and II, chipmunk I to III, rat I to V, deer mouse I to IV, avian I to IV, goose I–IV, snake I and II, tortoise I and II, and piscine II to VIII. These species/genotypes biologically, morphologically, and phylogenetically belong to three groups: intestinal, gastric, and piscine species/genotypes (Ryan et al. 2014; Xiao and Feng 2008). Oocysts of intestinal and piscine species are generally spherical and 4–6 μm in size. In contrast, oocysts of gastric species are more elongated and 6–9 μm in size. Only some of these *Cryptosporidium* species and genotypes have been found in humans (Table 5.1).

**Table 5.1** Size and shape index of established *Cryptosporidium* species

| Species | Major host | Human pathogen[a] | Size (μm) L × W | Shape index | Reference |
|---|---|---|---|---|---|
| Gastric species | | | | | |
| *C. andersoni* | Cattle | Yes (+) | 7.4 ± 0.1 × 5.5 ± 0.1 (6.0–8.1 × 5.0–6.5 ($n$ = 50) | 1.35 (1.07–1.50) | Lindsay et al. (2000) |
| *C. muris* | Rodents | Yes (++) | 6.1 ± 0.3 × 8.4 ± 0.3 (5.6–6.4 × 8.0–9.0) ($n$ = 25) | 1.38 (1.25–1.61) | Palmer et al. (2003) |
| *C. proliferans* | Rodents | No | 7.7 (6.8–8.8) × 5.3 (4.8–6.2) ($n$ = 100) | 1.48 | Kvac et al. (2016) |
| Intestinal species | | | | | |
| *C. avium* | Birds | No | 6.26 (5.30–6.90) × 4.86 (4.30–5.50) ($n$ = 100) | 1.29 (1.14–1.47) | Holubova et al. (2016) |
| *C. baileyi* | Birds | No | 6.2 × 4.6 (5.6–6.3 × 4.5–4.8) ($n$ = 25) | 1.4 (1.2–1.4) | Current et al. (1986) |
| *C. bovis* | Cattle | Yes (+) | 4.89 (4.76–5.35) × 4.63 (4.17–4.76) ($n$ = 50) | 1.06 | Fayer et al. (2005) |
| *C. canis* | Dogs | Yes (++) | 4.95 (3.68–5.88) × 4.71(3.68–5.88) ($n$ = 50) | 1.05 (1.04–1.06) | Fayer et al. (2001) |
| *C. cuniculus* | Rabbits | Yes (++) | 5.98 (5.55–6.40) × 5.38 (5.02–5.92) ($n$ = 50) | 1.1 | Robinson et al. (2010a) |
| *C. erinaci* | Hedgehogs, horses | Yes (+) | 4.9 (4.5–5.8) × 4.4 (4.0–4.8) ($n$ = 100) | 1.13 (1.02–1.35) | Kvac et al. (2014a) |
| *C. fayeri* | Marsupials | Yes (+) | 4.9 (4.5–5.1) × 4.3 (3.8–5.0) ($n$ = 50) | 1.14 (1.02–1.18) | Ryan et al. (2008) |
| *C. felis* | Cats | Yes (++) | 4.6 (3.2–5.1) × 4.0 (3.0–4.0) ($n$ = 40?) | 1.1 (1.0–1.2) | Sreter et al. (2000) |
| *C. fragile* | Toads | No | 6.2 (5.5–7.0) × 5.5 (5.0–6.5) ($n$ = 50) | 1.13 (1.00–1.30) | Jirku et al. (2008) |
| *C. galli* | Birds | No | 8.25 (8.0–8.5) × 6.3 (6.2–6.4) ($n$ = 50) | 1.30 | Ryan et al. (2003) |
| *C. hominis* | Humans | Yes (+++) | 5.2 (4.4–5.9) × 4.9 (4.4–5.4) ($n$ = 100). | 1.07 (1.0–1.09) | Morgan-Ryan et al. (2002) |
| *C. macropodum* | Marsupials | No | 5.4 (5.0–6.0) × 4.9 (4.5–6.0) ($n$ = 50) | 1.1 | Power and Ryan (2008) |
| *C. meleagridis* | Birds | Yes (++) | 5.0 (4.5–6.0) × 4.4 (4.2–5.3) | 1.1 (1.0–1.3) | Sreter et al. (2000) |
| *C. parvum* | Cattle, sheep, goats, horses, humans | Yes (+++) | 5.0 (4.5–5.4) × 4.5 (4.2–5.0) ($n$ = 50) | 1.1 (1.0–1.3) | Upton and Current (1985) |
| *C. rubeyi* | Squirrels | No | 4.67 (4.4–5.0) × 4.34 (4.0–5.0) ($n$ = 220) | 1.08 | Li et al. (2015b) |
| *C. ryanae* | Cattle | No | 3.73 (2.94–4.41) × 3.16 (2.94–3.68) ($n$ = 0) | 1.18 | Fayer et al. (2008) |
| *C. scrofarum* | Pigs | Yes (+) | 5.16 (4.81–5.96) × 4.83 (4.23–5.29) ($n$ = 400) | 1.07 ± 0.06 | Kvac et al. (2013) |

(continued)

**Table 5.1** (continued)

| Species | Major host | Human pathogen[a] | Size (μm) L × W | Shape index | Reference |
|---|---|---|---|---|---|
| *C. serpentis* | Snakes | No | 5.94 (5.82–6.06) × 5.11(5.03–5.19) ($n$ = 37) | 1.17 (1.14–1.20) | Xiao et al. (2004b) |
| *C. suis* | Pigs | Yes (+) | 4.6 (4.4–4.9) × 4.2 (4.0–4.3) ($n$ = 50) | 1.1 | Ryan et al. (2004) |
| *C. tyzzeri* | Mice | Yes (+) | 4.64 ± 0.05 × 4.19 ± 0.06 ($n$ = 69) | 1.11 ± 0.02 | Ren et al. (2012) |
| *C. ubiquitum* | Sheep, deer, rodents, primates | Yes (++) | 5.04 (4.71–5.32) × 4.66 (4.33–4.98) ($n$ = 50) | 1.08 | Fayer et al. (2010) |
| *C. varanii* | Lizards | No | 4.94 (4.81–5.07) × 4.49 (4.35–4.63) ($n$ = 20) | 1.14 (1.11–1.17) | Xiao et al. (2004b) |
| *C. viatorum* | Humans | Yes (++) | 5.35 (4.87–5.87) × 4.72 (4.15–5.20) ($n$ = 50) | 1.14 (1.03–1.32) | Elwin et al. (2012b) |
| *C. wrairi* | Guinea pigs | No | 5.4 (4.8–5.6) × 4.6 (4.0–5.0) | 1.17 (1.04–1.33) | Tilley et al. (1991) |
| *C. xiaoi* | Sheep | Yes (+) | 3.94 (2.94–4.41) × 3.44 (2.94–4.41) ($n$ = 25). | 1.15 | Fayer and Santin (2009) |
| Piscine species | | | | | |
| *C. huwi* | Fish | No | 4.6 (4.4–4.9) × 4.4 (4.0–4.8) ($n$ = 50) | 1.04 (0.92–1.35) | Ryan et al. (2015) |
| *C. molnari* | Fish | No | 4.72 (3.23–5.45) × 4.47 (3.02–5.04) ($n$ = 22) | 1.05 (1.0–1.17) | Alvarez-Pellitero and Sitja-Bobadilla (2002) |

[a]Note: +++, major human pathogen; ++, relatively common in humans; +, confirmed infections have been detected in less than a handful patients

## 5.3 Biology

*Cryptosporidium* spp. are intracellular parasites that primarily infect epithelial cells of the stomach or intestine. The infection site varies according to the parasite species, but almost the entire development of *Cryptosporidium* spp. occurs between the two lipoprotein layers of the membrane of the epithelial cells, with the exception in *C. molnari* and *C. huwi*, for which oogonial and sporogonial stages are located deeply within the epithelial cells (Alvarez-Pellitero et al. 2004; Ren et al. 2012; Ryan et al. 2015). *Cryptosporidium* infections in humans or other susceptible hosts start with the ingestion of viable oocysts, the infectious stage that is environmentally resistant. Upon gastric and duodenal digestions, four sporozoites are liberated from each excysted oocyst, invade the epithelial cells, and develop to trophozoites surrounded by a parasitophorous vacuole. Within the epithelial cells, trophozoites undergo several generations of asexual amplification called merogony, leading to the formation of different types of meronts. The types of meronts depend on *Cryptosporidium* species. For *C. parvum*, there are two types of meronts. The type 1 meront develops six to eight nuclei, giving rise to six to eight merozoites. These stages are morphologically similar to sporozoites and can infect neighboring epithelial cells, forming more type 1 meronts or the new type 2 meronts. The latter develop four nuclei, forming four merozoites. As with type 1 merozoites, these merozoites are released and infect new cells to generate more type 2 meronts or can differentiate into sexually distinct stages called macro- and microgametocytes in a process called gametogony. New oocysts are formed in the epithelial cells from the fusion of a macro- and a microgametocyte. The new fused cell evolves and sporulates in situ in a process called sporogony, becoming oocysts containing four sporozoites. It is believed by some that about 20% of the oocysts produced are "thin walled" and may excyst within the digestive tract of the host, leading to the infection of new cells (autoinfection). The remaining 80% of oocysts are excreted into the environment; are resistant to low temperature, high salinity, and most disinfectants; and can initiate infection in a new host upon ingestion. Thus, the only extracellular stages in the life cycle of *Cryptosporidium* are released sporozoites, merozoites, and microgametes, which are very briefly in the lumen of the digestive tract (Fayer 2008). However, recently, a gregarine-like stage has been described in *C. andersoni* and *C. parvum*, which undergo multiplication through syzygy, a sexual reproduction process involving the end-to-end fusion of two or more parasites (Ryan and Hijjawi 2015).

Like other members of the Apicomplexa, sporozoites and merozoites of *Cryptosporidium* use the apical complex structure for cell invasion. Unlike other apicomplexan parasites, *Cryptosporidium* spp. have no polar rings and the conoid as part of the apical complex, only a relict mitochondrion, no plastids, and no flagelles in microgametes. At the contact site between host cells and *Cryptosporidium* developmental stages, there is also a unique electron-dense attachment or feeder organelle, which is supposedly involved in selective transport of nutrients from host cells into developing parasites (Ren et al. 2012). The prepatent period (time from

ingestion of infective oocysts to the completion of endogenous development and excretion of new oocysts) varies with species, hosts, and infection doses. This is usually between 4 and 14 days (Fayer 2008).

The genomes of three *Cryptosporidium* species have been sequenced using the traditional Sanger sequencing technology, including *C. parvum*, *C. hominis*, and *C. muris* (Abrahamsen et al. 2004; Xu et al. 2004). With the recent development in next-generation sequencing technologies, several other *Cryptosporidium* species and genotypes such as *C. andersoni*, *C. baileyi*, *C. meleagridis*, *C. tyzzeri*, *C. ubiquitum*, and chipmunk genotype I and the host-adapted *C. parvum* IIc subtype family have been sequenced using 454 and Illumina technologies (Guo et al. 2015a; Guo et al. 2015b; Li et al. 2014; Widmer et al. 2012), and data of them are available in CryptoDB (http://cryptodb.org/cryptodb/). *Cryptosporidium* genomes are around 9.1 Mb in eight chromosomes. They have 30% G + C content and are much smaller than genomes of *Plasmodium falciparum* (22.8 Mb), *Toxoplasma gondii* (63 Mb), and *Ascogregarina taiwanensis* (>20 Mb) but slightly larger than those of *Theileria* spp. (8.3 Mb) (Table 5.2). The compact *C. parvum* genome is largely a result of the loss of many metabolic pathways, such as major de novo biosynthesis for amino acids, nucleotides, and fatty acids, as well as the small intergenic regions and the rarity of introns (the only gene with confirmed introns is the β-tubulin gene) (Abrahamsen et al. 2004). Additionally, the parasite has only a single copy of most genes. Because of the lack of de novo biosynthesis capacities, *C. parvum* has an array of expanded families of transporters to acquire nutrients from the host, including at least 11 transporters for amino acids, 20 for sugars, and 19 annotated ATP-binding cassettes (ABCs) for transporting various metabolites, lipids/sterols, and drugs (Zhu and Xiao 2011). These transporters, especially ABC transporters, are probably under selection pressure, as they are among the most polymorphic genes in the *C. parvum* genome (Widmer et al. 2012). There is an apparent difference in energy metabolism between gastric and intestinal *Cryptosporidium* species, with the latter having no Krebs cycle and the conventional cytochrome-based respiratory chain (Mogi and Kita 2010).

The genetic basis for differences in host specificity among *Cryptosporidium* spp. is not clear. Comparative genomic analysis has shown that *C. parvum* and *C. hominis* genomes are highly similar, with ~97% nucleotide sequence identity and complete synteny in gene organization. This indicates that differences in phenotypic characteristics between the two species, such as host range and virulence, may be caused by subtle sequence variations in certain coding regions or differences in expression levels of key genes rather than genome rearrangements and structural alterations (Mazurie et al. 2013). Recently, it has been shown that *C. parvum* and *C. hominis* differ from each other in the composition of ten genes, with *C. parvum* having nine species-specific genes and *C. hominis* having one species-specific gene (Table 5.3). It was suggested that sub-telomeric gene duplications and deletions in two gene families (MEDLE and insulinase-like proteins) in chromosomes 5 and 6 could be responsible for some of the observed biologic differences between the two species (Guo et al. 2015c). Recently, a genetic manipulation tool has been developed for *C. parvum* based on the new CRISPR/Cas9 technology, which should greatly facilitate functional and genetic studies of *Cryptosporidium* spp.(Vinayak et al. 2015).

**Table 5.2** Genomic features of *Cryptosporidium* spp. in comparison with other apicomplexan parasites

| | *P. falciparum* | *T. gondii* | *C. parvum* | *C. ubiquitum* | *C. andersoni* |
|---|---|---|---|---|---|
| Total length (Mb) | 22.85 | 65.67 | 9.10 | 8.97 | 9.09 |
| No. of super contigs | 16 | 2263 | 8 | 39 | 135 |
| GC content (%) | 20.0 | 48.5 | 30.3 | 30.8 | 28.5 |
| No. of genes | 5542 | 8322 | 3805 | 3767 | 3905 |
| Total length of CDS (Mb) | 12.58 | 20.03 | 6.83 | 6.94 | 6.86 |
| GC content in CDS (%) | 25.0 | 56.0 | 31.9 | 33.0 | 30.1 |
| GC content at 3rd position in codons (%) | 18.0 | 59.0 | 18.0 | 20.0 | 14.0 |
| Mean length of genes (bp) | 2271 | 2407 | 1720 | 1841 | 1757 |
| Gene density (gene/Mb) | 242.5 | 126.7 | 418.1 | 420.0 | 429.6 |
| Percent coding (%) | 55.1 | 30.5 | 75.0 | 77.4 | 75.5 |
| No. of genes with intron | 3055 | 6729 | 163 | 758 | 832 |
| Genes with intron (%) | 55.1 | 80.9 | 4.2 | 20.1 | 21.3 |
| No. of tRNA | 72 | 174 | 45 | 45 | 44 |
| No. of $tRNA^{met}$ | 2 | 8 | 2 | 2 | 2 |
| Proteins with signal peptide | 638 | 759 | 397 | 399 | 309 |
| Proteins with transmembrane domain | 1754 | 1103 | 832 | 772 | 839 |
| Proteins with GPI anchor | 62 | 255 | 63 | 50 | 47 |

**Table 5.3** Major insertions in the genomes of *C. parvum* and *C. hominis* and the associated species-specific genes

| Chromosome | Length (bp) | Gene | Specificity |
|---|---|---|---|
| 8 | 19,048 | cgd8_680, cgd8_690 | *C. parvum* |
| 6 | 15,314 | cgd6_5480, cgd6_5490, cgd6_5510, cgd6_5520 | *C. parvum* |
| 5 | 5620 | cgd5_4580, cgd5_4590, cgd5_4610 | *C. parvum* |
| 3 | ~4800 | Chro. 50011 | *C. hominis* |

Notes:
1. cgd6_5480, cgd6_5490, cgd5_4580, cgd5_4590, cgd5_4610: genes for telomeric MEDLE family of secreted proteins
2. cgd6_5510, cgd6_5520: genes for telomeric insulinase-like proteases

Because of the existence of a sexual phase in *Cryptosporidium* life cycle, genetic recombination is possible between two related subtypes. Thus, new subtypes can emerge as a result of sexual recombination during concurrent infection of mixed populations, which was demonstrated by experimental infection of mice with different subtypes of *C. parvum* (Feng et al. 2002). Indeed, multilocus characterizations of field isolates have mostly identified a panmictic population structure of *C. parvum* in cattle in many areas (De Waele et al. 2013; Feng et al. 2013;

Herges et al. 2012; Mallon et al. 2003a; Ramo et al. 2016). In contrast, a clonal population genetic structure is more commonly seen in *C. hominis* and *C. meleagridis* (Ramo et al. 2015; Wang et al. 2014). Genetic recombination has been shown recently to a major driving force for the emergence of virulent and highly transmissible *C. hominis* and *C. parvum* subtypes (Feng et al. 2013, 2014; Guo et al. 2015c; Li et al. 2013). Geographically segregated subpopulations of *C. hominis* are often seen in developing countries where intercountry population exchange is limited (Gatei et al. 2008; Gatei et al. 2003). No significant segregation in parasite population is seen in *C. parvum* in animals in some studies (Herges et al. 2012; Ramo et al. 2016). A recent comparison of bovine isolates from Italy, Ireland, and Scotland, however, has shown the occurrence of different populations of *C. parvum* in each country (Caccio et al. 2015).

Host adaptation has been seen in *C. parvum* and *C. ubiquitum*, which infect several species of mammals, indicating the occurrence of genetic isolation within some parasite lineages. The presence of host-adapted *C. parvum* subtype families is well known at the 60 kDa glycoprotein (gp60, also known as gp15/45/60, gp40/15) locus, including IIa in cattle, IId in sheep and goats, and IIc and IIe in humans (Xiao 2010). The existence of these host-adapted *C. parvum* subpopulations has been confirmed by multilocus characterization of parasites in several European countries (Drumo et al. 2012; Grinberg et al. 2008; Leoni et al. 2007; Quilez et al. 2013). Host adaptation at the gp60 locus has recently been shown also to occur in *C. ubiquitum* in sheep and rodents (Li et al. 2014).

## 5.4 Epidemiology and Transmission

### *5.4.1* Cryptosporidium *spp. in Humans*

Currently, about 20 *Cryptosporidium* species and genotypes have been reported in humans, including *C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, *C. canis*, *C. ubiquitum*, *C. cuniculus*, *C. viatorum*, *C. muris*, *C. suis*, *C. andersoni*, *C. tyzzeri*, *C. fayeri*, *C. bovis*, *C. xiaoi*, *C. scrofarum*, *C. erinacei*, and *Cryptosporidium* horse, skunk genotypes, and chipmunk genotype I (Table 5.1). Humans are most frequently infected with *C. hominis* and *C. parvum*. The former infects almost exclusively humans and nonhuman primates, thus is transmitted anthroponotically, whereas the latter mostly infects humans, ruminants, and horses, thus is transmitted both anthroponotically and zoonotically (Fig. 5.1). Other species, such as *C. meleagridis*, *C. felis*, *C. canis*, *C. ubiquitum*, *C. cuniculus*, *C. viatorum*, *C. muris*, and chipmunk genotype I, are less common. The remaining species and genotypes have been only found in a few human cases (Elwin et al. 2012a, b; Guo et al. 2015a; Kvac et al. 2014b; Li et al. 2014; Nichols et al. 2006; Raskova et al. 2013; Stensvold et al. 2015a; Wang et al. 2014; Xiao 2010). These *Cryptosporidium* spp. infect both immunocompetent and immunocompromised

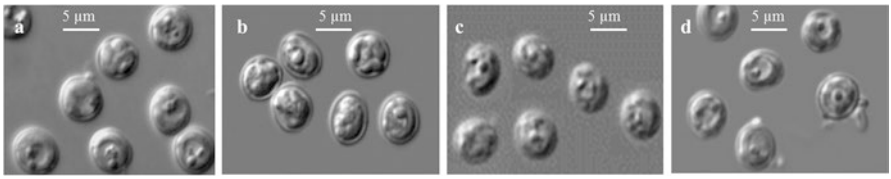


**Fig. 5.1** Differences in transmission of *Cryptosporidium hominis* (*left panel*) and *C. parvum* (*right panel*) (Image courtesy of DPDx (http://www.cdc.gov/dpdx/) from the Centers for Disease Control and Prevention)

persons. The distribution of these species in humans is different among geographic areas and socioeconomic conditions, with *C. meleagridis*, *C. canis*, and *C. felis* mostly seen in humans in developing countries, *C. ubiquitum* and chipmunk genotype I in industrialized nations, and *C. cuniculus* mostly in the United Kingdom. This is probably the results of differences in infection sources and transmission routes (Xiao 2010). *Cryptosporidium* species differ from each other in virulence and clinical presentations, with *C. hominis* more virulent and causing more severe symptoms than other species (Adamu et al. 2014; Cama et al. 2007, 2008; Dey et al. 2016).

### *5.4.2 Cryptosporidiosis in Immunocompetent Persons*

In developing countries, human *Cryptosporidium* infection occurs mostly in children younger than 5 years old, with peak occurrence of infections and diarrhea in children less than 2 years of age (Kotloff et al. 2013). Frequent symptoms include diarrhea, abdominal cramps, vomiting, headache, fatigue, and low-grade fever (Chalmers and Davies 2010). The diarrhea can be voluminous and watery but usually resolves within 1–2 weeks without treatment. Not all infected children have diarrhea or other gastrointestinal symptoms, and the occurrence of diarrhea in children with cryptosporidiosis can be as low as 30% in community-based studies (Bern et al. 2002; Sarkar et al. 2013; Xiao et al. 2001). Even subclinical cryptosporidiosis exerts a significant adverse effect on child growth, as infected children with no clinical symptoms experience growth faltering, both in weight and in height (Mondal et al. 2012; Opintan et al. 2010; Quihui-Cota et al. 2015; Sarkar et al. 2013; Yones et al. 2015). *Cryptosporidium*-infected children may never have enough catch-up growth covered for the growth retardation (Checkley et al. 2015). Children can have multiple episodes of cryptosporidiosis, implying that the anti-*Cryptosporidium* immunity in children acquired is short-lived or incomplete (Bern et al. 2002; Sarkar et al. 2013; Xiao et al. 2001). Cryptosporidiosis has been associated with increased child mortality in developing countries (Kotloff et al. 2013; Sarkar et al. 2014; Tumwine et al. 2003).

In developed countries, *Cryptosporidium* infection occurs later in life of children than in developing countries, probably due to later exposures to contaminated environment as a result of better hygiene (Network 2010; Nichols et al. 2006; Painter et al. 2015; Semenza and Nichols 2007; Stensvold et al. 2015b; Yoder et al. 2012). The peak occurrence of cryptosporidiosis in these countries is usually in children of 1–4 years in age. Children in these countries frequently acquire *Cryptosporidium* infection from another infected child attending the same day care or school, probably via person-to-person transmissions (Artieda et al. 2012; Davies et al. 2009; Johansen et al. 2015; Mateo et al. 2014). In industrialized nations, there is a second peak of cryptosporidiosis incidence in women of reproductive age, probably as a result of frequent exposure to infected children (Network 2010; Nichols et al. 2006; Painter et al. 2015; Stensvold et al. 2015b; Yoder et al. 2012). Cryptosporidiosis is also common in elderly attending nursing homes where person-to-person transmission probably also plays a major role in the spread of *Cryptosporidium* infections (Mor et al. 2009), and in recent years, there has been an increased incidence of cryptosporidiosis in the elderly in the United States (Painter et al. 2015). In the general population, a substantial number of adults are probably susceptible to *Cryptosporidium* infection, as sporadic infections occur in all age groups in the United States and United Kingdom, and traveling to developing countries and consumption of contaminated food or water can frequently lead to infection (Nichols et al. 2006; Roy et al. 2004; Yoder and Beach 2010). Unlike in developing countries, immunocompetent persons with sporadic cryptosporidiosis in industrialized nations usually have diarrhea (98%). Other common symptoms include abdominal pain (60–96%), vomiting (49–65%), low-grade fever (36–59%), and nausea (35%) (Chalmers and Davies 2010).

### *5.4.3 Cryptosporidiosis in Immunocompromised Persons*

Cryptosporidiosis is common in immunocompromised persons, such as AIDS patients, persons with primary immunodeficiency, and cancer and transplant patients undergoing through immunosuppressive therapy, and renal patients (Acikgoz et al. 2012; Hunter and Nichols 2002; Krause et al. 2012; Raja et al. 2014). It is frequently associated with chronic, life-threatening diarrhea (Hunter and Nichols 2002). In HIV+ persons, the occurrence of cryptosporidiosis increases as the CD4+ lymphocyte cell counts fall, especially below 200 cells/L (Ayinmode et al. 2014; Girma et al. 2014; Hunter and Nichols 2002; Rashmi and Ravi Kumar 2013). Symptoms of cryptosporidiosis in AIDS patients vary in severity, duration, and responses to drug treatment. Much of this variation can be explained by the degree of immunosuppression (Chalmers and Davies 2010). Sclerosing cholangitis and pulmonary involvement are common in HIV/AIDS patients with cryptosporidiosis (Netor Velasquez et al. 2012; Sullivan et al. 2013; Tali et al. 2011). Cryptosporidiosis in AIDS patients is associated with increased mortality and shortened survival (Bern et al. 2005).

### 5.4.4 *Infection Sources: Anthroponotic Versus Zoonotic Transmission*

In the United States and Europe, contact with persons with diarrhea has been identified as a major risk factor for sporadic cryptosporidiosis (Hunter et al. 2004; Pintar et al. 2009; Pollock et al. 2010; Roy et al. 2004; Valderrama et al. 2009). The importance of direct person-to-person transmission is exemplified by the high prevalence of cryptosporidiosis in childcare facilities, nursing homes, and mothers with young children in these countries. In most of these case-control studies, contact with farm animals (especially calves) is also a major risk factor for human cryptosporidiosis (Hunter et al. 2004; Lake et al. 2007; Pollock et al. 2010; Roy et al. 2004; Snel et al. 2009; Yoder and Beach 2010).

Differences in the distribution of *Cryptosporidium* genotypes in humans are a reflection of differences in infection sources (Chalmers et al. 2009; Elwin et al. 2012a; Learmonth et al. 2004; Snel et al. 2009; Xiao 2010). The occurrence of *C. hominis* in humans is most likely due to anthroponotic transmission, whereas *C. parvum* in a population can be the result of both anthroponotic and zoonotic transmissions. Thus far, studies conducted in developing countries have shown a predominance of *C. hominis* in children or HIV+ adults. This is also true for most areas in the United States, Canada, Australia, and Japan. In Europe and New Zealand, however, several studies have shown almost equal prevalence of *C. parvum* and *C. hominis* in both immunocompetent and immunocompromised persons (Xiao 2010). Thus, in most developing countries, anthroponotic transmission of *Cryptosporidium* plays a major role in human cryptosporidiosis, whereas in Europe, New Zealand, and rural areas of the United States, both anthroponotic and zoonotic transmissions are important. In Mideast countries, children are mostly infected with *C. parvum*, but the significance of this is not clear (Nazemalhosseini-Mojarad et al. 2012). One obvious exception is *Cryptosporidium* infections in AIDS patients in Addis Ababa, Ethiopia, where *C. parvum* is highly endemic and calf contact is a major risk factor for cryptosporidiosis occurrence (Adamu et al. 2014).

Sequence analyses of the gp60 gene have shown that *C. parvum* infections in humans in developing countries may mainly the result of anthroponotic transmission (Xiao 2010). Among several *C. parvum* subtype families identified in humans, IIa and IIc are the two most common families. The former has been identified in both humans and calves, thus can be a zoonotic pathogen, whereas the latter has been seen mainly in humans (Widmer 2009; Xiao 2010; Xiao and Feng 2008), thus is an anthroponotic pathogen. In developing countries, most *C. parvum* infections in children and HIV+ persons are caused by the subtype family IIc, with IIa largely absent, indicating that anthroponotic transmission of *C. parvum* is common in these areas (Xiao 2010; Xiao and Feng 2008). In contrast, IIa subtype families are commonly seen in humans in industrialized nations, where their occurrence in humans is often associated with calf contact (Hunter et al. 2007). Another *C. parvum* subtype family commonly found in sheep and goats, IId, is the dominant *C. parvum* subtype family in humans in Mideast countries (Nazemalhosseini-Mojarad et al. 2012) and is

occasionally found in humans in some European countries such as Sweden, where it is commonly seen in pre-weaned dairy calves (Insulander et al. 2013). Results of multilocus subtyping support the conclusions of gp60 subtyping studies (Grinberg et al. 2008; Mallon et al. 2003b).

### *5.4.5 Waterborne Transmission*

Epidemiologic studies have frequently identified water as a major route of *Cryptosporidium* transmission in industrialized nations. Numerous outbreaks of cryptosporidiosis have been associated with drinking water in these countries (Baldursson and Karanis 2011). Seasonal variations in the incidence of human *Cryptosporidium* infection in these countries have been partially attributed to waterborne transmission. Thus, in the United States and European countries, there is a late summer peak in sporadic cases of cryptosporidiosis (Painter et al. 2015; Semenza and Nichols 2007), which is largely due to recreational activities such as swimming and water sports (Hlavsa et al. 2011). In the United States and Canada, swimming in a lake or river was identified as a risk factor (Pintar et al. 2009; Roy et al. 2004).

The role of drinking water in sporadic *Cryptosporidium* infection is not clear. In England, there is an association between numbers of glasses of tap water drunk at home each day and the occurrence of sporadic cryptosporidiosis (Hunter et al. 2004). In the United States, drinking untreated surface water was identified as a risk factor for the acquisition of *Cryptosporidium* in a small case-control study (Gallaher et al. 1989), and residents living in cities with surface-derived drinking water generally have higher blood antibody levels against *Cryptosporidium* than those living in cities with groundwater as drinking water, indicating drinking water plays a role in the transmission of human cryptosporidiosis (Frost et al. 2003). Nevertheless, case-control studies conducted in the United States and Canada have failed to show a direct linkage of *Cryptosporidium* infection to drinking water (Khalakdina et al. 2003; Pintar et al. 2012; Sorvillo et al. 1994).

Numerous waterborne outbreaks of cryptosporidiosis have occurred in the United States, Canada, the United Kingdom, France, Australia, Japan, and other industrialized nations (Baldursson and Karanis 2011; Chalmers 2012; Hlavsa et al. 2011). These include outbreaks associated with both drinking water and recreational water (swimming pools and water parks). After the massive cryptosporidiosis outbreak in Milwaukee in 1993, several new drinking water regulations have been implemented in the United States, the United Kingdom, and other industrialized nations (Gostin et al. 2000; May 2006; USEPA 2006). As a result of more stringent treatment of source water, the number of drinking water-associated outbreaks is in decline in these countries, and most outbreaks in the United States are associated with recreational water (Hlavsa et al. 2011; Yoder and Beach 2010). Even though five *Cryptosporidium* species are commonly found in humans, *C. parvum* and *C. hominis* are responsible for most cryptosporidiosis outbreaks, with *C. hominis* responsible for more outbreaks

than *C. parvum* (Xiao 2010). This is even the case for the United Kingdom, where *C. parvum* and *C. hominis* are both common in the general population. Recently, there was one drinking water-associated cryptosporidiosis outbreak caused by *C. cuniculus* (Puleston et al. 2014). The importance of waterborne transmission in cryptosporidiosis epidemiology is probably smaller in developing countries (Sarkar et al. 2013).

### *5.4.6 Foodborne Transmission*

The importance of foodborne transmission in cryptosporidiosis epidemiology is less clear. *Cryptosporidium* oocysts have been isolated from several foodstuffs, and these have mainly been associated with fruits, vegetables, and shellfish (Table 5.4) (Amoros et al. 2010; Budu-Amoako et al. 2011; Duedu et al. 2014; Fayer et al. 2004; Giangaspero et al. 2009; Ranjbar-Bahadori et al. 2013; Robertson and Gjerde 2001; Rzezutka et al. 2010; Srisuphanunt et al. 2009). Direct contamination of food by fecal materials from animals or food handlers has been implicated in several foodborne outbreaks of cryptosporidiosis in industrialized nations. In most instances, human infections were acquired through consumption of contaminated fresh produce, unpasteurized apple cider, or milk (Aberg et al. 2015; Blackburn et al. 2006; Centers for Disease and Prevention 2011; Ethelberg et al. 2009; Gherasim et al. 2012; McKerr et al. 2015; Ponka et al. 2009; Rosenthal et al. 2015). Most of these outbreaks were caused by *C. parvum* IIa subtypes, except for several recent ones in Finland and Sweden, which were caused by *C. parvum* IId subtypes (Aberg et al. 2015; Gherasim et al. 2012). It is estimated that about 8% of *Cryptosporidium* infections in the United States are foodborne (Scallan et al. 2011).

## 5.5 Detection and Diagnosis

### *5.5.1 Methods for Detection of* Cryptosporidium *in Stool Specimens*

*Cryptosporidium* infections are usually diagnosed by analysis of stool specimens (Chalmers et al. 2015; Le Govic et al. 2016). Stool specimens are collected fresh or in fixative solutions such as 10% buffered formalin and polyvinyl alcohol (PVA). However, stool specimens fixed in formalin- and mercury-based preservatives (such as LV-PVA) cannot be used for PCR analysis, which requires the use of fresh or frozen stool specimens, stools kept in transport media such as Cary-Blair or Amies, or stools preserved in Total-Fix, zinc PVA, or 2.5% potassium dichromate. In clinical laboratories, *Cryptosporidium* spp. in stool specimens are commonly detected by microscopic examinations of oocysts or immunologic detection of antigens (Chalmers et al. 2015). PCR-based typing methods, however, are increasingly used in investigations of outbreaks, surveillance, and diagnosis of cryptosporidiosis (Xiao 2010).

**Table 5.4** Prevalence of *Cryptosporidium* in raw fruits, vegetables, and shellfish

| Food type | Country | Prevalence | Species | Reference |
|---|---|---|---|---|
| *Vegetables* | | | | |
| Vegetables | Ghana | Vegetables (cabbage, sweet bell pepper, carrot, lettuce, tomato, onion): 67/395 | | Duedu et al. (2014) |
| Vegetables | Canada | Ready to eat leafy greens: 32/544 | | Dixon et al. (2013) |
| Vegetables | Spain | Leafy greens: Chinese cabbage, 2/6; Lollo Rosso lettuce, 3/4; romaine lettuce, 7/9 | | Amoros et al. (2010) |
| Vegetables | Canada | Spinach: 1/324 | | Bohaychuk et al. (2009) |
| Vegetables | Costa Rica | Cilantro leaves: 4/80<br>Cilantro roots: 7/80<br>Lettuce: 2/80<br>Radish: 1/80<br>Carrot: 1/80<br>Tomato: 1/80<br>Cucumber: 1/80<br>Cabbage: 0/80 | | Monge and Arias (1996), Monge et al. (1996) |
| Vegetables | Peru | Vegetables (cabbage, celery, cilantro, green onion, ground green chili, leek, lettuce, parsley, yerba buena, huacatay): 28/172 | | Ortega et al. (1997) |
| Fruits and vegetables | Norway | Alfalfa: 0/16<br>Dill: 0/7<br>Lettuce: 5/125<br>Mung bean sprouts: 14/149<br>Mushrooms: 0/55<br>Parsley: 0/7<br>Precut salad: 0/38<br>Radish sprouts: 0/6<br>Raspberries: 0/10<br>Strawberries: 0/62 | | Robertson and Gjerde (2001), Robertson et al. (2002) |
| Sprout | Norway | | | |
| *Shellfish* | | | | |
| Clams | Spain and Italy | *Dosinia exoleta*, *Ruditapes philippinarum*, *Venerupis pullastra*, *Venerupis rhomboideus*, *Venus verrucosa*: 10/17 | | Freire-Santos et al. (2000) |
| | Spain | *Dosinia exoleta*, *Venerupis pullastra*, *Venerupis rhomboideus*, *Venus verrucosa*: 10/18 | *C. parvum* and *C. hominis* | Gomez-Couso et al. (2004) |

(continued)

**Table 5.4** (continued)

| | | | | |
|---|---|---|---|---|
| | Spain and EU countries | *Dosinia exoleta*, *Venerupis pullastra*, *Venerupis rhomboideus*, *Venus verrucosa*: 20/68 | | Gomez-Couso et al. (2003) |
| | Italy | *Chamelea gallina*: 2 of 16 pooled clams (30 clams/pool) | *C. parvum* | Traversa et al. (2004) |
| | Eastern United States and Canada | Clams: 3/375 (0.8) | | Fayer et al. (2003) |
| Cockles | Spain | *Cerastoderma edule*: positive/6 | *C. parvum* | Gomez-Bautista et al. (2000) |
| | Spain and EU countries | *Cerastoderma edule*: 5/24 | | Gomez-Couso et al. (2003) |
| Mussels | United States | *Mytilus* spp.: 7/26 | *C. parvum* | Staggs et al. (2015) |
| | United States | *Mytilus californianus*:0/961 | *C. parvum* | Adell et al. (2014) |
| | Thailand | *Perna viridis*: 7/56 | *C. parvum* | Srisuphanunt et al. (2009) |
| | Spain | *Mytilus galloprovincialis*: positive/180 | | (Gomez-Bautista et al. 2000) |
| | Spain | *Mytilus galloprovincialis*: 12/22 | *C. parvum* | Gomez-Couso et al. (2004) |
| | Spain | *Mytilus galloprovincialis*: 6/15 | | Freire-Santos et al. (2000) |
| | Spain and EU countries | *Mytilus galloprovincialis*:35/107 | | Gomez-Couso et al. (2003) |
| | Northern Ireland | *Mytilus edulis*: 2/16 | *C. hominis* | Lowery et al. (2001) |
| | Canada | Zebra mussel (*Dreissena polymorpha*): 32/32 pools (514 mussels total) | *C. hominis* | Graczyk et al. (2001) |
| | United States | Bent mussel (*Ischadium recurvum*): 14/16 | | Graczyk et al. (1999) |
| | Ireland | *Mytilus edulis*: 3/26 pools (10 mussels/pool) | | Chalmers et al. (1997) |
| Oysters | Chesapeake Bay, United States | *Crassostrea virginica*:142/360 | *C. parvum* and *C. hominis* | Fayer et al. (1998) |
| | Chesapeake Bay, United States | Commercial *Crassostrea virginica*:182/510 | *C. parvum* and *C. hominis* | Fayer et al. (1999) |
| | Chesapeake Bay, United States | *Crassostrea virginica*:331/1590 | *C. parvum* and *C. hominis* | Fayer et al. (2002) |

(continued)

**Table 5.4** (continued)

| Food type | Country | Prevalence | Species | Reference |
|---|---|---|---|---|
| | Eastern United States and Canada | *Crassostrea virginica*: 32/550 (5.8%) | *C. parvum*, *C. hominis*, *C. meleagridis* | Fayer et al. (2003) |
| | Spain | *Ostrea edulis*: 5/6 | | Freire-Santos et al. (2000) |
| | Spain | *Ostrea edulis*: 6/9 | *C. parvum* and *C. hominis* | Gomez-Couso et al. (2004) |
| | Spain and EU countries | *Ostrea edulis*: 23/42 | | Gomez-Couso et al. (2003) |

#### 5.5.1.1 Microscopy

Stool specimens can be examined for *Cryptosporidium* oocysts by microscopy of direct wet mount, after oocyst concentration by traditional ethyl acetate or Weber-modified ethyl acetate concentration methods (Smith 2008). Bright-field or differential interference contrast (DIC) microscopy of direct wet mounts is generally used. This allows the observation of oocyst morphology and more accurate measurement of oocysts (Fig. 5.2). Most *Cryptosporidium* species look similar under microscopes and have similar morphometric measurements, although *Cryptosporidium* oocysts in humans are generally 4–6 μm in size (Xiao et al. 2004a) (Table 5.1).

More often, *Cryptosporidium* oocysts in concentrated stool specimens are detected by microscopy after staining of the fecal smears. Many special stains have been used in the detection of *Cryptosporidium* oocysts, but modified acid-fast stains are the most commonly used (Smith 2008), especially in developing countries, because of their low cost and simultaneous detection of several other pathogens such as *Cystoisospora* and *Cyclospora*. Two stains widely used are the modified Ziehl-Neelsen acid-fast stain and modified Kinyoun's acid-fast stain (Smith 2008). Oocysts are stained bright red to purple against blue or green background (Fig. 5.3).

Direct immunofluorescence assays (DFA) are used increasingly in *Cryptosporidium* oocyst detection, especially in industrialized nations. Compared to acid-fast staining, DFA has higher sensitivity and specificity (Johnston et al. 2003). Many commercial DFA kits are marketed for the diagnosis of *Cryptosporidium*, most of which include reagents allowing simultaneous detection of *Giardia* cysts. Oocysts appear apple green against a dark background in immunofluorescence microscopy. It has been shown that most antibodies in commercial DFA kits react with oocysts of almost all *Cryptosporidium*, making species-specific diagnosis impossible (Yu et al. 2002).

#### 5.5.1.2 Antigen Detection by Immunoassays

Cryptosporidiosis can also be diagnosed by the detection of *Cryptosporidium* antigens in stool specimens by immunoassays (Chalmers et al. 2015). Antigen-capture-based enzyme immunoassays (EIAs) have been used in the diagnosis of cryptosporidiosis since 1990. In recent years, EIAs have gained popularity because

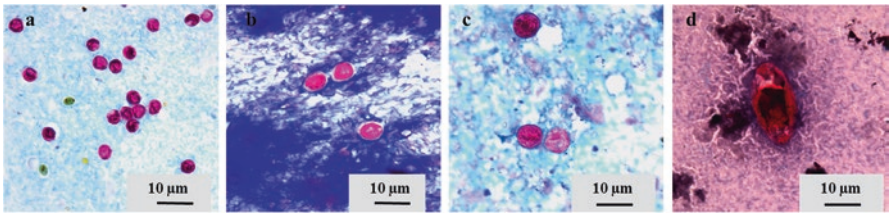


**Fig 5.2** Oocysts of *Cryptosporidium parvum* (**a**), *C. hominis* (**b**), *C. meleagridis* (**c**), and *C. suis* (**d**) under differential interference contrast microscopy

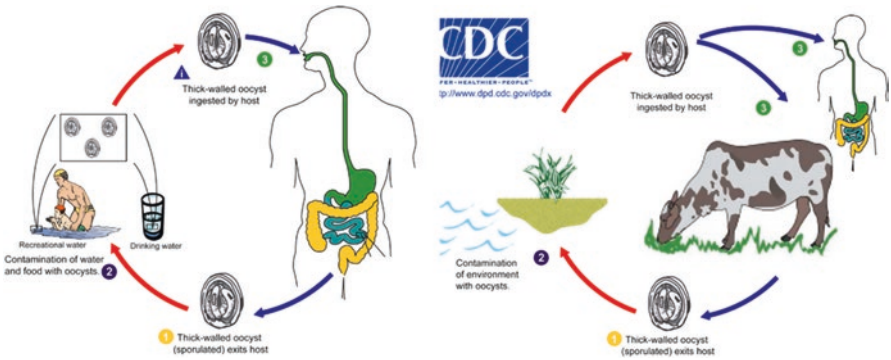


**Fig. 5.3** Acid-fast stained oocysts of *Cryptosporidium hominis* (**a**), *C. muris* (**b**), *Cystoisospora belli* (**c**), and *Cyclospora cayetanensis* (**d**) under bright-field microscopy

they do not require experienced microscopists and can be used to screen a large number of samples (Church et al. 2005). Several commercial EIA kits are commonly used in clinical laboratories. High specificity (99–100%) has been generally reported for these EIA kits (El-Moamly and El-Sweify 2012; Gaafar 2011; Sadaka et al. 2015). Sensitivities, however, have been reported to range from 70% (Johnston et al. 2003) to 94–100% (Bialek et al. 2002; Chalmers et al. 2011; Garcia and Shimizu 1997; Srijan et al. 2005). Most EIA kits have been evaluated only with human stool specimens presumably from patients infected with *C. hominis* or *C. parvum* (Chalmers et al. 2011). Their usefulness in the detection of *Cryptosporidium* spp. in animals may be compromised by the specificity of the antibodies.

In recent years, lateral flow immunochromatographic assays have gained popularity in rapid detection of *Cryptosporidium* in stool specimens. In evaluation studies, these assays have been shown to have high specificities (>90%) and sensitivity (98–100%) (Abdel Hameed et al. 2008; El-Moamly and El-Sweify 2012; Johnston et al. 2003; Regnath et al. 2006; Sadaka et al. 2015). However, sensitivities of 68–75% were shown in some studies for some assays (Agnamey et al. 2011; Goni et al. 2012; Johnston et al. 2003; Weitzel et al. 2006). High false-positive rates (positive predictive value = 56%) of several rapid assays have been recently reported in clinical diagnosis of cryptosporidiosis in the Unites States (Robinson et al. 2010b). This has prompted the Council of State and Territorial Epidemiologists to change the case definition of rapid assay-positive

cases from confirmed cases to probable cases. It has also been shown recently that some rapid assay kits have low sensitivity (<35%) in detecting some *Cryptosporidium* species other than *C. hominis* and *C. parvum* (Agnamey et al. 2011).

#### 5.5.1.3 Molecular Methods

Molecular techniques, especially PCR and PCR-related methods, have been developed and used in the detection and differentiation of *Cryptosporidium* spp. for many years. Several genus-specific PCR-RFLP-based genotyping tools have been developed for the detection and differentiation of *Cryptosporidium* at the species level (Amar et al. 2004; Coupe et al. 2005; Nichols et al. 2003; Sturbaum et al. 2001; Xiao et al. 1999). Most of these techniques are based on the small subunit (SSU) rRNA gene. Other genotyping techniques are designed mostly for the differentiation of *C. parvum* and *C. hominis*, thus cannot detect and differentiate other *Cryptosporidium* spp. or genotypes (Xiao 2010). Their usefulness in the analysis of human stool specimens is compromised by their inability to detect *C. canis*, *C. felis*, *C. suis*, *C. muris*, and other species/genotypes divergent from *C. parvum* and *C. hominis* (Jiang and Xiao 2003; Le Govic et al. 2016). In recent years, several FDA-approved multiplex commercial PCR assays are available for the detection of a broad range of enteric pathogens, including *Cryptosporidium* and *Giardia*, such as the xTAG® Gastrointestinal Pathogen Panel, BioFire FilmArray Gastrointestinal (GI) Panel, and BD Max Enteric Parasite Panel (Buss et al. 2015; Duong et al. 2016; Molling et al. 2016). Their use is expected to fundamentally change the diagnosis of cryptosporidiosis in clinical microbiology laboratories (van Lieshout and Roestenberg 2015).

Several subtyping tools have been developed to characterize the diversity within *C. parvum* or *C. hominis* (Xiao 2010). One of the most commonly used techniques is DNA sequence analysis of the gp60 gene (Alves et al. 2003; Feng et al. 2011a; Ryan et al. 2014; Widmer 2009). Most of the genetic heterogeneity in this gene is present in the number of a trinucleotide repeat (TCA, TCG, or TCT), although extensive sequence differences in non-repeat regions are also present between subtype families. One major advantage of this subtyping tool is the association of *C. parvum* and *C. ubiquitum* subtype families with host adaptation (Li et al. 2014; Widmer and Lee 2010) and *C. hominis* subtype families with virulence (Cama et al. 2008; Li et al. 2013). The usefulness of subtyping tools has been demonstrated by the analysis of samples from foodborne and waterborne outbreaks of cryptosporidiosis (Blackburn et al. 2006; Centers for Disease and Prevention, 2011; Chalmers et al. 2005, 2010; Feng et al. 2012, 2014; Fournet et al. 2013; Gherasim et al. 2012; Glaberman et al. 2002; Insulander et al. 2013; Leoni et al. 2003, 2007; Mayne et al. 2011; Ng et al. 2010; Valderrama et al. 2009; Waldron et al. 2011; Xiao and Ryan 2008). Currently, there are 20 gp60 subtype families of *C. parvum* and 10 each for *C. hominis* and *C. meleagridis* (Table 5.5). In recent years, species-specific gp60 subtyping tools have been developing for characterizations of the transmission of emerging human pathogenic *Cryptosporidium* species and genotypes, such as *C. ubiquitum*, *C. viatorum*, and

**Table 5.5** Subtype families of *C. parvum*, *C. hominis*, and other related species based on sequence analysis of the gp60 gene

| Species | Subtype family | Dominant trinucleotide repeat | Other repeat (R) | GenBank accession no. |
|---|---|---|---|---|
| *C. hominis* | Ia | TCA | AAGACGGTGGTAAGG | AF164502 (IaA23R4) |
| | Ib | TCA, TCG, TCT | – | AY262031 (IbA10G2) DQ665688 (IbA9G3) |
| | Id | TCA, TCG | – | DQ665692 (IdA16) |
| | Ie | TCA, TCG, TCT | – | AY738184 (IeA11G3T3) |
| | If | TCA, TCG | AAGAAGGCAAAGAAG | AF440638 (IfA19G1R5), FJ153244 (IfA22G1R4) |
| | Ig | TCA | – | EF208067 (IgA24) |
| | Ih | TCA, TCG | – | FJ971716 (IhA14G1) |
| | Ii | TCA | – | HM234173 (IiA17) |
| | Ij | TCA | | JF681174 (IjA14) |
| | Ik | | | KJ941148 (IkA15G1) |
| *C. parvum* | IIa | TCA, TCG | ACATCA | AY262034 (IIaA15G2R1), DQ192501 (IIaA15G2R2) |
| | IIb | TCA | – | AF402285 (IIbA14) |
| | IIc | TCA, TCG | – | AF164491 (IIcA5G3a), AF164501 (IIcA5G3b) |
| | | | | EU095267 (IIcA5G3c) |
| | | | | AF440636 (IIcA5G3d) |
| | | | | HM234172 (IIcA5G3e) |
| | | | | HM234171 (IIcA5G3f) |
| | | | | AJ973154 (IIcA5G3h) |
| | | | | AM947935 (IIcA5G3i) |
| | | | | GQ259136 (IIcA5G3j) |
| | | | | JF802123 (IIcA5G3k) |
| | | | | KU670809 (IIcA5G3l) |
| | | | | KU670810 (IIcA5G3m) |
| | | | | KU670811 (IIcA5G3n) |
| | | | | KU670812 (IIcA5G3o) |
| | | | | JN867336 (IIcA5G3p) |
| | IId | TCA, TCG | – | AY738194 (IIdA18G1) |
| | IIe | TCA, TCG | – | AY382675 (IIeA12G1) |
| | IIf | TCA | – | AY738188 (IIfA6) |
| | IIg | TCA | – | AY873780 (IIgA9) |
| | IIh | TCA, TCG | – | AY873781 (IIhA7G4) |
| | IIi | TCA | – | AY873782 (IIiA10) |
| | IIk | TCA | – | AB237137 (IIkA14) |
| | IIl | TCA | – | AM937006 (IIlA18) |
| | IIm | TCA, TCG | | AY700401(IImA7G1) |
| | IIn | TCA | | FJ897787 (IInA8) |

(continued)

**Table 5.5** (continued)

| Species | Subtype family | Dominant trinucleotide repeat | Other repeat (R) | GenBank accession no. |
|---|---|---|---|---|
| | IIo | TCA, TCG | | JN867335 (IIoA16G1) |
| | IIp | TCA | | KC885904 (IIpA9) |
| | IIq | TCA | | KU670813 (IIqA6R2) |
| | IIr | TCA, TCG | | KU852719 (IIrA5G1) |
| | IIs | TCA, TCG | | KU852720 (IIsA14G1) |
| | IIt | TCA | | KU852718 (IItA13R1) |
| *C. meleagridis* | IIIa | TCA, TCG | – | AF401499 (IIIaA24G3) |
| | IIIb | TCA, TCG | – | AB539720 (IIIbA20G1) |
| | IIIc | TCA | – | AF401497 (IIIcA6) |
| | IIId | TCA | – | DQ067570 (IIIdA6) |
| | IIIe | TCA, TCG | – | AB539721 (IIIeA20G1) |
| | IIIf | TCA, TCG | | EU164813 (IIIfA16G2) |
| | IIIg | TCA, TCG | | JX878614 (IIIgA19G5) |
| | IIIh | TCA | | KU831548 (IIIhA7) |
| | IIIi | TCA | | KP730324 (IIIiA13) |
| | IIIj | TCA | | KP730324 (partial at 5′) |
| *C. fayeri* | IVa | TCA, TCG, TCT | – | FJ490060 (IVaA11G3T1) |
| | IVb | TCA, TCG, TCT | – | FJ490087 (IVbA9G1T1) |
| | IVc | TCA, TCG, TCT | – | FJ490069 (IVcA8G1T1) |
| | IVd | TCA, TCG, TCT | | FJ490058 (IVdA7G1T1) |
| | IVe | TCA, TCG, TCT | – | FJ490071 (IVeA7G1T1) |
| | IVf | TCA, TCG, TCT | – | FJ490076 (IVfA12G1T1) |
| Opossum genotype | XIa | TCA, TCG, TCT | – | HM234181 (XIaA4G1T1) |
| *C. cuniculus* | Va | TCA | – | FJ262730 (VaA18) |
| | Vb | TCA | – | FJ262734 (VbA29) |
| Horse genotype | VIa | TCA, TCG | – | FJ435960 (VIaA11G3), DQ648547 (IIjA15G4) |
| | VIb | TCA | | FJ435961 (VIbA13) |
| | VIc | TCA | | KU852738 (VIcA16) |
| *C. wrairi* | VIIa | TCA, TCT | – | GQ121020 (VIIaA17T1) |
| Ferret genotype | VIIIa | TCA, TCG | – | GQ121029 (VIIIaA5G2) |
| *C. tyzzeri* | IXa | TCA | ATTCTGGTACTGAAGATA | GQ121030 (IXaA6R3), AY378188 (IIfA6R2) |
| | IXb | TCA | – | HM234177 (IXbA6) |

(continued)

**Table 5.5** (continued)

| Species | Subtype family | Dominant trinucleotide repeat | Other repeat (R) | GenBank accession no. |
|---|---|---|---|---|
| Mink genotype | Xa | TCA, TCG | – | HM234174 (XaA5G1) |
| Opossum genotype I | XIa | TCA, TCG, TCT | | HM234181 (XIaA4G1T1) |
| *C. ubiquitum*** | XIIa | – | | JX412915 |
| | XIIb | – | | JX412926 |
| | XIIc | – | | JX412925 |
| | XIId | – | | JX412922 |
| | XIIe | – | | KC204983 |
| | XIIf | – | | KC204984 |
| *C. erinaci* | XIIIa | TCA | ACATCA | KF055453 (XIIIaA20R10) |
| Chipmunk genotype I | XIVa | TCA, TCG, TCT | | KP099082 (XIVaA18G2T1a), KP099086 (XIVaA18G2T1b), KP099085 (XIVa19G2T2a), KP099084 (XIVa19G2T2b), KP099083 (XIVaA20G2T2) |
| *C. viatorum* | XVa | TCA | | KP115936 (XVaA3a), KP115937 (XVaA3b), KP115938 (XVaA3c), KP115939 (XVaA3d), KP115940 (XVaA3e), KP115941 (XVaA3f) |
| Skunk genotype | XVIa | TCA | | KP099095 (XVIa14a) |

(continued)

chipmunk genotype I (Guo et al. 2015a; Li et al. 2014; Stensvold et al. 2015a). Some of the gp60 subtypes are highly virulent and transmissible, such as *C. parvum* subtype IIaA15G2R1 and *C. hominis* subtype IIbA10G2 (Feng et al. 2013; Li et al. 2013; Segura et al. 2015).

Multilocus subtyping tools for *C. parvum* and *C. hominis* have also been developed (Gatei et al. 2006; Grinberg et al. 2008; Mallon et al. 2003a; Ramo et al. 2015; Tanriverdi et al. 2008; Tanriverdi et al. 2006). They are currently mostly used in population genetic studies and characterizations of mechanisms for the emergence of virulent or highly transmissible *C. parvum* and *C. hominis* subtypes (Feng et al. 2013, 2014; Li et al. 2013; Ramo et al. 2015). Recently, procedures have been developed for rapid isolation and enrichment of *Cryptosporidium* DNA from stool specimens for whole genome sequencing

(Guo et al. 2015b; Hadfield et al. 2015). They have been used recently in a study on the role of genetic recombination in the emergence of virulent *C. hominis* subtypes in the United States (Guo et al. 2015c).

## 5.5.2 *Methods for Detection of* Cryptosporidium *Oocysts in Environmental Samples*

### 5.5.2.1 Detection of *Cryptosporidium* Oocysts in Water Samples

The detection of *Cryptosporidium* oocysts in water samples is now mostly done using the standard EPA Method 1623 and its equivalent in other countries (USEPA 2012; Weintraub 2006). It involves the concentration of oocysts in 10–100 liters of water, immunomagnetic isolation of oocysts from water concentrates, immunofluorescence and DAPI staining of isolated oocysts, and examination of oocysts under epifluorescence and DIC microscopy. Method 1623 has four certified sample concentration systems to capture oocysts in water, including Envirochek filters (Pall Corporation, Ann Arbor, MI), Envirochek HV filters (Pall Corporation), Filta-Max filters (IDEXX, Westbrook, MA), or continuous flow centrifugation (Haemonetics, Braintree, MA). More recently, in response to the need for simultaneous detection of viral pathogens of biodefense importance, hollow fiber ultrafiltration has been used as an alternative oocyst concentration technique in the conventional EPA Method 1623, using either the tangential flow or dead-end filtration format (Kahler et al. 2015; Liu et al. 2012; Rhodes et al. 2012).

Because most *Cryptosporidium* oocysts in surface water samples are not from human pathogenic species, genotyping *Cryptosporidium* oocysts has been used in Method 1623 downstream analysis. This allows the assessment of the human infective potential of oocysts in source or drinking water and contamination sources in watersheds (Feng et al. 2011b; Hu et al. 2014; Kothavade 2012; Li et al. 2015a; Prystajecky et al. 2014; Ruecker et al. 2012; Van Dyke et al. 2012; Yang et al. 2008). This can be done using either residual water concentrates or microscopy-positive slides from Method 1623 (Ruecker et al. 2007; Ware et al. 2013; Yang et al. 2008). In resource-limited countries, calcium carbonate flocculation can be used to replace the filtration in oocyst concentration, and PCR analysis of DNA extraction from water concentrates can be used as an alternative to immunomagnetic separation and fluorescence microscopy to reduce the high cost associated with Method 1623 (Feng et al. 2011b; Hu et al. 2014).

### 5.5.2.2 Detection of *Cryptosporidium* Oocysts in Food Samples

The detection of parasites in food matrices is a major challenge to parasitologists and food safety professionals for many years. First, there is a wide range of sample matrices. Second, the volume of materials needed to be analyzed is often huge

comparing to the technical abilities of most traditional methods. Third, the load of parasites likely present is usually low. As a result, the recovery rate of detection methods for parasites in foodstuff can be very low (Bier 1991).

No standard methods are available for the detection of *Cryptosporidium* oocysts in food (Smith and Nichols 2010). Current methods in research laboratories for the detection of oocysts in fresh produces, meat products, and other food generally include steps for oocyst elution, concentration, separation, and detection, some of which are adapted from Method 1623 for water samples (Chandra et al. 2014; Robertson and Huang 2012; Rzezutka et al. 2010; Shields et al. 2012). The recovery rates for these methods are reportedly high but have not been verified through vigorous interlaboratory trials. Transparent double-sided adhesive tape has been used recently in combination with DFA and PCR for the recovery of *Cryptosporidium* oocysts from the surface of fresh produce (Fayer et al. 2013). DNA aptamers are used in the capture of *C. parvum* oocysts in fresh produce samples and detection of the oocysts by an electrochemical sensor (Iqbal et al. 2015).

The detection of *Cryptosporidium* oocysts in shellfish is relatively easy compared with their detection in vegetables, largely because the amount of materials for analysis is smaller and the number of oocysts potentially present is generally higher (Hohweyer et al. 2013). In large mollusks such as oysters, mussels, and large clams, gills are usually removed with scissors and washed by vortexing and centrifugation. *Cryptosporidium* oocysts present are examined and quantitated by microscopy after immunofluorescence staining. Sometimes, hemolymph is also harvested, and *Cryptosporidium* oocysts in hemocytes are examined by immunofluorescence (Fayer et al. 1998). With smaller mollusks such as small mussels and clams, the hemolymph or homogenized whole shellfish or gastrointestinal tract is generally examined individually or in pools (Marquis et al. 2015). In a comparative study, the most sensitive method for the detection of *C. parvum* in mussels was IMS concentration with DFA analysis of pooled digestive gland samples (Miller et al. 2006).

PCR is increasingly used in the analysis of fresh produce. In theory, IMS-purified oocysts from fresh produces can be genotyped by molecular techniques using the same procedures developed for the analysis of water samples (Chandra et al. 2014; Dixon et al. 2013; Keserue et al. 2012). Many studies have used PCR to genotype *Cryptosporidium* oocysts found in shellfish (Adell et al. 2014; Giangaspero et al. 2014), which is useful in tracking the sources of contamination.

## 5.6 Treatment

Numerous pharmaceutical compounds have been screened for anti-*Cryptosporidium* activities in vitro or in laboratory animals (Miyamoto and Eckmann 2015). Some of those showing some promises have been used in the experimental treatment of cryptosporidiosis in humans, but few have been shown

to be effective in controlled clinical trials (Abubakar et al. 2007b; Hunter and Nichols 2002; Sparks et al. 2015). Oral or intravenous rehydration is used whenever severe diarrhea is associated with *Cryptosporidium* infection. Nitazoxanide (NTZ) is the only FDA-approved drug for the treatment of cryptosporidiosis. Clinical trials have demonstrated that NTZ can shorten clinical disease and reduce parasite load (Bailey and Erramouspe 2004; Hussien et al. 2013; McLeod et al. 2014; Rossignol 2010). This drug, however, is not effective for the treatment of *Cryptosporidium* infections in immunodeficient people (Abubakar et al. 2007b; Amadi et al. 2009; Bailey and Erramouspe 2004). Thus, rehydration is still the major supportive treatment in AIDS patients (Abubakar et al. 2007a).

In industrialized nations, the most effective treatment and prophylaxis for cryptosporidiosis in AIDS patients is the use of highly active antiretroviral therapy (HAART) (Pantenburg et al. 2009; Rossignol 2010; Sparks et al. 2015; Zardi et al. 2005). It is also an effective prevention for cryptosporidiosis in HIV+ persons in developing countries (Werneck-Silva and Prado 2009). It is believed that the eradication and prevention of the infection are related to the replenishment of CD4+ cells in treated persons and the antiparasitic activities of the protease inhibitors used in HAART (Pantenburg et al. 2009; Zardi et al. 2005). Relapse of cryptosporidiosis is common in AIDS patients who have stopped taking HAART (Maggi et al. 2000). In developing countries, protease inhibitors are generally not included in the HAART regimens. Limited reports have shown that cryptosporidiosis is still common in HIV-positive patients receiving HAART in developing countries, although at lower frequencies than those generally reported in untreated HIV patients (Akinbo et al. 2013; Certad et al. 2005; Girma et al. 2014; Tuli et al. 2008; Werneck-Silva and Prado 2009).

## 5.7 Control of *Cryptosporidium* Contamination in Water and Food

*Cryptosporidium* oocysts are very environmentally robust, with the capability for long-term survival in a variety of natural environments and resistance to most disinfectants. Unlike the majority of bacteria and viruses, *Cryptosporidium* spp. have an environmentally resistant resting stage as part of its complex life cycle in the form of the oocyst. The wall of oocysts allows the organism to remain viable for considerable period, resist various harsh environmental challenges, and await the opportunity to infect a new susceptible host. Table 5.6 summarizes the survival of *Cryptosporidium* oocysts in a variety of matrices under controlled conditions in selected studies. *Cryptosporidium* oocysts can survival for months in soil, freshwater, and seawater. Thus, natural contamination of the environment can accumulate over time, and the contaminated environment may be reservoir of viable oocysts for long periods of time. For example, Tamburrini and Pozio (Tamburrini and Pozio 1999) reported that oocysts remain infective in seawater for up to 1 year and can be filtered out by benthic mussels in which they retain their infectivity.

**Table 5.6** Percentage reduction in *Cryptosporidium* oocyst viability with different treatments

| | Treatment | % reduction | Reference |
|---|---|---|---|
| Water | 60 days at natural condition (*C. parvum*) | 54 | Kato et al. (2001) |
| | 120 days at natural condition (*C. parvum*) | 89 | Kato et al. (2001) |
| Soil | 60 days at natural condition (*C. parvum*) | 61 | Kato et al. (2001) |
| | 120 days at natural condition (*C. parvum*) | 90 | Kato et al. (2001) |
| Silage | 106 days (*C. parvum*) | 46–62 | Merry et al. (1997) |
| Oysters | e-Beam irradiation 1.0 kGy | 46–57 | Collins et al. (2005) |
| | e-Beam irradiation 1.5 kGy | 63–73 | Collins et al. (2005) |
| | e-Beam irradiation 2.0 kGy | 100 | Collins et al. (2005) |
| | Microwave 1 s (43.2 °C) | 29 | Collins et al. (2005) |
| | Microwave 2 s (54.0 °C) | 33 | Collins et al. (2005) |
| | Microwave 3 s (62.5 °C) | 46 | Collins et al. (2005) |
| Mineral water | 4 °C for 12 weeks (*C. parvum*) | 1–11 | Nichols et al. (2004) |
| | 20 °C for 12 weeks (*C. parvum*) | 22–59 | Nichols et al. (2004) |
| Food and beverage treatment | 4·5% NaCl at 22 °C for 8 days (*C. hominis*) | 77 | Dawson et al. (2004) |
| | 4·5% NaCl 9 days at 22 °C (*C. parvum*) | 57 | Dawson et al. (2004) |
| | 9% ethanol 7 days at 22 °C (*C. hominis*) | 77 | Dawson et al. (2004) |
| | 9% ethanol 8 days at 22 °C (*C. hominis*) | 66 | Dawson et al. (2004) |
| | 40% ethanol 8 days at 22 °C (*C. hominis*) | 72 | Dawson et al. (2004) |
| | 20% glycerol 7 days at 4 °C (*C. hominis*) | 57 | Dawson et al. (2004) |
| | 20% glycerol 13 days at 4 °C (*C. parvum*) | 85 | Dawson et al. (2004) |
| | 20% glycerol 13 days at 22 °C(*C. parvum*) | 87 | Dawson et al. (2004) |
| | 20% glycerol 14 days at 4 °C (*C. parvum*) | 53 | Dawson et al. (2004) |
| | 50% sucrose 7 days at 22 °C (*C. hominis*) | 100 | Dawson et al. (2004) |
| | 50% sucrose 8 days at 22 °C (*C. hominis*) | 86 | Dawson et al. (2004) |
| | 50% sucrose 9 days at 22 °C (*C. parvum*) | 90 | Dawson et al. (2004) |
| Fruit juices | 1–5% organic acids (malic, citric, tartaric) (*C. parvum*) | ≤88 | Kniel et al. (2003) |
| | 0.025–0.03% $H_2O_2$ (*C. parvum*) | 0.04–0.6 log | Kniel et al. (2003) |
| Water and water treatment | Frozen at −22 °C for 297 h (*C. parvum*) | 86 | Robertson et al. (1992) |
| | 4 °C for 176 days (*C. parvum*) | 57–66 | Robertson et al. (1992) |
| | In seawater at 4 °C for 35 days (*C. parvum*) | 22–31 | Robertson et al. (1992) |
| | 1.5 ppm aluminum at room temperature for 7 mins (*C. parvum*) | 3–4 | Robertson et al. (1992) |
| | 16 ppm ferric sulfate at room temperature for 1 h (*C. parvum*) | 37 | Robertson et al. (1992) |
| | 0.2% calcium hydroxide (lime) at room temperature for 1 h (*C. parvum*) | 30 | Robertson et al. (1992) |
| | 250–270 nm UV radiation at 2 mJ/cm$^2$ (*C. parvum*) | 1.8–2.3 log | Linden et al. (2001) |
| | UV irradiation 0.5 mWs/ cm$^2$ (*C. parvum*) | 0.98–1.01 log | Morita et al. (2002) |
| | UV irradiation 1.0 mWs/ cm$^2$ (*C. parvum*) | 1.97–2.0 log | Morita et al. (2002) |
| | UV irradiation 1.5 mWs/ cm$^2$ (*C. parvum*) | 3.13–3.15 log | Morita et al. (2002) |
| | 60s pulsed UV light xenon lamp at 200–300 nm (278 mJ/cm$^2$) (*C. parvum)* | 6 log | Lee et al. (2008) |

A combination of filtration and disinfection is required in controlling *Cryptosporidium* oocysts in water, which also helps to reduce the contamination in food and beverage. Physical removal of oocysts from drinking water through coagulation, sedimentation, and filtration is the primary barrier against waterborne cryptosporidiosis (Rose 1997). Deficiencies in any one of these processes have been shown to directly affect the efficiency of overall oocyst removal (Medema et al. 2003). Properly operated conventional treatment (coagulation/flocculation, sedimentation, filtration, and disinfection) can remove 99% or more of oocysts (Hashimoto et al. 2001; Hijnen et al. 2004; Hsu and Yeh 2003). One of the critical times when oocysts can breach the filtration barrier is following backwash (Karanis et al. 1996). For this reason, optimization of the backwash procedure, including addition of coagulants, or filtering the waste can minimize the passage of oocysts.

Chlorination alone has not been successful for eliminating waterborne *Cryptosporidium* oocysts. As much as 80 mg/l of free chlorine or monochloramine required 90 minutes to produce 90% oocyst inactivation (Korich et al. 1990). Chlorine dioxide, on the other hand, seems to be more effective than free chlorine. Peeters et al. (Peeters et al. 1989) reported that 0.43 mg/l of chlorine dioxide ($ClO_2$) reduced infectivity within 15 mins, although some oocysts remained viable. Korich et al. (Korich et al. 1990) reported approximately 90% inactivation of oocysts exposed to 1.3 mg/l of chlorine dioxide for 60 mins. In contrast, ozone and ultraviolet (UV) radiations have shown the most promise as effective inactivation practices. An initial concentration of 1.11 mg/l ozone for 6 mins was shown to inactivate viable oocysts at a concentration of $10^4$ oocysts/ml (Peeters et al. 1989). Korich et al (Korich et al. 1990) reported that exposure to 1 mg/l ozone inactivated between 90% and 99% of oocysts ($2.8 \times 10^5$/ml) in water at 25 °C. Inactivation of 99 to 99.9% was achieved when the exposure time was increased to 10 mins. In addition to ozone treatment, UV radiation is now rapidly adopted by the water industry in inactivation of *Cryptosporidium* oocysts in water (Bukhari et al. 2004; Lorenzo-Lorenzo et al. 1993). UV light between 250 and 270 nm in wavelengths has been shown to reduce *C. parvum* oocyst infectivity at 2 mJ/cm$^2$ (Linden et al. 2001). Higher doses can lead to higher inactivation rates (Craik et al. 2001). Most chemicals used in flocculation during the first step of water treatment have only limited effect on viability of *Cryptosporidium* oocysts at the practical concentrations (Robertson et al. 1992).

*Cryptosporidium* oocysts can contaminate food through many pathways. These include (i) introduction to the foodstuff through contaminated raw ingredients, e.g., unwashed lettuce destined for ready-to-eat salads; (ii) introduction during food processing due to addition of contaminated water, as an important ingredient of the foodstuff, e.g., in soft drink production; (iii) introduction during food processing as a contaminant of cleaning of equipment with non-potable water; (iv) introduction of the parasite through pest infestations, e.g., cockroaches, houseflies, mice, and rats; and (v) introduction of the parasite to processed foodstuffs from positive food handlers. The associated risk from

each of these potential routes of entry of oocysts into the foodstuff should be controlled through an integrated HACCP (hazard analysis and critical control point) management.

The effect of food processing and storage practices on the viability of potentially contaminated *Cryptosporidium* oocysts in food and beverage depends on the nature of the treatment. Snap freezing is detrimental to the survival of *Cryptosporidium* oocysts (Fayer and Nerad 1996; Robertson et al. 1992), but if suspended in water, stored at −20 ° C for 24 h, and then transferred to −70 °, *C. muris* oocysts can survive for at least 15 months (Rhee and Park 1996). Some *C. parvum* oocysts can survive freezing in water at higher temperatures (−20 to −22 °C) for 1–32 days (Deng and Cliver 1999; Fayer and Nerad 1996; Robertson et al. 1992). As expected, air-drying for 4 h kills almost all *C. parvum* oocysts (Deng and Cliver 1999; Robertson et al. 1992). *Cryptosporidium* oocysts can survive high temperature for only short durations. Oocysts of *C. parvum* lose infectivity at 72.4 °C or higher within 1 min or when the temperature is held at 64.2 °C or higher for 2 mins (Fayer 1994). The high-temperature short-time conditions (71.7 degrees C for 15 s) used in commercial pasteurization are sufficient to destroy infectivity of *C. parvum* oocysts in milk and apple cider (Deng and Cliver 2001; Harp et al. 1996). Most oocysts of *C. parvum* can survive for at least 10 days during the process of yogurt making and storage but cannot survive the ice cream making process (Deng and Cliver 1999).

Not much is known on the survival of *Cryptosporidium* oocysts in beverage. Although high salinities reduce the survival of *C. parvum* in water (Fayer et al. 1998), oocysts can maintain viability for months in natural mineral water, especially at low temperatures (Nichols et al. 2004). There is some reduction in oocyst viability in acidified and carbonated beverages (Friedman et al. 1997). Oocysts of *C. hominis* stored in 9% or 40% ethanol for 7 or 8 days at 22 °C suffer 66–77% reductions in viability (Dawson et al. 2004). However, their ability for long-term survival in ethanol is not clear.

Not all food preservatives have detrimental effects on the viability of *Cryptosporidium* oocysts. Considerable viability is maintained when *C. parvum* oocysts are stored at 4 °C or 22 °C in media containing citric, acetic, or lactic acid (Dawson et al. 2004). Oocysts of *C. parvum* and *C. hominis* kept in 4.5% sodium chloride at 22 °C for 8 or 9 days have 57–77% reduction in viability. Similar losses in viability also occur in oocysts stored in 20% glycerol at 4 °C or 22 °C for 13 or 14 days. Storage in 50% sucrose at 22 °C, however, is detrimental to most *C. parvum* and *C. hominis* oocysts (Dawson et al. 2004).

Presently, there is no solid recommendation regarding to the management of *Cryptosporidium*-positive food handlers within the food processing sector. The mean duration of the illness has previously been reported as 12.2 days; however, the range in duration is 2–26 days (Jokipii and Jokipii 1986). Oocyst excretion times have varied widely from 6.9 days (range 1 to 15 days) after the cessation of symptoms to 2 months and greater in a small proportion of patients. Thus, it is impossible to predict the carrier status of persons based on cessation of symptoms. In addition, microbiological screening for carrier status in

infected persons is problematic as symptomatic patients may have intermittently negative stool specimens (Jokipii and Jokipii 1986). Other studies have shown that asymptomatic carriers are found in 0.4% of the general population in Australia (Hellard et al. 2000) and 6.4% of immunocompetent children in the United States (Pettoello-Mantovani et al. 1995). Thus, processors of ready-to-eat foodstuffs are potentially vulnerable to potential contamination of products by food handlers with both symptomatic and asymptomatic cryptosporidioses (Quiroz et al. 2000). Therefore, it is important that other general hygienic practices such as handwashing and glove wearing are also implemented as part of the HACCP management to minimize foodborne disease due to cryptosporidiosis and other pathogens.

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# Chapter 6
# *Toxoplasma gondii*

**D.E. Hill and J.P. Dubey**

## 6.1 Introduction

*Toxoplasma gondii* is a coccidian parasite with an unusually wide range of intermediate hosts. Felids serve as definitive hosts and produce the environmentally resistant oocyst stage. *Toxoplasma* is one of the most common parasitic infections of man, though its prevalence varies widely from place to place. Toxoplasmosis continues to be a significant public health problem in the USA, where 8–22% of people are infected; a similar prevalence is seen in the UK (Dubey and Beattie 1988; Dubey and Jones 2008; Jones et al. 2001, 2003, 2007). In Central America, South America, and continental Europe, estimates of infection range from 30% to 90% (Dubey and Jones 2008; Dubey 2010; Minbaeva et al. 2013). Most infections in humans are asymptomatic, but at times the parasite can produce devastating disease. Infection may be congenitally or postnatally acquired. In the USA, nationwide serological surveys demonstrated that seroprevalence in people remained stable at 23% from 1990 until 1998 (Jones et al. 2001), while recent surveys have demonstrated a significant decrease in seroprevalence to 10.8% over the last decade (Jones et al. 2007). Similar decreases in seroprevalence have been observed in many European countries (Dubey 2010).

It is estimated that 1,075,242 persons are infected with *T. gondii* each year in the USA, and approximately 2839 persons develop symptomatic ocular disease annually (Jones and Holland 2010). The cost of illness in the USA caused by *Toxoplasma* has been estimated to be nearly 3 billion dollars and an 11,000 quality-adjusted life year (QALY) loss annually (Batz et al. 2012; Hoffmann et al. 2012).

D.E. Hill, PhD (✉) • J.P. Dubey
United States Department of Agriculture, Agricultural Research Service, Northeast Area, Animal Parasitic Diseases Laboratory, Beltsville Agricultural Research Center-East, Center Road, BLDG. 307-C, Beltsville, MD 20705, USA
e-mail: dolores.hill@ars.usda.gov



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Recent publications have linked suicide and schizophrenia to *Toxoplasma* infection (Pedersen et al. 2012; Torrey et al. 2012).

*Toxoplasma gondii* also infects food animals, including sheep, goats, pigs, chickens, and many game animal species. Infected animals harbor tissue cysts, and human consumers can be infected by ingestion of these cysts in raw or undercooked meat. Virtually all edible portions of an animal can harbor viable *T. gondii* tissue cysts (Dubey et al. 1986), and tissue cysts can survive in food animals for years. The relative contribution of foodborne (meat) sources of *Toxoplasma* infection versus oocyst transmission of *Toxoplasma* to human infection is unknown, and various studies have suggested widely disparate estimates of foodborne transmission. Mead et al. (1999) suggested that *T. gondii* is one of three pathogens (along with *Salmonella* and *Listeria*) which account for >75% of all deaths due to foodborne disease in the USA. Roghmann et al. (1999) suggested that 50% of *Toxoplasma* infections in the USA could be ascribed to foodborne transmission. Scallan et al. (2011) estimated that *Toxoplasma* caused 8% of hospitalizations and 24% of deaths resulting from foodborne illnesses. In contrast, Dubey et al. (2005), in a nationwide survey of retail meats (beef, chicken, and pork), found no viable *Toxoplasma* in any of 2094 beef or 2094 chicken samples and 7 positive pork samples out of 2094 samples assayed, concluding that there was not enough viable *Toxoplasma* present in retail meats to account for the level of *Toxoplasma* infection found in the US population. Recent studies (Hill et al. 2011; Boyer et al. 2011) have suggested that oocyst exposure is the predominate route of *Toxoplasma* transmission in the USA. Despite the uncertainty of human infection source, *Toxoplasma* is recognized as a foodborne risk. Animal infections with *Toxoplasma*, especially infections in nonmeat-eating ruminants, birds, and pigs raised in confinement, also likely result from environmental exposure to *T. gondii* oocysts. Oocyst contamination of the environment is widespread as a result of fecal contamination of soil and groundwater by the estimated 140 million domestic and feral cats in the USA, each of which can deposit hundreds of millions of oocysts in feces during infection (www.avma.org; Levy and Crawford 2004; Dubey 2010). Oocyst-contaminated runoff surface water entering the marine environment has resulted in devastating disease in endangered sea otters off the west coast of the USA (Miller et al. 2002; Conrad et al. 2005), and even wild herbivores have been shown to have very high seroprevalence as a result of exposure to the environmentally resistant oocysts (Hill et al. 2005).

## 6.2 Morphology and Classification

*Toxoplasma gondii* belongs to phylum Apicomplexa, class Sporozoasida, subclass Coccidiasina, order Eimeriorina, and family Toxoplasmatidae (Dubey, 2010). There is only one species of *Toxoplasma*, *T. gondii*. Coccidia in general have complicated life cycles. Most coccidia are host specific and are transmitted via a fecal–oral route. Transmission of *T. gondii* occurs via the fecal–oral route (Fig. 6.1), as well as through consumption of infected meat, and by transplacental transfer from mother to fetus (Frenkel et al. 1970; Dubey and Beattie 1988).

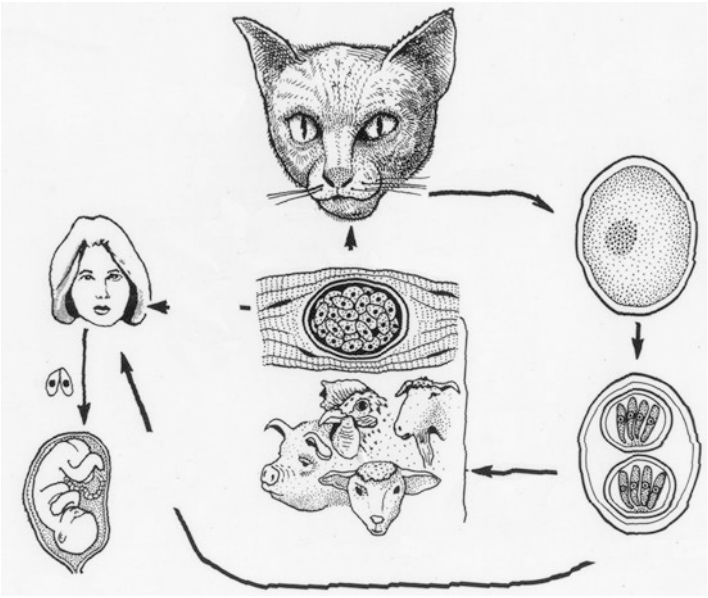

**Fig. 6.1** Life cycle of *Toxoplasma gondii*

## 6.3 Structure and Life Cycle

The name *Toxoplasma* (toxon = arc, plasma = form) is derived from the crescent shape of the tachyzoite stage (Fig. 6.2). There are three infectious stages of *T. gondii*: the tachyzoites (in groups) (Fig. 6.3a), the bradyzoites (in tissue cysts) (Fig. 6.3b, c), and the sporozoites (in oocysts) (Fig. 6.3g). The tachyzoite is often crescent shaped and is approximately the size (2 × 6 μm) of a red blood cell (Fig. 6.4). The anterior end of the tachyzoite is pointed, and the posterior end is round. It has a pellicle (outer covering), several organelles including subpellicular microtubules, mitochondrium, smooth and rough endoplasmic reticulum, a Golgi apparatus, apicoplast, ribosomes, a micropore, and a well-defined nucleus. The nucleus is usually situated toward the posterior end or in the central area of the cell. The tachyzoite enters the host cell by active penetration of the host cell membrane and can tilt, extend, and retract, as it searches for a host cell. After entering the host cell, the tachyzoite becomes ovoid in shape and becomes surrounded by a parasitophorous vacuole (pv; Fig. 6.4). *Toxoplasma gondii* in a parasitophorous vacuole is protected from host defense mechanisms. The tachyzoite multiplies asexually within the host cell by repeated divisions in which two progenies form within the parent parasite, consuming it (Fig. 6.5a–d). Tachyzoites continue to divide until the host cell is filled with parasites.

After a few divisions, *T. gondii* forms tissue cysts that vary in size from 5 to 70 μm and remain intracellular (Fig. 6.6a–f). The tissue cyst wall is elastic, thin (<0.5 μm), and may enclose hundreds of the crescent-shaped, slender *T. gondii*

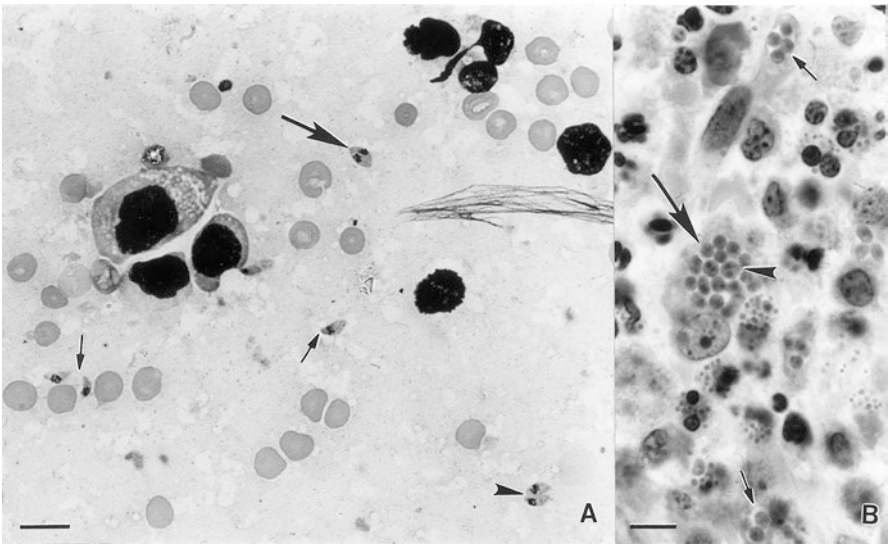


**Fig. 6.2** Tachyzoites of *T. gondii*. Bar = 10 μm. (**A**) Individual (*small arrows*), binucleate (*large arrow*), and divided (*arrowhead*) tachyzoites. Impression smear of the lung. Compare size with red blood cells and leukocytes. Giemsa stain. (**B**) Tachyzoites in a group (*large arrow*) and in pairs (*small arrows*) in section of a mesenteric lymph node. Note organisms are located in parasitophorous vacuoles and some are dividing (*arrowhead*). Hematoxylin and eosin stain (H & E)

stage known as bradyzoites (Fig. 6.7). The bradyzoites are approximately 7 × 1.5 μm in size and differ structurally only slightly from tachyzoites. They have a nucleus situated toward the posterior end, whereas the nucleus in tachyzoites is more centrally located. Bradyzoites are more slender and less susceptible to destruction by proteolytic enzymes than tachyzoites. Although tissue cysts containing bradyzoites may develop in visceral organs, including the lungs, liver, and kidneys, they are more prevalent in muscular and neural tissues, including the brain (Fig. 6.6a–f), eye, and skeletal and cardiac muscle. Intact tissue cysts probably do not cause any harm and can persist for the life of the host.

After the ingestion of tissue cysts by cats, the tissue cyst wall is dissolved by proteolytic enzymes in the stomach and small intestine. The released bradyzoites penetrate the epithelial cells of the small intestine and initiate development of numerous generations of asexual and sexual cycles of *T. gondii* (Dubey and Frenkel 1972). Bradyzoites penetrate the lamina propria of the feline intestine and multiply as tachyzoites. Within a few hours after infection of cats, *T. gondii* may disseminate to extraintestinal tissues. *Toxoplasma gondii* persists in intestinal and extraintestinal tissues of cats for at least several months and possibly for the life of the cat.

As the enteroepithelial cycle progresses, *T. gondii* multiplies profusely in intestinal epithelial cells of cats (enteroepithelial cycle), and these stages, represented by five distinct morphological types (Types A–E), are known as schizonts (Fig. 6.3d). Several generations of each type are produced, and daughter organisms known as

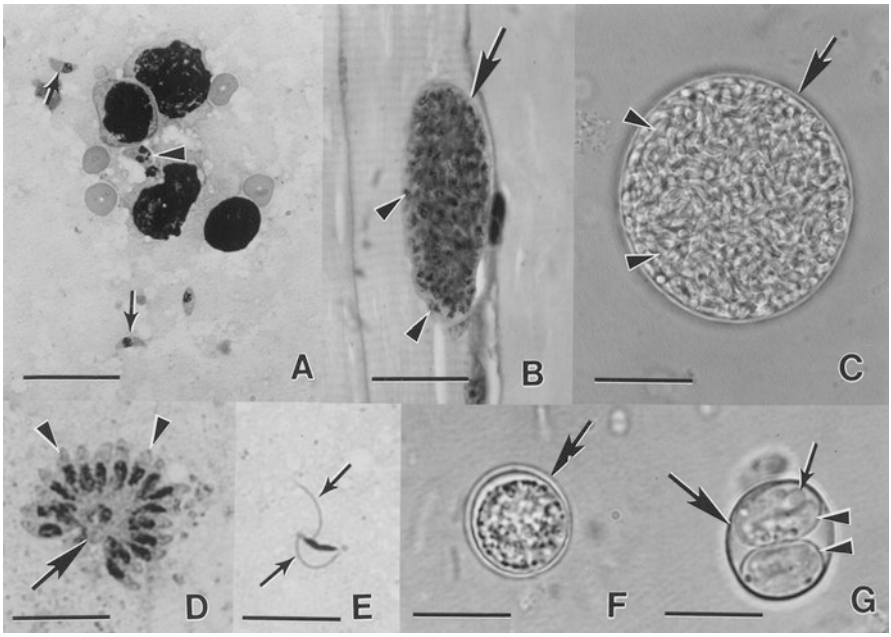


**Fig. 6.3** Stages of *Toxoplasma gondii*. Scale bar in (**A**)–(**D**)= 20 μm, in (**E**)–(**G**) = 10 μm. (**A**) Tachyzoites in impression smear of the lung. Note crescent-shaped individual tachyzoites (*arrows*) and dividing tachyzoites (*arrowheads*) compared with the size of host red blood cells and leukocytes. Giemsa stain. (**B**) Tissue cysts in section of muscle. The tissue cyst wall is very thin (*arrow*) and encloses many tiny bradyzoites (*arrowheads*). H & E stain. (**C**) Tissue cyst separated from host tissue by homogenization of infected brain. Note tissue cyst wall (*arrow*) and hundreds of bradyzoites (*arrowheads*). Unstained. (**D**) Schizont (*arrow*) with several merozoites (*arrowheads*) separating from the main mass. Impression smear of infected cat intestine. Giemsa stain. (**E**) A male gamete with two flagella (*arrows*). Impression smear of infected cat intestine. Giemsa stain. (**F**) Unsporulated oocyst in fecal float of cat feces. Unstained. Note double-layered oocyst wall (*arrow*) enclosing a central undivided mass. (**G**) Sporulated oocyst with a thin oocyst wall (*large arrow*), two sporocysts (*arrowheads*). Each sporocyst has four sporozoites (*small arrow*) which are not in complete focus. Unstained

merozoites are formed coincident with the last nuclear division. Merozoites give rise to gametes, the sexual stages of the organism. The microgamont (male gamont) has two flagella (Fig. 6.3e), and it swims to, enters, and fertilizes the macrogamont (female gamont), forming a zygote. After fertilization, oocyst wall formation begins around the zygote. Oocysts are discharged into the intestinal lumen by the rupture of intestinal epithelial cells.

Oocysts are environmentally resistant and are formed only in felids, probably in all members of the Felidae (Fig. 6.3g). Cats shed oocysts after ingesting any of the three infectious stages of *T. gondii*, i.e., tachyzoites, bradyzoites, and sporozoites (Dubey and Frenkel 1972, 1976; Dubey 1996, 2002). Prepatent periods (time to the shedding of oocysts after initial infection) and frequency of oocyst shedding vary according to the stage of *T. gondii* ingested. Prepatent periods are 3–10 days after

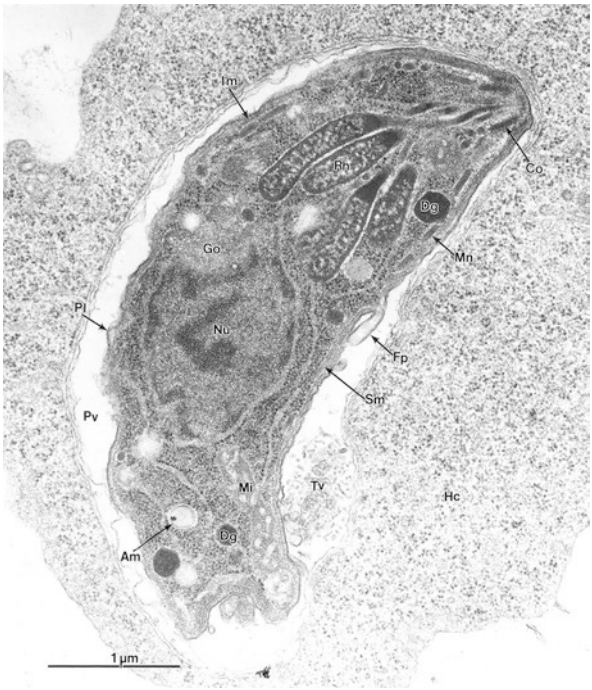


**Fig. 6.4** Transmission electron micrograph (TEM) of a tachyzoite of *T. gondii* in a mouse peritoneal exudate cell; *Am* amylopectin granule, *Co* conoid, *Dg* electron-dense granule, *Fp* fingerlike projection of tachyzoite plasmalemma, *Go* Golgi complex, *Hc* host cell cytoplasm, *Im* inner membrane complex, *Mi* mitochondrion, *Mn* microneme, *Nu* nucleus, *Pl* plasmalemma, *Pv* parasitophorous vacuole, *Rh* rhoptry, *Sm* subpellicular microtubule, *Tv* tubulovesicular membranes. Bar = 1 µm

ingesting tissue cysts and 19 days or more after ingesting tachyzoites or oocysts (Dubey and Frenkel 1972, 1976; Dubey 1996). Less than 50% of cats shed oocysts after ingesting tachyzoites or oocysts, whereas nearly all cats shed oocysts after ingesting tissue cysts (Dubey and Frenkel 1976). In freshly passed feces, oocysts are unsporulated (noninfective; Fig. 6.3f). Unsporulated oocysts are subspherical to spherical and are 10 × 12 µm in diameter. They sporulate (become infectious) outside the cat within 1–5 days depending upon aeration and temperature. Sporulated oocysts contain two ellipsoidal sporocysts (Fig. 6.3g), and each sporocyst contains four sporozoites. The sporozoites are 2 × 6–8 µm in size.

## 6.4 Biology, Genetics, and Genomics

The nucleus of *T. gondii* is haploid except during sexual recombination in the gut of the cat. Fourteen chromosomes are present, encompassing a 65 Mb genome (Khan et al. 2007 Dubey 2010). Most *T. gondii* isolates from human and animal sources in North America, Europe, and Africa have been grouped into one of three clonal lineages, Types I, II, and III (Dardé et al. 1992; Howe and Sibley 1995; Ajzenberg et al. 2002a, b; Su et al. 2003; Khan et al. 2007; Velmurugan et al. 2009), and are biologically and genetically different from isolates from Brazil and Columbia (Dubey et al. 2002, 2007; Lehmann et al. 2006; Dubey and Su 2009; Su et al. 2012). A number of recent studies suggest that only a few ancestral strains have given rise

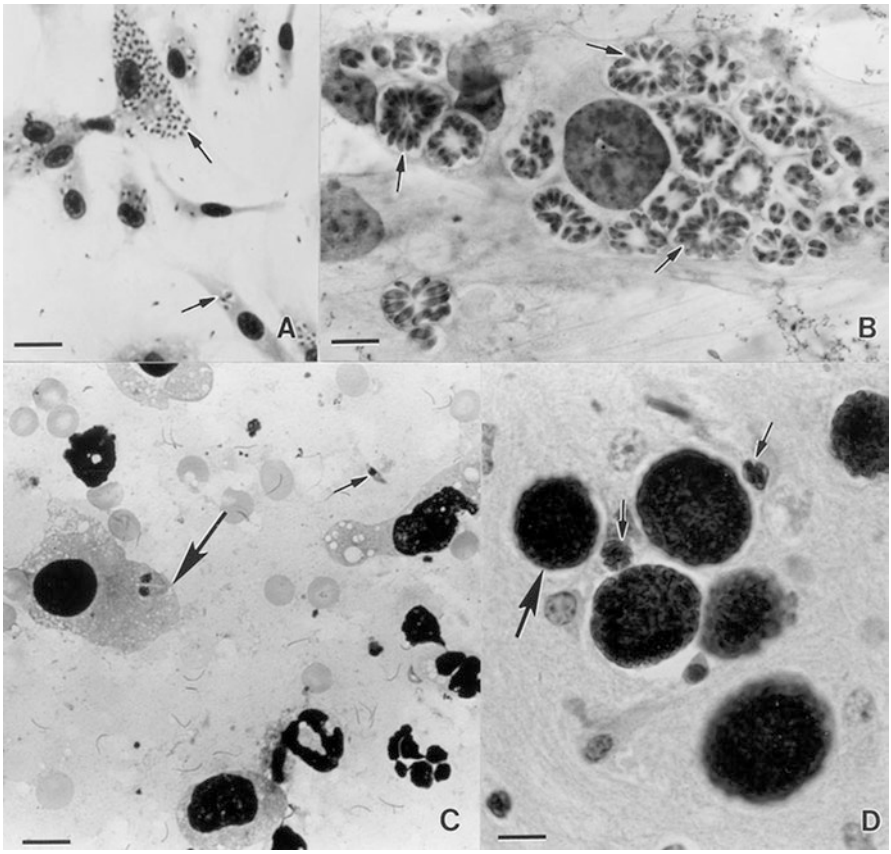


**Fig. 6.5** *Toxoplasma gondii* stages in in vitro and in in vivo preparations. (**A**) Tachyzoites in culture of human foreskin fibroblasts cells. Giemsa stain. Bar = 25 μm. (**B**) Rosettes of tachyzoites in human foreskin fibroblasts. Immunohistochemical stain with anti-tachyzoite-specific antibody. Smear. Bar = 10 μm. (**C**) Tachyzoites in a cytospin smear of pleural fluid from a cat with pneumonia. Giemsa stain. Compare the size of tachyzoites (*arrow*) with host cells. Giemsa stain. Bar = 10 μm. (**D**) Tachyzoites (*arrows*) and tissue cysts (*large arrow*) in section of mouse brain. Immunohistochemical stain with *T. gondii*-specific antibody. Bar = 10 μm

to the three dominant clonal lineages and the existing genetic diversity seen in various geographic regions through a process of limited, mostly asexual, recombination (Howe and Sibley 1995; Grigg et al. 2001; Su et al. 2003, 2012). Recently, Su et al. (2012) demonstrated a biphasic pattern where a few clonal lineages dominate the population in the Northern Hemisphere, while, particularly in South America, populations are much more genetically diverse. Recent genotyping studies of isolates from pigs, lambs, and goats demonstrate that the Type II lineage predominates in food animals in the USA, followed by Type III isolates and atypical genotypes; Type I isolates have rarely been found in farm animals (Dubey et al. 2008a, b, 2011; Velmurugan et al. 2009).

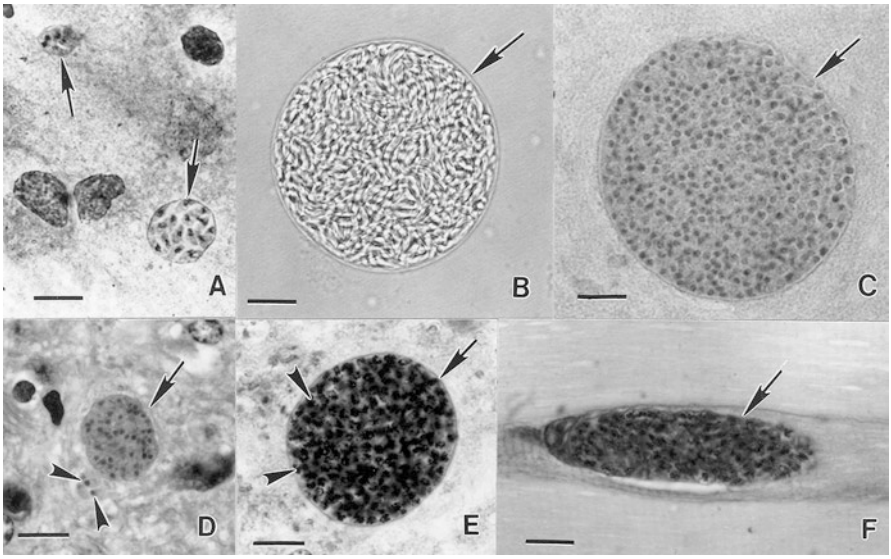


**Fig. 6.6** Tissue cysts of *T. gondii*. Bar = 10 μm. (**A**) Two tissue cysts (*arrows*). Note thin cyst wall enclosing bradyzoites. Impression smear of mouse brain. Silver impregnation and Giemsa stain. (**B**) A tissue cyst freed from mouse brain by homogenization in saline. Note thin cyst wall (*arrow*) enclosing many bradyzoites. Unstained. (**C**) A large tissue cyst in section of rat brain 14 months postinfection. Note thin cyst wall (*arrow*). H & E stain. (**D**) A small tissue cyst with intact cyst wall (*arrow*) and four bradyzoites (*arrowheads*) with terminal nuclei adjacent to it. Section of mouse brain 8 months postinfection. H & E stain. (**E**) A tissue cyst in section of mouse brain. Note PAS-negative cyst wall (*arrow*) enclosing many PAS-positive bradyzoites (*arrowheads*). The bradyzoites stain bright red with PAS but they appear black in this photograph. Periodic acid Schiff hematoxylin stain (PASH). (**F**) An elongated tissue cyst (*arrow*) in section of skeletal muscle of a mouse. PASH stain

## 6.5 Diagnosis

Diagnosis is made by biologic, serologic, or histologic methods or by some combination of the above. Clinical signs of toxoplasmosis are nonspecific and are not sufficiently characteristic for a definite diagnosis. Toxoplasmosis in fact mimics several other infectious diseases.

Detection of *T. gondii* antibody in patients may aid diagnosis. There are numerous serologic procedures available for the detection of humoral antibodies; these include the Sabin–Feldman dye test (DT), the modified agglutination test (MAT), the indirect hemagglutination test (IHAT), the indirect fluorescent antibody assay (IFA), the direct agglutination test, the latex agglutination test (LAT), the ELISA, and the immunosorbent agglutination assay test (IAAT). The IFA, IAAT, and ELISA have been modified to detect IgM antibodies (Remington et al. 1995). The IgM antibodies appear sooner after infection than the IgG antibodies and disappear faster than IgG antibodies after recovery, though a small percentage of infected people produce IgG first (Remington et al. 1995; Fricker-Hidalgo et al. 2013). Progress has

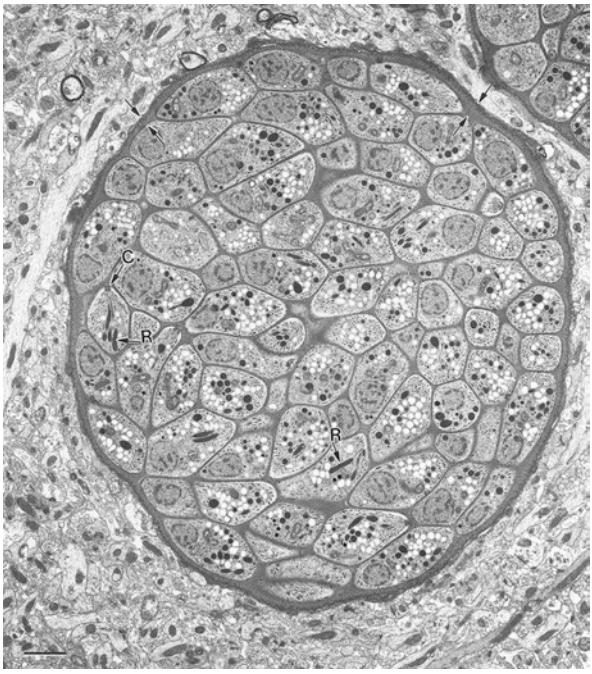


**Fig. 6.7** TEM) of a tissue cyst in the brain of a mouse 6 months postinfection. Note thin cyst wall (*opposing arrows*), numerous bradyzoites each with a conoid (C), and electron-dense rhoptries (R). Bar = 3.0 μm

been made in the diagnosis of human infection with *Toxoplasma* using PCR (Rahumatullah et al. 2012). Infection has been diagnosed using nested, stage-specific primers and cerebrospinal fluid from AIDS patients with suspected toxoplasmic encephalitis (Contini et al. 2002; Joseph et al. 2002), in immunocompromised patients undergoing hematopoietic stem cell transplantation (Lewis et al. 2002), and in suspected cases of fetal toxoplasmosis using amniotic fluid (Antsaklis et al. 2002). Improved sensitivity and performance standards for in-house methods and commercially available PCR kits are needed, as recent studies have shown that these PCR tests may not perform well using experimental or clinical samples (Hill et al. 2006a; Morelle et al. 2012; Mikita et al. 2013).

## 6.6 Epidemiology

Toxoplasmosis may be acquired by ingestion of oocysts or by ingestion of tissue-inhabiting stages of the parasite. Contamination of the environment by oocysts is widespread as oocysts are shed by domestic cats and other members of the Felidae (Frenkel et al. 1970; Dubey and Beattie 1988). Domestic cats are probably the major source of contamination as oocyst formation is greatest in domestic cats, and cats are extremely common. Widespread natural infection of the environment is possible since a cat may excrete millions of oocysts after ingesting as few as one bradyzoite

or one tissue cyst, and many tissue cysts may be present in one infected mouse (Frenkel et al. 1970; Dubey 2001). Sporulated oocysts survive for long periods under most ordinary environmental conditions and even in harsh environments for months. They can survive in moist soil, for example, for months and even years (Dubey and Beattie 1988).

Oocysts in soil can be mechanically transmitted by invertebrates such as flies, cockroaches, dung beetles, and earthworms, which can spread oocysts into human food and animal feeds.

Infection rates in cats are determined by the rate of infection in local avian and rodent populations because cats typically become infected by eating these animals. The more oocysts in the environment, the more likely it is that prey animals would be infected, and this in turn would increase the infection rate in cats. In certain areas of Brazil, up to 50% of 6–8-year-old children have antibodies to *T. gondii* linked to the ingestion of oocysts from the environment heavily contaminated with *T. gondii* oocysts (Bahia-Oliveira et al. 2003; Dubey et al. 2012). The largest recorded outbreak of clinical toxoplasmosis in humans in North America was epidemiologically linked to drinking water from a municipal water reservoir in British Columbia, Canada (Aramini et al. 1998, 1999). This water reservoir was supposedly contaminated with *T. gondii* oocysts excreted by cougars (*Felis concolor*). Although attempts to recover *T. gondii* oocysts from water samples in the British Columbia outbreak were unsuccessful, methods to detect oocysts were reported (Isaac-Renton et al. 1998). At present, there are no commercial reagents available to reliably detect *T. gondii* oocysts in the environment.

Widespread infection in aquatic mammals indicates contamination and survival of oocysts in seawater (Cole et al. 2000; Lindsay and Dubey 2009). Wild populations of southern sea otters have been significantly impacted by exposure to *Toxoplasma* oocysts, presumably by eating filter-feeding mollusks in near-shore environments (Miller et al. 2002, 2008).

Transmission of *Toxoplasma* from consumption of infected meat products is difficult to quantify, since meat from infected animals may undergo postharvest treatments such as heating, freezing, salting, or pumping (injection of water- and salt-based solutions to retard microbial growth) that can render the parasite nonviable (Hill et al. 2004, 2006b), and few comprehensive assessments have been completed in meat available for retail purchase. Complicating matters is the fact that the number of *T. gondii* organisms in meat from naturally infected food animals is very low, making the parasite difficult and expensive to detect by direct methods. It is estimated that as few as one tissue cyst may be present in 100 g of meat (Dubey 2010). In addition, there is no predilection site for *Toxoplasma* in meat animals; virtually all edible portions of an animal can harbor viable *T. gondii* tissue cysts (Dubey et al. 1986), and tissue cysts can remain viable in food animals for years.

Beef, chicken, and pork are the main meat types consumed in the USA. In a case control study of 148 recently (<6 months) infected individuals, Jones et al. (2009) identified an elevated risk of infection associated with eating raw ground beef, rare lamb, locally produced cured, dried, or smoked meat, raw oysters, clams, or mussels, working with meat, and drinking unpasteurized goat's milk.

The relative risk to US consumers of acquiring *T. gondii* infection from undercooked meat was recently determined in a nationwide survey of retail chicken, beef, and pork. The survey of 698 retail outlets in 28 metropolitan statistical areas (MSAs as defined by the US Census Bureau) covered 80% of the US population. Only pork was found to harbor viable *T. gondii* tissue cysts, which were isolated from 0.38% of samples (7/2094) by cat bioassay, and 0.57% of pork samples were suspected to be infected based on positive enzyme-linked immunosorbent assay (ELISA) results. No beef samples were positive by bioassay or by ELISA, while 1.4% of chickens were positive by ELISA only. The northeastern USA had a higher number of positive pork samples than other regions of the country, reflecting the higher risk of pig infection due to regional management practices (outdoor vs confinement rearing; Dubey et al. 2005). Thus, while the extent of human infection resulting from meat sources remains undetermined, the lack of viable organisms in beef and chicken and the low prevalence of *T. gondii* infection in market pigs found in this comprehensive study would not account for the estimated incidence and measured seroprevalence in humans in the USA.

## 6.7 Pathogenesis and Clinical Features

*Toxoplasma gondii* usually parasitizes the host, definitive and intermediate, without producing clinical disease. Only rarely does it produce severe clinical manifestations. The bradyzoites from the tissue cysts or sporozoites from the oocyst penetrate intestinal epithelial cells and multiply in the intestine as tachyzoites within 24 h of infection. *Toxoplasma gondii* may spread first to mesenteric lymph nodes and then to distant organs by invasion of lymphatics and blood and can multiply in virtually any cell in the body. All extracellular forms of the parasite are directly affected by antibody, but intracellular forms are not. More virulent strains of *Toxoplasma* have developed effective defensive mechanisms using ROP18, a rhoptry-associated serine/threonine kinase, to inactivate p47 GTPases, which are generated by the infected cell to rupture the vacuole containing the parasite, resulting in digestion of the organism (Fentress and Sibley 2011). It is believed that cellular factors, including lymphocytes and lymphokines, are more important than humoral factors in immune-mediated destruction of *T. gondii* (Gigley et al. 2009; Vouldoukis et al. 2011; Koshy et al. 2012). Immunity does not eradicate infection. *Toxoplasma gondii* tissue cysts persist for years after acute infection. The fate of tissue cysts is not fully known. Whether bradyzoites can form new tissue cysts directly without transforming into tachyzoites is not known. It has been proposed that tissue cysts may at times rupture during the life of the host. The released bradyzoites may be destroyed by the host's immune responses, or there may be formation of new tissue cysts. In immunosuppressed patients, such as those given large doses of immunosuppressive agents in preparation for organ transplants and in those with acquired immunodeficiency syndrome (AIDS), rupture of a tissue cyst may result in transformation of bradyzoites into tachyzoites and renewed multiplication. The immunosuppressed host may die

from toxoplasmosis unless treated. It is not known how corticosteroids cause relapse, but it is unlikely that they directly cause rupture of the tissue cysts. Pathogenicity of *T. gondii* is determined by the virulence of the strain and the susceptibility of the host species (Hunter and Sibley 2012). *Toxoplasma gondii* strains may vary in their pathogenicity in a given host. Certain strains of mice are more susceptible than others, and the severity of infection in individual mice within the same strain may vary. Mice of any age are susceptible to clinical *T. gondii* infection (Dubey 2010). However, adult rats do not become ill, while young rats can die of toxoplasmosis. Adult dogs, like adult rats, are resistant, whereas puppies are fully susceptible to clinical toxoplasmosis. Certain species are genetically resistant to clinical toxoplasmosis. Cattle and horses are among the hosts more resistant to clinical toxoplasmosis, whereas certain marsupials and New World monkeys are highly susceptible to *T. gondii* infection (Dubey and Beattie 1988; Dubey 2010). Nothing is known concerning genetically determined susceptibility to clinical toxoplasmosis in higher mammals, including humans.

Infection in humans may be congenitally or postnatally acquired. Congenital infection occurs only when a woman becomes infected during pregnancy. Congenital infections acquired during the first trimester are more severe than those acquired in the second and third trimesters (Desmonts and Couvreur 1974; Remington et al. 1995). While the mother rarely has symptoms of infection, she does have a temporary parasitemia. Focal lesions develop in the placenta, and the fetus may become infected. At first there is generalized infection in the fetus. Later, infection is cleared from the visceral tissues and may localize in the central nervous system. A wide spectrum of clinical diseases occurs in congenitally infected children (Desmonts and Couvreur 1974). Mild disease may consist of slightly diminished vision, whereas severely diseased children may have the full tetrad of lesions of the eye, hydrocephalus, convulsions, and intracerebral calcification. Of these, hydrocephalus is the least common but most significant lesion of toxoplasmosis. So far the most common sequela of congenital toxoplasmosis is ocular disease (Desmonts and Couvreur 1974; Remington et al. 1995). The socioeconomic impact of toxoplasmosis in human suffering and the cost of care of sick children, especially those with mental retardation and blindness, are enormous (Roberts and Frenkel 1990; Roberts et al. 1994). The testing of all pregnant women for *T. gondii* infection is compulsory in some European countries, including France and Austria (Thiebaut et al. 2007; Petersen 2007). The cost benefits of such mass screening are being debated in many other countries (Cortina-Borja et al. 2010; Remington et al. 1995, 2001). Recently, Stillwaggon et al. (2011) provided an extensive guideline for estimating costs of preventive maternal screening for and the social costs resulting from toxoplasmosis based on studies in Europe and the USA. While estimating these costs, the value of all resources used or lost should be considered, including the cost of medical and nonmedical services, wages lost, cost of in-home care, and indirect costs of psychological impacts borne by the family for lifetime care of a substantially cognitively impaired child; cost of a fetal death was estimated to be $5 million dollars (Stillwaggon et al. 2011).

Postnatally acquired infection may be localized or generalized. Infection may occur in any organ. Oocyst-transmitted infections may be more severe than tissue

**Table 6.1** Frequency of symptoms in people with postnatally acquired toxoplasmosis

| Patients with symptoms (%) | | |
|---|---|---|
| Symptoms | Atlanta outbreak[a] (35 patients) | Panama outbreak[b] (35 patients) |
| Fever | 94 | 90 |
| Lymphadenopathy | 88 | 77 |
| Headache | 88 | 77 |
| Myalgia | 63 | 68 |
| Stiff neck | 57 | 55 |
| Anorexia | 57 | NR[c] |
| Sore throat | 46 | NR |
| Arthralgia | 26 | 29 |
| Rash | 23 | 0 |
| Confusion | 20 | NR |
| Earache | 17 | NR |
| Nausea | 17 | 36 |
| Eye pain | 14 | 26 |
| Abdominal pain | 11 | 55 |

[a]From Teutsch et al. 1979
[b]From Benenson et al. 1982
[c]Not reported

cyst-induced infections (Teutsch et al. 1979; Benenson et al. 1982; Dubey and Beattie 1988; Smith 1993; Bowie et al. 1997; Burnett et al. 1998). Enlarged lymph nodes are the most frequently observed clinical form of toxoplasmosis in humans (Table 6.1). Lymphadenopathy may be associated with fever, fatigue, muscle pain, sore throat, and headache. Although the condition may be benign, its diagnosis is vital in pregnant women because of the risk to the fetus. In a British Columbia outbreak, of the 100 people who were diagnosed with acute infection, 51 had lymphadenopathy, and 20 had retinitis (Aramini et al. 1998, 1999). Encephalitis is the most important manifestation of toxoplasmosis in immunosuppressed patients as it causes the most severe damage to the patient (Dubey and Beattie 1988; Luft and Remington 1992). Patients may have headache, disorientation, drowsiness, hemiparesis, reflex changes, and convulsions, and many become comatose. Encephalitis caused by *T. gondii* is now recognized with great frequency in patients treated with immunosuppressive agents.

Toxoplasmosis ranked high on the list of diseases which lead to death of patients with AIDS and approximately 10% of AIDS patients in the USA, and up to 30% in Europe have died from toxoplasmosis (Luft and Remington 1992). In AIDS patients, although any organ may be involved, including the testis, dermis, and the spinal cord, infection of the brain is most frequently reported. Most AIDS patients suffering from toxoplasmosis have bilateral, severe, and persistent headaches, which respond poorly to analgesics. As the disease progresses, the headaches may give way to a condition characterized by confusion, lethargy, ataxia, and coma. The predominant lesion in the brain is necrosis, especially of the thalamus (Renold et al. 1992). Since the advent of highly active antiretroviral therapy (HAART) in the mid-

1990s, the number of AIDS patients suffering from toxoplasmic encephalitis has fallen dramatically, at least partially due to the impact of protease inhibitors used in HAART on *Toxoplasma* proteases (Palella et al. 1998; Pozio 2004; Pozio and Morales 2005).

## 6.8 Treatment and Prevention

Sulfadiazine and pyrimethamine (Daraprim) are two drugs widely used for treatment of toxoplasmosis (Guerina et al. 1994; Chirgwin et al. 2002). While these drugs have a beneficial action when given in the acute stage of the disease process when there is active multiplication of the parasite, they will not usually eradicate infection. It is believed that these drugs have little effect on subclinical infections, but the growth of tissue cysts in mice has been restrained with sulfonamides. Certain other drugs, like diaminodiphenylsulfone, atovaquone, spiramycin, and clindamycin, are also used to treat toxoplasmosis in difficult cases. To prevent infection of human beings by *T. gondii*, the hands of people handling meat should be washed thoroughly with soap and water before they go to other tasks (Dubey and Beattie 1988; Lopez et al. 2000). All cutting boards, sink tops, knives, and other materials coming in contact with uncooked meat should be washed with soap and water. Washing is effective because the stages of *T. gondii* in meat are killed by contact with soap and water (Dubey and Beattie 1988). *Toxoplasma gondii* organisms in meat can be killed by exposure to extreme cold or heat. Tissue cysts in meat are killed by heating the meat throughout to 67 °C (Dubey et al. 1990) and by cooling to −13 °C (Kotula et al. 1991). *Toxoplasma* in tissue cysts are also killed by exposure to 0.5 kilorads of gamma irradiation (Dubey and Thayer 1994). Meat of any animal should be cooked to 67 °C before consumption, and tasting meat while cooking or while seasoning should be avoided. Pregnant women, especially, should avoid contact with cats, soil, and raw meat. Pet cats should be fed only dry, canned, or cooked food. The cat litter box should be emptied every day, preferably not by a pregnant woman. Gloves should be worn while gardening. Vegetables should be washed thoroughly before eating because they may have been contaminated with cat feces. Expectant mothers should be aware of the dangers of toxoplasmosis (Foulon et al. 1994, 2000). At present there is no vaccine to prevent toxoplasmosis in humans.

## 6.9 Future Directions and Trends

Toxoplasmosis continues to be a significant public health problem worldwide. Although toxoplasmosis is estimated to have a disease burden and economic impact comparable to that of campylobacteriosis and salmonellosis, there are presently no explicit monitoring programs to screen animals entering the food chain and no

standardized reporting of human toxoplasmosis between different countries. The increasing demand for food safety together with the potential economic impact of legislation aimed at risk reduction has brought attention to the need for development and standardization of diagnostic tests for *Toxoplasma* infection. Such tests will need to provide an accurate estimate of risks of transmission of *Toxoplasma* to humans and must perform with comparable specificity and sensitivity across a range of animal species.

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# Chapter 7
# *Angiostrongylus* spp. of Public Health Importance

**Alexandre da Silva and Blaine A. Mathison**

## 7.1 Introduction

The genus *Angiostrongylus* includes two species with significant human public health impact, *A. cantonensis* and *A. costaricensis*. *Angiostrongylus cantonensis* is primarily associated with eosinophilic meningitis also known as cerebral angiostrongyliasis worldwide, whereas *A. costaricensis* has been reported in the Americas and is associated with abdominal angiostrongyliasis. The knowledge about the geographic distribution, some biological and epidemiological aspects that include prevalence in newly discovered intermediate hosts, as well as trends of angiostrongyliasis in different parts of the world has expanded dramatically in the last decades, especially in the case of *A. cantonensis*, with several thousand cases being reported worldwide showing high fatality rates in some countries (Wang et al. 2008, 2012; Epelboin et al. 2016). Cerebral angiostrongyliasis was thought to be restricted to Southeast Asia for decades, but outbreaks have been reported in countries where the disease was not previously considered endemic, becoming a potential global public health concern (Wang et al. 2008; Lindo et al. 2011; Thiengo et al. 2013; Dorta-Contreras et al. 2011; Espírito-Santo et al. 2013; Prociv and Carlisle 2001; Kwon et al. 2013). As a consequence, more attention has been given to cerebral angiostrongyliasis, and advanced studies focused on molecular diagnostics, genomics, and proteomics have been pursued by researchers worldwide. New molecular

A. da Silva, MSc, PhD (✉)
Foodborne Parasitology Laboratory – Office of Applied Research and Safety Assessment, Division of Food and Environmental Microbiology, CFSAN, U.S. Food and Drug Administration, Laurel, MD 240-402-3608, USA
e-mail: alexandre.dasilva@fda.hhs.gov

B.A. Mathison, BS, M(ASCP)
Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA 404-718-4103, USA
e-mail: gqa4@cdc.gov



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methods have been developed, and environmental studies using such tools were crucial to describe the presence of *A. cantonensis* in novel intermediate hosts. In addition, a draft of the *A. cantonensis* genome became available recently (Yong et al. 2015) which could be of crucial importance to better understand the parasite's biology and the pathology of cerebral angiostrongyliasis. These developments may have a significant impact on diagnosis, and novel diagnostic targets will likely be discovered. As the disease is expanding to urban areas (Espírito-Santo et al. 2013; Morton et al. 2013; Robinson et al. 2013), concerns about transmission of the disease through consumption of contaminated leafy greens or infected paratenic hosts will rise. In this chapter we summarize and discuss the trends in angiostrongyliasis with a strong focus on cerebral angiostrongyliasis due to the large public health impact that it may cause in the coming years.

## 7.2 Biology and Life Cycle

*Angiostrongylus cantonensis* has a complex life cycle requiring a mammalian definitive host (normally rats in the genus *Rattus*) and mollusks as intermediate hosts. Figure 7.1 illustrates the life cycle of *A. cantonensis* and *A. costaricensis.* When an infected mollusk is consumed by a rat, the third-stage larvae within the mollusk are released in the rat's stomach. From the stomach, the nematode larvae quickly move down the intestine, where they move through the walls of the small intestine into the blood and then enter the lungs and kidneys. The third-stage larvae are carried passively to the brain and molt to the fourth stage. After that they molt to the adult form, leave the brain, and enter the bloodstream where sexual maturation occurs. Mating occurs shortly thereafter, followed by production of eggs, which are released into the blood. The eggs lodge in the capillaries of the lungs and hatch, and then the first-stage nematodes break into the respiratory tract, migrate to the trachea, and are swallowed. After 40 to 50 days after being ingested, first-stage larvae are released in the rat's feces. The first-stage larvae are consumed by different species of mollusks, such as freshwater snails, land snails, and slugs or semi-slugs. The nematode larvae soon molt to the second and then third stage in the mollusk host. Several different species of mollusks have been identified as potential intermediate hosts for *A. cantonensis*, and this list is likely to increase as more research is conducted in different parts of the world. Humans are considered incidental hosts for *Angiostrongylus* spp. and may acquire the infection by eating raw or undercooked infected snails or slugs, paratenic (transport) hosts such as freshwater shrimp, frogs, monitor lizards, and flatworms (Planaria), or vegetables contaminated with these hosts or their secretions (Wang et al. 2008). The life cycle of *A. costaricensis* is similar, except that the adult worms reside in the arterioles of the ileocecal area of the definitive host. In humans, *A. costaricensis* often reaches sexual maturity and releases eggs into the intestinal tissues. The eggs and larvae degenerate and cause intense local inflammatory reactions and do not appear to be shed in the stool. Various animals can also act as paratenic (transport) hosts for *A. costaricensis* (Wang et al. 2008).

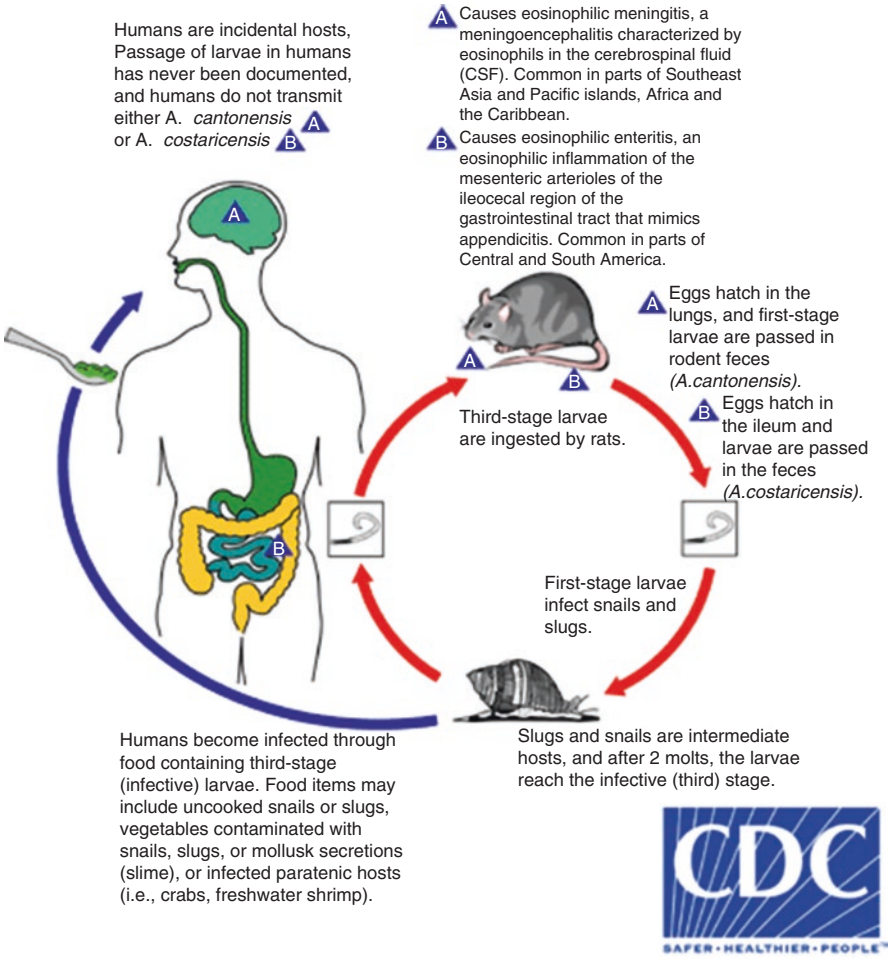


**Fig. 7.1** A simplified life cycle of *Angiostrongylus cantonensis* and *A. costaricensis* (Image is courtesy of the DPDx website from the Center for Disease Control and Prevention (DPDx, http://www.cdc.gov/dpdx/angiostrongyliasis/index.html))

The most recent findings on mollusks that have been identified worldwide as intermediate host for both *A. cantonensis* and *A. costaricensis* are summarized in Table 7.1. The dynamic of this emerging aspect is not well understood, and introduction of potential intermediate hosts in areas where the disease had not been reported is of concern. Also, addressing the transportation of infected definitive hosts from areas where the disease is considered endemic to areas where the disease has not yet been established should be the focus of public health efforts to control this disease globally.

**Table 7.1** List of mollusks (genera or species) that have been identified as being naturally infected with *A. cantonensis* in different geographic regions and potential association with outbreaks or sporadic cases if human disease

| Mollusk species | Region of the world or country | Associated with outbreaks or sporadic cases of infections | Type of mollusk (snail, slug or semi-slug) | Method used to identify the nematode | Reference(s) |
|---|---|---|---|---|---|
| *Achatina fulica*[a] | Brazil, China, and USA (Hawaii) and other Latin | Yes (in Brazil, China, and USA) | Land snail | Molecular and morphologic | Wang et al. (2008), Carvalho et al. (2012), Caldeira et al. (2007) |
| *Bradybaena circulus* | Not defined | No | Land snail | Molecular | Puthiyakunnon and Chen (2015) |
| *Bradybaena ravida* | Not defined | No | Land snail | Molecular | Puthiyakunnon and Chen (2015) |
| *Bradybaena similaris* | Latin America Australia, | No | Land snail | Molecular | Thiengo et al. (2013), Carvalho et al. (2012), Oehler et al. (2014) |
| *Cornu aspersum* | Australia | Yes | Land snail | Molecular | Oehler et al. (2014) |
| *Cyclophorus* sp. | Not defined | No | Land snail | | Puthiyakunnon and Chen (2015) |
| *Laevicaulis alte* | USA | No | Slug | Not defined | Rosen et al. (1962) |
| *Parmarion martensi* | USA (Hawaii) | Yes | Semi-slug | Molecular and morphologic | Dorta-Contreras et al. (2011), Rosen et al. (1962) |
| *Pili asp.* | Thailand | Yes | Freshwater snail | Morphologic | Puthiyakunnon and Chen (2015) |
| *Pleurodonte* sp. | Jamaica | No | Land snail | Morphologic | Robinson et al. (2013) |
| *Pomacea canaliculata* | China, Taiwan, and USA | Yes (China and Taiwan) | Freshwater snail | Molecular | Teem et al. (2013), Rosen et al. (1962), Yang et al. (2013), |
| *Pomacea insularum* | USA | No | Freshwater snail | Molecular | Rosen et al. (1962), Teem et al. (2013) |
| *Poteria* sp. | Jamaica | No | Land snail | Morphologic | Robinson et al. (2013) |
| *Sarasinula linguaeformis* | Brazil | Yes | Slug | Molecular | Carvalho et al. (2012) |
| *Sarasinula marginata* | Latin America (Brazil) | No | Slug | Molecular | Carvalho et al. (2012) |

(continued)

**Table 7.1** (continued)

| Mollusk species | Region of the world or country | Associated with outbreaks or sporadic cases of infections | Type of mollusk (snail, slug or semi-slug) | Method used to identify the nematode | Reference(s) |
|---|---|---|---|---|---|
| *Subulina octona*[a] | Brazil, Dominican Republic | Yes (case of *A. costaricensis* in Dominican Republic) | Snail | Molecular and morphologic | Carvalho et al. (2012), Vargas et al. (1992) |
| *Thelidomus aspera* | Jamaica | No | Land snail | Morphologic | Robinson et al. (2013) |
| *Veronicella cubensis* | USA (Hawaii), Jamaica | No | Slug | Molecular | Robinson et al. (2013), Dorta-Contreras et al. (2011) |

[a]Hosts also documented as being naturally infected with *A. costaricensis*)

## 7.3 Clinical Manifestations, Pathology, and Therapy

### *7.3.1* Angiostrongylus cantonensis

In humans the central nervous system (CNS) is the primary site for angiostrongyliasis caused by *A. cantonensis*. Cerebral angiostrongyliasis is often described as a mild, self-limited condition. However, severe cases including fatalities have been reported even associated with pediatric cases (Koo et al. 1988; Espírito-Santo et al. 2013; Qvarnstrom et al. 2016). It has been postulated that the severity of infection may be related to the size of the inoculum of infective larvae relative to the mass of the patient (Mackerras and Sandars 1955). The disease is well understood in the definitive host. The third-stage larvae (L3) that are ingested migrate to the brain and spinal cord where they molt to L4 larvae and then to L5 young adult worms (days 11–13). During this development they wander through the brain, sometimes emerging in the subarachnoid space. Young adult worms migrate to the pulmonary arteries via the cerebral venous system (Tangchai et al. 1967; Lindo et al. 2004). In humans most worms presumably die in the CNS before reaching the lungs, and therefore they do not complete the life cycle (Wang et al. 2008; Tangchai et al. 1967). Symptoms and clinical features in human disease are probably associated with the movement and death of the third-stage larva in the CNS as well as the immune response they provoke (Tangchai et al. 1967). There is a spectrum of disease produced when *A. cantonensis* invades the human CNS, but cases may go undiagnosed or present mild symptoms or even no symptoms. In cases where the infection produces clear symptoms, most patients will present with a meningitis

characterized by eosinophils in the CSF (Wang et al. 2008, 2012). In fact, *A. cantonensis* is considered the most common cause of eosinophilic meningoencephalitis in many countries like Taiwan (Tsai et al. 2001; Tsai et al. 2003), and infestations can produce encephalitis characterized by severe neurological symptoms, coma, and even death (Qvarnstrom et al. 2016, Yii 1976). Peripheral eosinophilia is also common in both angiostrongyliasis caused by *A. cantonensis* and *A. costaricensis*, and it is observed in up to 80% of patients with CNS infections associated with *A. cantonensis* and other neurotropic nematodes according to different reports (Yii 1976; Kwon et al. 2013). However, cerebral angiostrongyliasis is often suspected when eosinophilic pleocytosis is observed in cases of acute meningitis, particularly if accompanying risk factors, such as residence or travel to an area known to be endemic for the parasite, are also present. The main symptoms for adult patients with eosinophilic meningitis are headache, neck stiffness, paresthesias, vomiting, and nausea. Studies conducted in several different countries have shown that 95% of patients suffered from headache, 46% had mild neck stiffness, 44% suffered from persistent paresthesia, 38% had vomiting, and 28% had nausea (Wang et al. 2012; Puthiyakunnon and Chen 2015; Howe 2013). In addition, these symptoms could be accompanied by face or limb paralysis, photophobia, and diplopia. Infection of the optical nerve has been an important feature described in cases identified in various countries (see New Trends in Geographic Distribution of *A. cantonensis* in this chapter). Ocular infections may result in sequela such as loss of vision depending on the severity of the infection (Thiengo et al. 2013; Caldeira et al. 2007; Howe 2013). Continuous high intracranial pressure and corresponding damage to the brain and lung may precipitate unconsciousness, coma, and even death in severe cases (Graeff-Teixeira et al. 2009). The symptoms in children differ greatly from those in adults. Stiff neck and paresthesias are observed less frequently in children, but a high occurrence of nausea and vomiting is found, with 82% of pediatric patients having nausea and vomiting. The incidences of fever (up to 80%), somnolence (82%), constipation (76%), and abdominal pain (34.2%) are relatively higher in children than among adults.

Although the human infective dose is not known, 50 infective larvae have been used to infect rodents in experimental models. *Achatina fulica* were found to harbor a median of 5200 larvae in a study from American Samoa, and one snail contained 90,800 larvae (Sawanyawisuth and Kitthaweesin 2008).

Sometimes the acute clinical features may be helpful to distinguish cerebral infections caused by *A. cantonensis* and other nematodes that are associated with CNS infections, such as *Gnathostoma* spp. (Thiengo et al. 2013, Graeff-Teixeira et al. 2009, Ramirez-Avila et al. 2009a, b, Punyagupta et al. 1990). In cases of human gnathostomiasis, larvae invade human tissues; a classical presentation is a migratory panniculitis that consists of a nodule accompanied by itching. Due to the erratic migration of the *Gnathostoma* L3 larvae, the organism may cause CNS and eye infections. CNS gnathostomiasis may be even more severe than infections caused by *Angiostrongylus* sp. Usually CNS gnathostomiasis presents with radiculomyelitis, paresthesias of the trunk and extremities. Paresis or paralysis may occur in some cases. Another important aspect of the CNS gnathostomiasis is the presence

of severe headache, neck stiffness, convulsions, and vomiting (Graeff-Teixeira et al. 2009). However in general, gnathostomiasis often presents with migratory cutaneous symptoms prior to CNS invasion (and sometimes with radicular symptoms) where in angiostrongyliasis symptoms may start with headache or with paresthesias. Brain imaging may be helpful to differentiate the two diseases. Even though most meningitic angiostrongyliasis cases do not exhibit pathognomonic brain imaging signs, certain abnormal signs that are not specific for angiostrongyliasis may be found, such as periventricular linear hypersignal lesions, small nodules, or small hemorrhagic tracts (Graeff-Teixeira et al. 2009).

Unfortunately, symptoms are often not specific enough to differentiate between the diseases. Treatment for cerebral angiostrongyliasis is usually supportive with the use of analgesics for pain and corticosteroids to limit the inflammatory reaction. Careful removal of CSF at frequent intervals can help to relieve headache in patients with elevated intracranial pressure (Kwon et al. 2013). However, it is an invasive procedure with potentially serious complications, such as CSF leak, worsening headache, and brain herniation. The risks versus benefits of repeated collection of CSF must be carefully considered for each patient before recommended. Multiple collections of CSF may also benefit laboratory diagnosis. It is believed that testing of multiple CFS samples collected during the course of the infection may result in a more specific and sensitive diagnostic testing results (Qvarnstrom et al. 2016). No antihelminthic drugs have been proven to be effective in treatment, and there is concern that antihelminthics could exacerbate neurological symptoms due to a systemic response to dying worms. Additional symptomatic treatment may also be required for nausea, vomiting, and in some cases chronic pain due to nerve damage and muscle atrophy.

Mild cases may resolve spontaneously without specific therapy (Tu and Lai 2006; Slom and Johnson 2003; Sawanyawisuth and Sawanyawisuth 2008; Kwon et al. 2013). When recomended, more serious cases can be improved by careful removal of CSF at frequent intervals can help to relieve headache in patients with elevated intracranial pressure. Therapeutic LPs were helpful in one patient for symptomatic relief as described by Kwon et al. (2013). Severe cases can develop permanent, neurologic sequelae or progress to coma and death, so specific treatments to reduce morbidity and mortality would be welcome.

Evidently, the key issue in treatment of angiostrongyliasis is the control of inflammation. There is an urgent need for extensive studies of new approaches to chemotherapy and evaluation of neurological relapses. Steroids have been used to help decrease intracranial pressure and blunt the immune response (Punyagupta et al. 1975; Tu and Lai 2006; Chotmongkol et al. 2000; Chotmongkol et al. 2009).

Therapy of severe disease has not been studied systematically, and there are still a number of scientific knowledge gaps that need to be addressed (Tsai et al. 2001; WC and Lai, 2006; Jitpimolmard et al. 2007). It is possible that not all treatments described in the literature work for all patients, and this may be related to several different aspects: (1) burden of infection and size of the inoculum, (2) host-parasite interactions, and (3) genetics of the nematode. Studies focusing on the parasite's genome and transcriptome as well as proteomics studies will be crucial to address

several knowledge gaps under items 2 and 3. Yet, there are a few convincing studies of therapy for cerebral angiostrongyliasis that support the idea that populations demonstrate different severities of illness, related to the size of the inoculum of *A. cantonensis* L3 larvae ingested. For example, adult individuals from Thailand that consumed *Pila* or *Pomacea* snails, with a relatively low larval burden, tend to have milder disease, whereas ingestion of the giant African snail (*Achatina fulica*) with its high inoculum can lead to severe or fatal disease (Murphy and Johnson 2013; Caldeira et al. 2007).

### *7.3.2* Angiostrongylus costaricensis

*Angiostrongylus costaricensis* causes enteritis which may mimic appendicitis. The severity of clinical features is dependent on worm burden, location in the host, host susceptibility, and immune response. Infections can be fatal. Patients will present with fever but rarely chills. Pain is usually concentrated in the right iliac fossa, right flank, or right upper quadrant. Eosinophilia is usually present. There are two pathologic presentations with *A. costaricensis*. One is due to the adult worms living in the mesenteric arteries inducing thrombosis and necrosis of tissues formerly supported by the arteries. In the other, the presence of eggs, larvae, and metabolic by-products elicits inflammatory reactions. Unlike with *A. cantonensis*, adults of *A. costaricensis* will develop to sexual maturity in the human host, and lesions may contain adults, larvae, and eggs in all stages of development. Histologically, there are three hallmark findings: (1) massive infiltration of eosinophils in the mucosa and submucosa, (2) granuloma formation consisting of epithelioid cells, giant cells, and eosinophils, and (3) eosinophilic vasculitis (Kramer et al. 1998). Additional information about treatment of abdominal angiostrongyliasis can be found on the CDC website (DPDx, https://www.cdc.gov/dpdx/angiostrongyliasis/index.html).

Diagnosis of *A. costaricensis* is made primarily by the morphologic identification of adult and larval worms and eggs in tissue, in conjunction with clinical manifestations. Eggs and larvae are not seen in human stool (Morera et al. 2000; Orihel and Ash 1995).

## 7.4 Geographic Distribution of *Angiostrongylus* spp.

Based on recent reports it seems that disease caused by *A. costaricensis* has not significantly expanded to areas of the world outside the Central and South America, with few exceptions. Two cases of children who did not travel overseas were described in the USA in the early 1990s (Hubert et al. 1992). One case was in an 11-year-old boy who presented with fever and abdominal pain. Examination of histologic sections obtained from the patient's small intestine demonstrated the presence of degenerated nematodes. Despite the condition of the specimen, the worm

was identified as *Angiostrongylus* sp. based on the few morphological features available. This child also had a positive reaction in a serological test using crude antigen of *A. cantonensis* performed at the Centers for Disease Control and Prevention. In the second case the evidence of infection was provided by a positive serological testing result using the same test as in the first case, although the patient did not present any symptoms of abdominal angiostrongyliasis.

Although for many years it was believed that cerebral angiostrongyliasis was restricted to Asia and possibly Australia, a significant increase in the number of cases of cerebral angiostrongyliasis reported in different parts of the world started to take place in the 1980s. For several decades the public health concern in Latin American countries focused on the disease caused by *A. costaricensis*. Sporadic outbreaks of eosinophilic meningitis caused by *A. cantonensis* were first reported in Latin America in the late 1980s with five cases of cerebral angiostrongyliasis being described in Havana, Cuba (Pascual et al. 1981). Locally acquired human cases or infected rodents or mollusks have now been reported in countries from different continents including Cuba, Brazil, Dominican Republic, Ecuador, Haiti, Jamaica, South Africa, Australia, Tahiti, French Polynesia, and the USA (a few cases in continental USA and several cases in Hawaii) (Wang et al. 2008; Lindo et al. 2011; Prociv and Carlisle 2001; Kwon et al. 2013; Caldeira et al. 2007; Pascual et al. 1981; Raccurt et al. 2003; Vargas et al. 1992; Pincay et al. 2009a, b; Acha and Szyfres 2003; Thiengo et al. 2010; Cowie 2013; Evans-Gilbert et al. 2014). In Jamaica, investigations were pursued to assess the presence of *A. cantonensis* in the environment following a foodborne outbreak of eosinophilic meningitis that included severe cases of infection in the mid-2000s. Through these efforts, researchers examined 437 wild rats representing two species (i.e., *Rattus rattus* and *R. norvegicus*) and over 700 hundred snails and slugs representing 12 species. Adults of *A. cantonensis* were recovered from the cardiopulmonary system of 32.0% of the wild rats examined. Data from the investigation showed that *A. cantonensis* occurred significantly more frequently in *R. rattus*. From 777 snails and slugs examined, 12.5% harbored *A. cantonensis*, including *Thelidomus aspera* (18.7%, $n = 369$), *Pleurodonte* sp. (29%, $n = 86$), *Sagda* sp. (11%, $n = 18$), *Poteria* sp. (20%, $n = 5$), and veronicellid slugs (6%, $n = 34$) (Table 7.1). All four genera of snails represent newly reported hosts for *A. cantonensis*. The occurrence of the parasite, combined with reports over the last 20 years of infections in humans who never traveled abroad, indicates that autochthonous transmission is occurring and that *A. cantonensis* represents an emerging infection in Jamaica (Lindo et al. 2004, 2011; Robinson et al. 2013).

The first report of this zoonosis in Brazil was in the municipality of Cariacica, Espírito Santo State, with subsequent reports from two municipalities (Olinda and Escada) in Pernambuco State and in the city of São Paulo, São Paulo State (Thiengo et al. 2010, 2013; Espírito-Santo et al. 2013; Caldeira et al. 2007). In the first two states, naturally infected definitive and/or intermediate hosts had been discovered during the epidemiological investigation of the human cases. *Achatina fulica* was considered the vector in three of the four reported cases. One of the cases was attributed to ingestion of undercooked apple snails (*Pomacea lineata*). Specimens of *A. fulica* have been found infected with *A. cantonensis* larvae in south and southeastern Brazil

since 2007 and more recently from the Amazon region of northern Brazil. Cerebral angiostrongyliasis was reported for the first time in the city of São Paulo, Brazil, in an 11-year-old child in 2010. The case was confirmed using serological methods (Espírito-Santo et al. 2013).

In Ecuador the disease was first discovered in the late 2000s, and after that outbreaks and sporadic cases have been reported and investigated in the country (Pincay et al. 2009a, b; Dorta-Contreras et al. 2011). In Ecuador it is very common to eat raw mollusks and animals that can serve as paratenic hosts for the parasite mostly prepared as *ceviche*. The cases reported in Ecuador included severe cases with some being fatal. In one of the severe cases, the L5 larva was identified in the subarachnoid space of a deceased patient (Pincay et al. 2009a). Because the disease was not well studied until recently, health authorities and researchers believe that the disease is more prevalent than initially estimated.

A few cases of *A. cantonensis* infection acquired in the continental USA have been reported (New et al. 1995; Al Hammoud et al. 2017; Cowie 2017; Flerlage et al. 2017). In contrast, it has been recognized in Hawaii since the late 1950s, and the parasite has been considered endemic there since. An epidemic of eosinophilic meningitis occurred in Hawaii in 1958 and was presumed to have been caused by a parasitic infection, but the actual cause of the cases was not determined. In the course of this investigation, it was reported that two patients had died at the State Mental Health Hospital in O'ahu due to eosinophilic meningitis. The preserved brain of one patient yielded a number of young adult nematodes identified as *A. cantonensis*; the other contained possible nematode remnants (Rosen et al. 1962). However, infections were rarely reported until late 2004, when a case cluster was noted on the Island of Hawaii (Hochberg et al. 2007). Infection caused by *A. cantonensis* has continued to be diagnosed throughout the state, especially on the Island of Hawaii. After five cases of cerebral angiostrongyliasis were reported within a 4-month period, the health authorities at the state level made angiostrongyliasis a reportable disease in 2007 (Kwon et al. 2013; Howe 2013; Qvarnstrom et al. 2015). An important discovery in Hawaii was the novel intermediate host, the semi-slug *Parmarion martensi* (Hollingsworth et al. 2007). This semi-slug, which was probably introduced in Hawaii in the 1960s, is abundant in residential areas and seems to always be heavily infected with the nematode (Qvarnstrom et al. 2007, 2010). Education of physicians and the public on the prevention and control of this emerging infection is still needed (Hochberg et al. 2007).

Angiostrongyliasis may be quite widespread in Pacific islands, and more investigations are needed to ascertain the prevalence of the disease in this area of the world. Recently 42 cases of eosinophilic meningitis were investigated in French Polynesia (Oehler et al. 2014) that could be caused by *A. cantonensis*. The patients were either diagnosed using a serological test or had risk factors that supported *A. cantonensis* meningitis, e.g., consumption of or prolonged contact with an intermediate/paratenic host.

Rodents infected with *A. cantonensis* have been identified in South Africa. In a single study from 391 *Rattus norvegicus* and 7 *Rattus rattus*, 55 and 1, respectively, were found harbor the parasite. Adult worms were isolated from the heart,

pulmonary arteries, and arterioles, giving a prevalence of 14.1%. In this study the parasite was confirmed to the species level by the use of polymerase chain reaction (PCR) (Archer et al. 2011). Nevertheless, analysis of mollusks or paratenic hosts for the presence of the parasite has not been pursued, and human cases of cerebral angiostrongyliasis have yet been reported in South Africa.

In Australia, cerebral angiostrongyliasis had been documented since the late 1950s in Brisbane with increasing prevalence until the 1970s (Gutteridge et al. 1972). Recently, severe cases have been reported in areas in the country believed to be free of angiostrongyliasis. The first reported case of angiostrongyliasis in Sydney occurred in 2001 (Pryor et al. 2003), in a circumstance of a young man accepting a dare to eat a slug. A number of severe cases were recently reported, which prompted researchers to initiate studies to evaluate the prevalence of *A. cantonensis* in the mollusk population in Sydney (Pryor et al. 2003; Blair et al. 2013; Cooke-Yarborough et al. 1999; Chan et al. 2015). These surveys confirmed the presence of the parasite in terrestrial mollusks by identifying at least two species commonly found in Sydney area that could serve as intermediate host for *A. cantonensis*, i.e., *Bradybaena similaris* and *Cornu aspersum* (Chan et al. 2015).

## 7.5 Food Safety

The rat lungworm, *A. cantonensis*, uses various species of rats as its definitive hosts and a wide range of slug and snail species as its intermediate hosts (Wang et al. 2008). These infected animals when ingested by humans may cause mild to severe eosinophilic meningitis requiring medical care (Puthiyakunnon and Chen 2015). Although consumption of infected mollusks is still considered the major source of foodborne angiostrongyliasis in Southeast Asia, the parasite can also contaminate fresh produce and water, and these aspects have not been well studied. Therefore, there is a need to gather more data to fully understand the food safety impact that *Angiostrongylus* sp. might have, including (1) estimation of the parasite prevalence in areas of the world where the disease was not considered to be endemic, (2) systematic studies and surveys to show how frequently the parasite is found in foods, and (3) surveillance where the disease has recently been established. These three aspects are especially important to consider in areas of the world where uncooked mollusks that carry the parasite are not typically intentionally consumed as foods.

Due to food consumption trends and global transportation of food products, it is expected that the disease will be more prevalent worldwide in the future (Puthiyakunnon and Chen 2015; Howe 2013). Travelers to areas where the parasite is present can also be exposed to the parasite through contaminated mollusks or may acquire infection through consumption of foods or possibly water. Because the disease is now considered to be found worldwide, increasing the public awareness in areas even where the disease is not considered endemic, regarding the modes of transmission of *A. cantonensis* and its potential for damage to the health of the general population, is essential to control the spread of the disease.

Consumption of undercooked snails or slugs should always be considered as high risk for acquiring *A. cantonensis* or *A. costaricensis* infection. Recommending adequate cooking before eating snails, slugs, small mollusks, and paratenic hosts of *A. cantonensis*, such as frogs, shrimp, land crabs, and monitor lizards, and eradicating terrestrial mollusks that can serve as hosts near houses and vegetable gardens might reduce the spread of this parasite to humans living in endemic areas. Nevertheless, there is no consensus about the risk of acquiring angiostrongyliasis through consumption of foods that do not fall into the category of mollusks. In the USA the impact of *Angiostrongylus* as a foodborne pathogen has not yet been ascertained. However, cases of cerebral angiostrongyliasis reported in Hawaii seem to be associated with consumption of raw vegetables contaminated by infected terrestrial mollusks rather than consumption of the mollusks themselves (Hollyer 2013). It is also possible that irrigation of produce with water contaminated by infected mollusks could be a way for contamination of food commodities. Ascertaining all these aspects is very challenging because the disease is underdiagnosed and underreported worldwide. In addition, testing of foods for the presence of *Angiostrongylus* sp. is not performed routinely. Although there are molecular methods for the detection of *A. cantonensis*, none have been validated for detection of the parasite in food. Different food samples (e.g., different types of produce) need to be included in analytical studies to evaluate the usefulness of these methods for the detection of the parasite in foods.

However, consumers should exercise caution when consuming fresh produce from local growers; e.g., all vegetables should be inspected for signs of snail or slug activity and discarded if there is any damage. Intact plants, for leafy vegetables, such as lettuce and any leafy green vegetables that are generally consumed raw or lightly cooked, should be pulled apart and inspected. Slugs and snails, especially very small juveniles, can burrow deep among the leaves and stalks of the plant. Simply rinsing off the outer leaves of produce and then chopping the entire plant, for example, for a fresh salad, might not be enough to get rid of slugs/snails that might be present in the vegetable. In fact, this practice may lead to dicing up a live slug or snail; if infected, it will probably spread larva to other sections of the plant. Once the plant has been disassembled and inspected thoroughly, all surfaces should be well rinsed with drinkable, running, cool water. Although this is believed to be the most effective way to reduce risk, it is not absolutely guaranteed. If a water sanitizing or produce cleaning product is used, it may or may not reduce the risk of a slug, snail, or the nematodes themselves being on the food that has been prepared and is about to be served.

Unlike pesticides used against other crop and garden pests, which can be spread over an entire field or plant, pesticides for killing rats and slugs and snails are more targeted and come in bait forms. Therefore, the best way to control or eliminate the risk of infection is through the control of rats and mollusks in the production and processing areas. These procedures are generally less effective, and the baits can be highly susceptible to disintegrating in heavy rain (Hollyer 2013). Angiostrongyliasis may currently not be perceived as a major foodborne threat in the USA. This could change in the future as additional data about the prevalence of the disease becomes available through studies, surveys, and investigations conducted worldwide.

## 7.6 Trends in Laboratory Diagnosis

Diagnosis of angiostrongyliasis is complicated due to the difficulty of detecting the *Angiostrongylus* larvae themselves and will usually be made based on the presence of eosinophilic meningitis and history of exposure to snail hosts. Eosinophilic meningitis is generally characterized as a meningitis with ≥10 eosinophils/μL in CSF or at least 10% eosinophils in the total CSF leukocyte count. Morphology is not always practical for diagnosis of *A. cantonensis*. Because *A. cantonensis* is not reliably observed in the CSF of patients with angiostrongyliasis, except in some very severe cases (Pincay et al. 2009a; Petjom et al. 2002; Kuberski et al. 1979; Qvarnstrom et al. 2015), laboratory diagnosis has historically relied on immunodiagnostic methods to detect parasite-specific antibodies. Immunodiagnostic methods for angiostrongyliasis were employed in the 1960s soon after *A. cantonensis* was determined to be the probable etiologic agent of eosinophilic meningitis in Asia and the Pacific. Results were indicative of angiostrongyliasis if the reaction to adult *A. cantonensis* extracts was three times greater than the reactions to a phosphate buffer control and to *Gnathostoma spinigerum*, *Paragonimus westermani*, *Dirofilaria immitis*, and *Toxocara canis* antigens. Positive reactions were frequently elicited in asymptomatic individuals or patients with other parasitic infections (Welch et al. 1980). Qvarnstrom et al. (2015) reported a case of infection in which the parasite was found in CSF, with a positive result in PCR but negative by the serological method used in the study. The fact that the serological method missed that case shows that more than one technique should be recommended for diagnosis. In the 1970s a number of methods to detect *Angiostrongylus*-specific antibodies were developed, including indirect hemagglutination, complement fixation, indirect fluorescent antibody staining of frozen worm sections, lipid extracts of adult *A. cantonensis*, and latex agglutination tests (Welch et al. 1980). Enzyme-linked immunosorbent assay (ELISA) methods were developed in the late 1970s and used crude antigen extracts prepared from young adult *A. cantonensis* (Cross and Chi 1982). Specificity represented an issue in antibody detection and individual protein antigens that might be more specific than total worm extracts were identified. A number of immunoblot studies were pursued focusing on 29 kDa and 31 kDa proteins that were present in adult worm preparations (Akao et al. 1992; Eamsobhana et al. 1995; Nuamtanong 1996; Maleewong et al. 2001; Sawanyawisuth et al. 2011). Based on these few studies, the 31 kDa protein seemed to be more specific than the 29 kDa protein, with minimal cross reactivity. Serum or CSF specimens could be used for detection, but there is still some debate regarding which type of protein-based approach should be more adequate for the diagnosis of angiostrongyliasis. It is possible that antigen detection in serum or CSF may be more reliable than antibody detection for diagnosis of angiostrongyliasis, but antigen detection methods have not been adopted for clinical diagnostic use (Wilkins et al. 2013).

The genome of *A. cantonensis* has been sequenced, and this will provide new opportunities to identify new proteins that could be used to develop efficient serological tests (Yong et al. 2015). The lack of such diagnostic tools has hampered the ability of studying all the risk factors that could be associated with the transmission

of this disease. The specificity and sensitivity of the available techniques have not been evaluated and these methods have not been validated for clinical diagnosis, limiting the use of these methods to research only (Morassutti et al. 2013a). Serological tools with reasonable sensitivity and specificity should be developed that will allow studies to be conducted to estimate the level of exposure of the human population that is considered to have a high burden of the disease. In addition, a study using the dirty genome sequence approach, which is based on random genome sequencing, has identified novel protein targets that could be superior to the current antigens for diagnostic purpose (Morassutti et al. 2013b). Using the *A. cantonensis* genomic sequences, 156 putative open reading frames, matching peptide sequences obtained from previous proteomic studies, were considered novel, with no homology to existing sequences. Combined with the whole-genome data available, these findings can reveal a significant number of novel proteins that could be used in diagnostics. Studies need to be conducted to evaluate these protein targets for their usefulness in clinical diagnosis.

A conventional PCR technique that amplified a 1134 bp fragment of the 18S rRNA was developed to be used in environmental surveys for detection of the genus *Angiostrongylus*. The method was not specific for the identification to the species level and will amplify both *A. cantonensis* and *A. costaricensis*. It required DNA sequencing analysis to identify the *Angiostrongylus* to the species level (Qvarnstrom et al. 2007). Shortly after that, a qPCR assay targeting the internal transcribed spacer-1 (ITS1) was developed for the detection of *A. cantonensis* in invertebrate hosts, also with the purpose of use in environmental surveys (Qvarnstrom et al. 2010). Although the authors indicated in their study that the ITS1-qPCR was specific for the detection of *A. cantonensis*, another study has shown that this technique amplifies *A. mackerrasae*, which at least in Australia is associated with cerebral angiostrongyliasis (Chan et al. 2015). However, recent findings demonstrated that the two species are identical at the 18SrRNA gene sequence level with almost identical ITS1 sequences. Because of that, it will probably be very difficult to use these genetic markers to differentiate the two species. At the time Qvarnstrom et al. published their study in 2010, no *A. mackerrasae* sequences were available in GenBank, a major hurdle that was impossible to overcome. Further comparative genomics studies are needed to fully ascertain the level of genetic similarity between these two species. In addition, the ITS1-qPCR test has not yet been widely used for screening of intermediate and paratenic hosts for the presence of *A. cantonensis*. Its efficacy for this purpose will require additional environmental studies.

The qPCR method is advantageous because it can be used to provide a semiquantitative perspective of the infection. In one study it was shown that samples containing a single stage-three larva are positive after an average of 23 PCR cycles (i.e., average Ct value of 23) (Qvarnstrom et al. 2016). Human samples analyzed in this study had Ct values between 27 and 38, suggesting that these samples contained 1–4 $\log_{10}$ less DNA than that present in a specimen containing a single larva. This indicates that qPCR can actually detect DNA from remnants of larvae, such as individual cells, nuclei, or chromosomal fragments, which may leak into the CSF from

the brain. Presence of DNA-containing remnants is the likely reason why so many CSF samples where no intact parasites could be found can be positive by the use of PCR assays. Such assays are not fully validated for clinical use at this time, but DNA-based tests represent an improvement in diagnostic detection as was observed in some cases. Cerebral angiostrongyliasis is difficult to diagnose clinically, and in most cases, symptoms are nonspecific and the CSF reveals eosinophilia, but no larvae are detected. When available, serum samples should be tested for the presence of antibodies against *A. cantonensis* using a crude antigen ELISA and/or WB technique that detects the 31 kDa antigen to strengthen the diagnostic findings. In certain cases more than one molecular test can be used to confirm initial diagnostic findings when molecular and serologic test results do not match. For instance, the CSF samples from a patient that tested negative by qPCR were also tested by a DNA-based test targeting a polymorphic fragment the *A. cantonensis* cytochrome oxidase unit I gene (COI) (Qvarnstrom et al. 2016). The approach relied on conventional PCR amplification followed by DNA sequencing analysis of the amplified fragment. In this report one patient that tested positive in qPCR but negative for antibodies and was initially considered a potential false-positive was verified using the COI methodology, and the additional testing confirmed that this CSF sample contained *A. cantonensis* DNA, so the explanation for the discordance was more likely failed antibody detection. The study by Qvarnstrom et al. (2016) illustrates that combination of clinical diagnosis with the use of laboratory molecular and immunological tests should be recommended for a specific and sensitive diagnosis of cerebral angiostrongyliasis.

PCR has the potential to become positive earlier than serology because *A. cantonensis* DNA has the potential to be present in the CSF in the acute phase, prior to the development of antibodies, which is useful for diagnosis of human cases, especially severe cases and with a potential application in outbreak investigations. However, the sensitivity and specificity of PCR need to be compared to serological methods in order to ascertain its usefulness. In general, it is believed that serology will be more important for prevalence studies which will help to strengthen prevention.

On the other hand, the opposite can also be observed; i.e., patients can be negative by molecular tests and positive in antibody detection. Studies are needed to better estimate what is the actual immunological window and how long specific antibodies last in the bloodstream of infected individuals. These cases will be more challenging to confirm, considering that microscopic examination is much less sensitive than molecular tests. Thus, collection of additional CSF samples should be recommended if the initial test results are negative and the clinical suspicion remains high. That strategy combined with the use of multiple tests could provide very specific and sensitive diagnosis of cerebral angiostrongyliasis. More systematic studies, comparing standardized protocols, will be required to better understand the diagnostic value of different methodologies and how to generate robust diagnostic algorithms that will rely on multiple methodologies to provide sensitive and specific diagnosis of angiostrongyliasis.

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# Chapter 8
# Anisakiasis

**Blaine A. Mathison and Alexandre da Silva**

## 8.1 Introduction

Anisakiasis is a zoonotic infection caused by nematodes in the genera *Anisakis*, *Pseudoterranova*, and *Contracaecum*, with most human cases being caused by members of the first two. The first human cases were described in 1960 (Kuipers et al. 1960; Rodenburg and Wieslinga 1960), and the pathology in humans was first described by Asami et al. (1965). The definitive hosts in nature are marine mammals (all three genera) or fish-eating birds (*Contracaecum*). Humans are incidental, dead-end hosts for anisakid nematodes and become infected after eating undercooked fish or mollusks containing infective third-instar (L3) larvae. Anisakid nematodes occur nearly worldwide in fish in marine and brackish environments, and a low host specificity among paratenic fish and mollusk hosts means the risk of infection can be high in populations and individuals who engage in the ingestion of undercooked seafood.

B.A. Mathison, BS, M(ASCP) (✉)
Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, 1600 Clifton Road NE, Atlanta, GA 30329, USA
e-mail: blaine.mathison@aruplab.com

A. da Silva, MSc, PhD
Parasitology Laboratory – Office of Applied Research and Safety Assessment, Division of Virulence Assessment, CFSAN, U.S. Food and Drug Administration, 8301 Muirkirk Rd, Laurel, MD 20878, USA
e-mail: alexandre.dasilva@fda.hhs.gov



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## 8.2 Biology and Life Cycle

In the definitive hosts of anisakid nematodes, the adult worms attach to the gastric mucosa, and embryonated eggs containing third-instar (L3) larvae are shed in the feces (Køie et al. 1995). Eggs hatch in the water, and the free-swimming L3 larvae are ingested by crustaceans (e.g., copepod, krill), the intermediate host, in which they grow in the hemocoel (Mattiucci et al. 2015). Fish or mollusks become infected after eating infected crustaceans; the L3 larvae become encapsulated in the host tissue and do not develop further. Because parasite development does not occur in the piscine and molluscan hosts, they are considered paratenic hosts. Infected smaller fish may be eaten by larger fish, and the latter may serve as a new paratenic host. Definitive hosts become infected after eating infected intermediate or paratenic hosts. L3 larvae mature in the stomach (or intestine) of the definitive host and develop into sexually mature males and females. Humans become infected after ingesting improperly or undercooked infected paratenic hosts containing viable L3 larvae. Humans are dead-end hosts for anisakid nematodes and further development cannot take place in the human host (DPDx 2015). A large number of fish and mollusk species may serve as paratenic hosts, depending on the species of anisakid (Mattiucci et al. 2015), and common sources of human infection include salmon, cod, red snapper, grouper, and squid (see also Epidemiology, below) .

## 8.3 Clinical Manifestations

There are four main clinical presentations of anisakiasis: (1) gastric, (2) intestinal, (3) extra-gastrointestinal/ectopic, and (4) acute allergic response.

In cases of gastric anisakiasis, clinical symptoms usually develop when the worms attempt to invade the mucosa, often as early as within a few hours. In many cases, the worms migrate back up the esophagus and are expelled alive out of the nose or mouth, although they may lodge in the esophagus, causing pain and difficulty swallowing. While unpleasant for the patient, this is the best clinical outcome as removal of the worm is curative. Intestinal manifestations also do not usually occur unless the worms proceed to burrow into the intestinal mucosa. Symptoms may take up to a week or so to develop and include nausea, vomiting, abdominal and epigastric pain, fever, and diarrhea with blood and mucus (Rodenburg and Wieslinga 1960). Symptoms may also mimic angina-like chest pain (Machi et al. 1997). Leukocytosis and eosinophilia may develop within the first couple weeks of infection. Worms that penetrate the mucosa may cause peritonitis or bowel obstruction (Shweiki et al. 2014).

Worms may also migrate to ectopic sites, including the peritoneum (Akbar and Ghosh 2005), mesenteric mass (Cespedes et al. 2000), omental nodules (Cancrini et al. 1997; Takekawa et al. 2004), mesocolic lymph nodes (Kim et al. 1997), spleen (Testini et al. 2003), pancreas (Yamoto et al. 2014), and perimetrium (Ramanan

et al. 2013), where they are often discovered incidentally during the pursuit of other diagnoses (e.g., tumors). Usually within six months, the larvae die in these sites. The patients' history (onset of symptoms following ingestion of raw seafood) can be helpful in supporting the histopathologic diagnosis. Histologically, worms in tissue are usually surrounded by many inflammatory cells, including eosinophils (Andersen and Lichtenfels 2000).

There has been increased awareness in recent years to acute and chronic allergic reactions associated with eating seafood containing anisakid nematodes (Sakanari and McKerrow 1989; Audicana and Kennedy 2008; Daschner et al. 2012). Acute allergic anisakiasis may or may not be accompanied by gastrointestinal symptoms, and the worm need not be viable. Clinical manifestations may range from urticaria and angioedema (Daschner et al. 2012) to anaphylactic shock (Audicana et al. 2002). The relationship of anisakid nematodes to allergic reactions emphasizes the importance of obtaining accurate and complete patient food histories, and *Anisakis simplex* is now included in routine batteries for the investigation of food allergies (Audicana et al. 2002). However, it should be noted that ingestion is not always required, as fish handlers (Antibarro and Seoane 1998) and food preparers (Armentia et al. 1998) are at risk for conjunctivitis and protein contact dermatitis from handling infected fish.

Clinical diagnosis is usually made by the morphologic analysis of intact, often live, worms expelled out of the nose or mouth, or rarely, passed in stool. Endoscopy may be necessary to remove worms that have embedded in the gastric or intestinal mucosae. Worms in ectopic sites are usually found incidentally in biopsy sections collected for other diagnoses (DPDx 2015). Serologic assays are available to aid in the diagnosis of anisakiasis, although not readily available in the United States (Rodero et al. 2007; Arilla et al. 2008). Diagnosis of allergic reactions to *A. simplex* is a positive skin prick test, based on having compatible clinical manifestations following consumption of fish, and confirmed by specific IgE antibodies against *A. simplex* using additional assays (e.g., radioimmunoassay) and a lack of reaction to proteins from the host fish (Audicana et al. 2002). Molecular assays are useful for epidemiologic studies, but are not typically used for primary clinical diagnosis and patient management.

Identification to the genus or species level is not needed for patient management but may be employed for epidemiologic purposes. In most cases, removal is curative although there has been successful treatment reported with albendazole in three patients with a highly suspicious history or serologic results supporting a diagnosis of anisakiasis (Pacios et al. 2005; DPDx 2015).

## 8.4 Epidemiology

Human anisakiasis is usually attributed to *Anisakis simplex* and *Pseudoterranova decipiens*, and more often than not, simply *Anisakis* sp. As relatively few clinical cases are completely or accurately identified to the species level, the full range of

species that can cause clinical disease in the human host is unknown. Species-level delineation of anisakid nematodes is not fully understood, but molecular evidence, and multilocus alloenzyme electrophoresis in particular, supports the presence of at least nine distinct species of *Anisakis* (Mattiucci et al. 2005, 2015) and at least six distinct species of *Pseudoterranova* (Paggi et al. 1991; Nadler et al. 2005). There are over 100 described species of *Contracaecum*, although the taxonomic status of many remains unclear (Shamshi et al. 2009). It is clear that a better understanding of the precise parasite species, as well as the paratenic host source, from clinical cases is needed to better understand the epidemiology of this disease. For example, it is generally believed that *Pseudoterranova* spp. cause primarily gastric anisakiasis, while *Anisakis* spp. cause gastric, intestinal, and ectopic infections (Chai et al. 2005). A better understanding of the parasite species involved, and in turn its paratenic host(s), could also help better understand the risk of particular food items to the human consumer.

Anisakid nematodes are nearly cosmopolitan in distribution and in general have a low host specificity. Well over 100 species of fish and squid are known to serve as paratenic hosts for *Anisakis* spp. (Mattiucci et al. 2015), and over 70 species of fish and invertebrates are known paratenic hosts for *Pseudoterranova* spp. (ICES 2012).

In the continental United States, Alaska, and Canada, food sources for *Anisakis* include salmon (Deardorff and Kent 1989), Pacific herring (Moser and Hsieh 1992; Richard 2003), Atlantic cod (Chandra and Khan 1988; Richard 2003), Pacific cod (Munger 1983), Arctic cod (Køie et al. 2008), American shad (Shields et al. 2002), mackerel (Deardorff et al. 1991; Richard 2003), tuna (Richard 2003), halibut (Munger 1983; Deardorff et al. 1991), flounder (Munger 1983), sole (Munger 1983), rockfish (Munger 1983; Deardorff et al. 1991), sablefish (Munger 1983), squid (Deardorff et al. 1991), and clams (Shweiki et al. 2014). Cod and red snapper are the major sources of *Pseudoterranova* (Oshima 1987). In Mexico, sources of *Pseudoterranova* include barracuda and red grouper (Laffon-Leal et al. 2000).

*Pseudoterranova* has been reported in at least 40 species of marine fish off the coasts of South America (Hernández-Orts et al. 2013). Among them are sea robins (Hernández-Orts et al. 2013), Brazilian flathead (Hernández-Orts et al. 2013), Argentinian sea bass (Hernández-Orts et al. 2013), bluefish (Borges et al. 2015), flounder (Hernández-Orts et al. 2013), corvina drum (George-Nascimento and Urrutia 2000; Weitzel et al. 2015), Argentinean sand perch (Hernández-Orts et al. 2013), largehead hairtail (Borges et al. 2015), black cusk-eel (George-Nascimento and Urrutia 2000; Weitzel et al. 2015), hake (George-Nascimento and Urrutia 2000; Weitzel et al. 2015), and Lebranche mullet and white mullet (Espinoza 2014). Garbin et al. (2013) reported *Contracaecum* from anchovies off the coast of Argentina.

In Western Europe and the Mediterranean and surrounding seas, sources of *Anisakis* include anchovies (Tuncel 2003; Rello et al. 2009; Mladineo et al. 2012; Cavallero et al. 2015), European pilchard (Oğuz et al. 2000; Cavallero et al. 2015),

herring (Davey 1972; Verhamme and Ramboer 1988; Petrie et al. 2005), flounder (Marques et al. 2006), European sea bass (Bernardi et al. 2011), cod (Petrie et al. 2005), blue whiting (Cruz et al. 2007), Atlantic mackerel (Keser et al. 2007; Gutíerrez-Galindo et al. 2010; Ferrantelli et al. 2014), Atlantic horse mackerel (Oğuz et al. 2000; Cruz et al. 2005; Gutíerrez-Galindo et al. 2010), Mediterranean horse mackerel (Akmirza 1998), chub mackerel (Akmirza 1997), hake (Valero et al. 2006), gray gurnard (Levsen and Kari 2014), monkfish (Petrie et al. 2005), striped red mullet (Akmirza 2000a), annular seabream (Akmirza 2000b), common seabream (Akmirza 2000b), scabbardfish (Cruz et al. 2009), and squid (Abollo et al. 1998). Sources of *Pseudoterranova* in Europe include cod (des Clers and Andersen 1995; Mehrdana et al. 2014; Lunneryd et al. 2015), sculpin (Mitgaard et al. 2003); Lunneryd et al. 2015), four-bearded rockling (des Clers and Andersen 1995), plaice (Bristow and Berland 1992; des Clers and Andersen 1995), and dab (des Clers and Andersen 1995). Sources of *Contracaecum* in and around Europe include cod (Petrie et al. 2005; Mehrdana et al. 2014), Atlantic mackerel (Gutíerrez-Galindo et al. 2010), and monkfish (Petrie et al. 2005).

Kijewska et al. (2009) reported *Anisakis* species from chub mackerel, roughsnout grenadier, European pilchard, Benguela hake, Atlantic horse mackerel, and black slimehead off the coast of Africa between Morocco and Mauretania. In southern Africa, *Contracaecum* spp. have been found in freshwater fish such as catfish, tilapia, common carp, African tigerfish, largescale yellowfish, elephantfish, and largemouth bass (Barson 2004; Tavakol et al. 2015).

Ishikura (2003) reported 38 species of fish and cephalopods in Japan (where most cases of anisakiasis are diagnosed) that may serve as a source of anisakid nematodes, including tuna, mackerel, sardine, salmon, and octopus. Nagasawa and Moravec (1995) also reported *Anisakis* from Japanese flying squid from Japan. Purivirojkul (2009) reported *Anisakis* from grouper, cobia, and hairtail and *Contracaecum* from purple-spotted bigeye and bream from the Gulf of Thailand. Setyobudi et al. (2013) reported *Anisakis* from squid in Korea. Anshary et al. (2014) reported *Anisakis* from skipjack tuna, mackerel tuna, frigate tuna, bluespotted hind, and Indian mackerel off the coast of Indonesia.

In the waters around Australia, flathead gray mullet (Jabbar et al. 2013; Shamsi 2014), hardyheads (Shamsi 2014), mackerel (Shamsi and Butcher 2011), striped jack (Jabbar et al. 2013; Shamsi 2014), yellowtail amberjack (Hutson et al. 2007), and kingfish (Shamsi 2014) have been known to harbor *Anisakis* and *Contracaecum*. Cannon (1978) reported anisakid-type nematode larvae from razor clams, tiger cowrie, northern spiny oyster, and Queensland scallop in Australia. Hurst (1984) reported *Anisakis* from barracuda and *Pseudoterranova* from barracuda and subantarctic cod from New Zealand. Wharton et al. (1999) reported *Anisakis* from barracuda, tarakihi, red cod, red gurnard, and arrow squid from New Zealand.

## 8.5 Trends in Diagnosis in Food

### *8.5.1 Detection*

Currently, visual examination of the fish, extraction of the visible anisakid parasites, if found, and the removal of those fish that are heavily parasitized from the market are required based on European Community regulations for fish products (Directive 91/493/EC and Decision 93/140/EC). Detection of nematodes in fish is usually performed by visual examination, often with enhanced lighting procedures such as candling. Fish samples are generally placed on a light box or industrial candling table consisting of a white, translucent plastic sheet overlaying a cool white light source. Nematodes can be detected by the shadows they cause as light shines through the flesh of the fish. Slicing of larger fish samples may increase detection (Petrie et al. 2005). Celano et al. (2013) proposed the use of UV transillumination for a rapid and more reliable method to detect anisakids in fish.

Digestion methods may also be used to recover nematodes from fish samples. Jackson et al. (1981) recommend using a sodium chloride-pepsin solution consisting of 0.85% sodium chloride with pepsin added to a concentration of 10 mg/L. After covering the fish samples with the pepsin solution, the pH is adjusted to pH 2 using concentrated hydrochloric acid. The fish are then incubated in the adjusted solution at 37 °C overnight. The following day, the solution is sieved and the contents may be examined for parasites.

The International Organization for Standardization (ISO [http://www.iso.org/iso/home.htm]) plays an important role regarding publication of methods and standards for detection of microbes in food products. Despite the regulations regarding the quality of fish products, there are no methods currently under ISO for the detection of *Anisakis* spp. and *Pseudoterranova* spp. This might change in the future due to the impact that these parasites are causing, especially in European countries.

### *8.5.2 Morphologic Diagnosis*

A lack of specialized organs in L3 larvae makes morphologic analysis impossible for anything beyond a genus-level identification. L3 anisakid larvae are typically 10–50 mm long and cream-colored to pale pink when fresh (Fig. 8.1a, b). Members of all three genera have three anterior fleshy "lips" characteristic of ascarid nematodes and a triangular boring tooth (Fig. 8.1c). *Pseudoterranova* species and *Anisakis* Type I species possess a small spine-like mucron at the posterior end (Fig. 8.1d); *Anisakis* Type II species and *Contracaecum* species lack a mucron. The cuticle is multilayered. Lateral chords are prominent and may be Y-shaped with a narrow base (*Anisakis*, Fig. 8.2c) or broad and butterfly-shaped (*Pseudoterranova* and *Contracaecum*, Fig. 8.2b). Musculature is polymyarian/coelomyarian with many cells per quadrant. Intestinal cells are tall with a single basal nucleus and a microvillous brush border (Orihel and Ash 1995; Andersen and Lichtenfels 2000; Felizardo et al. 2009).

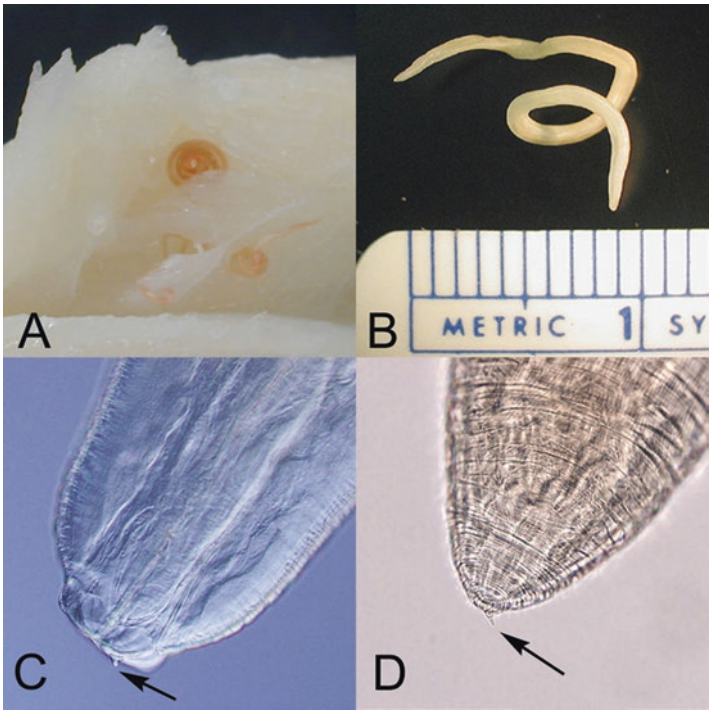


**Fig. 8.1** Characteristics of anisakid nematodes. (**A**) Nematode larvae within a filet of market fresh cod. (**B**) Individual anisakid nematode. (**C**) Anterior end of *Pseudoterranova* sp. showing the characteristic ascarid lips and boring tooth (*arrow*). (**D**) Posterior end of *Pseudoterranova* sp. showing the mucron (*arrow*) (Images courtesy of the CDC-DPDx)

The three genera can be separated by the structures of the alimentary canal. All three genera possess a ventriculus which connects the esophagus and the intestine. In *Anisakis*, this connection is simple with no extension of the intestine. In *Pseudoterranova* (Fig. 8.2a), there is an anteriorly directed intestinal cecum that lies adjacent to the ventriculus and esophagus. In *Contracaecum*, there is both an anteriorly directed intestinal cecum and a posteriorly directed ventricular appendix (Andersen and Lichtenfels 2000) [see also Table 8.1].

### *8.5.3 Molecular Analysis*

While molecular analysis is not widely available for routine clinical diagnosis, it can be a useful tool for epidemiologic investigations and, in general, understanding the host range of infectious agents and in turn the risk of infection for the consumer. Molecular methods have a great potential to be used for fish inspection for regulatory purposes.

PCR amplification of the ITS-1 and ITS-2 regions, followed by restriction fragment length polymorphism (PCR-RFLP), has been developed for the

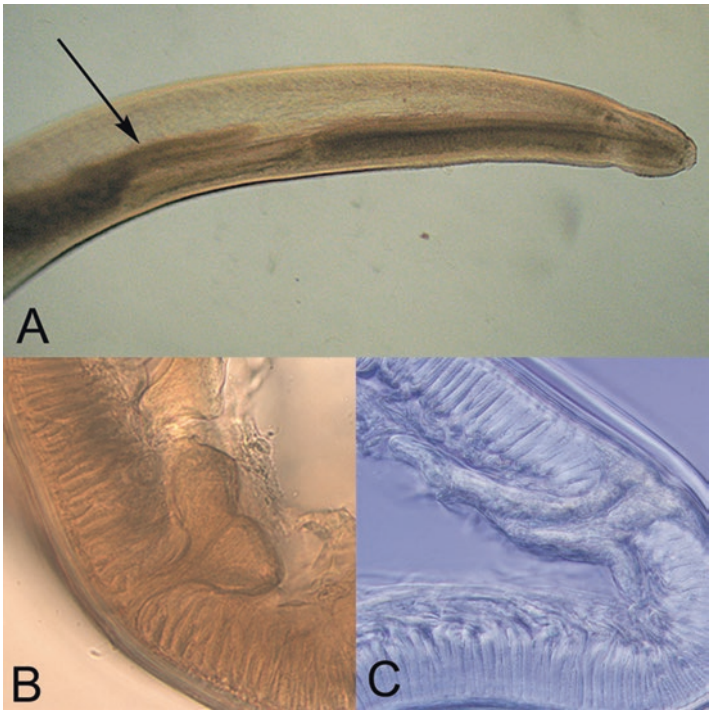


**Fig. 8.2** Characteristics of anisakid nematodes. (**A**) Anterior third of *Pseudoterranova* sp. showing the anteriorly directed intestinal cecum (*arrow*). (**B**) Butterfly-shaped lateral chords of *Pseudoterranova* sp. (**C**) Y-shaped lateral chords of *Anisakis* sp. (Images **B** and **C** also show characteristic tall, coelomyarian musculature. Images courtesy of the CDC-DPDx)

**Table 8.1** Morphologic comparison of *Anisakis*, *Pseudoterranova*, and *Contracaecum*

| Genus | Mucron | Ventriculus | Number of intestinal cells in cross section | Lateral chords |
|---|---|---|---|---|
| *Anisakis* | Type I, present Type II, absent | Simple; no cecum nor appendix | 60–80 | Y-shaped |
| *Pseudoterranova* | Present | Anteriorly directed cecum (only) | >100 | Butterfly-shaped |
| *Contracaecum* | Absent | Anteriorly directed cecum and posteriorly directed ventricular appendix | >100 | Butterfly-shaped |

species-level identification of *Anisakis* spp. (D'Amelio et al. 2000; Paggi et al. 1991), which have substantial ITS sequence diversity (Nadler et al. 2005). While PCR-RFLP has been used to identify *Pseudoterranova* to the species-level (La Rosa et al. 2005), this genus appears to have much less ITS sequence diversity, and therefore other methodologies may be employed, such as single-strand conformation polymorphism (SSCP) followed by sequencing analysis (Zhu et al. 2002; Nadler et al. 2005). Analysis of the ITS-1 and ITS-2 regions has also been used for the identification of *Contracaecum* spp. (Garbin et al. 2013).

Alloenzyme markers have also been used for the identification of *Pseudoterranova* spp. (Paggi et al. 2000).

The best use of molecular methods is in conjunction with morphologic analysis for sensitive and specific detection (Garbin et al. 2013). Morphologic examination allows precise estimation of the burden of infection in fish, which is quite challenging when using conventional PCR such as the ones described above. However, Mossali et al. (2010) have described a TaqMan qPCR test to detect and provide relative quantification of *Anisakis* spp. and *Pseudoterranova* spp. whenever they are present in the sample. DNA can be detected in fish and fish-derived products, including fish fillets, surimi, fish sticks, canned fish, as well as baby food. This methodology is based on a duplex TaqMan assay with one set of primers and TaqMan probe targeting the ITS-1 of the two anisakid genera for detection and quantification of the parasites and a generic TaqMan targeting the 18SrRNA of eukaryotic organisms for quantification of the total eukaryotic DNA present in the sample. There is no cross-amplification with the related species of *Ascaris* and *Contracaecum*, which can contaminate the same type of food tested for the presence of *Anisakis* spp. and *Pseudoterranova* spp. The quantitative results are based on plotting the fluorescence signal originated by the sample against a standard curve using DNA extracted with different concentrations of *Anisakis* spp. and *Pseudoterranova* spp. larvae. Results showed that this test is able to detect DNA from both genera in a proportion of $1{:}10^5$ in 1 ng of total DNA. Although methods such as the one described have potential applications for fish farms, fish markets, and food producers, the procedure requires laboratories with adequate infrastructure and adequately trained laboratory personnel for execution and interpretation of data. Incorporation of molecular data in regulatory mandates is not simple in some areas because of the validation process that the methods need to go through.

The complete mitochondrial genome for *A. simplex* (Kim et al. 2006) and *P. azarasi* (Liu et al. 2015) have been sequenced.

## 8.6 Prevention and Control

Unlike such pathogens as norovirus and enteric bacteria, the presence of anisakid nematodes in fish and shellfish is not due to negligence and contamination by human activities. Fish and mollusks are a natural part of the parasites' life cycle,

and their presence in these food sources, especially wild-caught, should be expected. The best protection against anisakiasis is to avoid eating raw or undercooked fish and mollusks. However, the consumption of such food items is a common practice throughout much of the world, and it is ultimately the responsibility of the food preparer and consumer to ensure the proper precautions are taken to avoid infection.

Adequately cooking and/or freezing the meat are the best way to kill larvae. The FDA (2012) recommends cooking fish fillets to an internal temperature of 62.8 °C (145 °F) for 15 s, 68.3 °C (155 °F) for comminuted fish (e.g., fish cakes), and 73.9 °C (165 °F) for stuffed fish. Some of the allergens, however, appear to be heat stable (Mattiucci et al. 2015). Freezing will also kill larvae, at the proper temperatures and for appropriate lengths of time. It is generally recommended to freeze fish at −4 °F (−20 °C) or below for 7 days **or** −31 °F (−35 °C) or below until solid, then storing at −31 °F (−35 °C) for 15 h **or** −31 °F (−35 °C) or below until solid and storing at −4 °F (−20 °C) or below for 24 h (Deardorff et al. 1991; FDA 2012; CDC 2015).

Cold smoking and marinating are generally not sufficient to kill larvae, although food-grade acetic acid may be used to kill larvae (ICMSF 1996). Dry salting will kill larvae, if evenly distributed and at the right concentrations (>20 baumè) (Brutti et al. 2010). Oh et al. (2014) reported inactivation of larvae in salt-fermented fish and squid using a concentration of 15% NaCl for 7 days and 20% NaCl for 6 days. High-hydrostatic pressure treatments have also been successfully used to inactivate larvae (Brutti et al. 2010; Oh et al. 2014).

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# Chapter 9
# *Trichinella* and Other Foodborne Nematodes

**Edoardo Pozio**

## 9.1 Introduction

Nematodes, or roundworms, are a highly diverse group of organisms (De Ley and Blaxter 2002). What nematodes lack in obvious morphological disparity, they make up for in abundance, accounting for 80% of all individual animals on earth, and diversity, with estimates ranging from 100,000 to 1 million extant species. They exploit a wide variety of niches and include free-living terrestrial and marine microbivores, meiofaunal predators, and herbivores, and only 7000 are plant and animal parasites. On the basis of small subunit ribosomal RNA (SSU rRNA) phylogenetics, nematodes have been divided into five clades, two of which, Dorylaimia (clade I) and Spirurina (clade III), include important foodborne-transmitted roundworms (Blaxter et al. 1998).

These worms complete their life cycle through four molts from first-stage larvae (L1) to L5 which mature to adults. As a rule, the infective stage for the final host is the L3, but parasites of the genera, *Trichinella* and *Baylisascaris*, use L1 and L2 stages, respectively. Most of parasitic nematodes transmitted to humans by food are zoonotic in origin, even if in some cases (e.g., *Ascaris* spp. and *Trichostrongylus* spp.) humans can act as reservoir in addition to animals.

Several foodborne parasitic infections are transmitted to humans because of improper animal breeding, food handling, or both (Torgerson et al. 2014). In this chapter, in addition to *Trichinella* spp., information on basic biology, distribution, epidemiology, disease, detection/diagnosis, treatment, pathology, and control measure of *Ascaris* spp., *Toxocara* spp., *Baylisascaris procyonis*, *Capillaria* spp., *Gnathostoma* spp., and *Trichostrongylus* spp. will be provided.

E. Pozio (✉)
Istituto Superiore di Sanità, viale Regina Elena 299, Rome 00161, Italy
e-mail: edoardo.pozio@iss.it



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## 9.2 *Trichinella* spp.

### *9.2.1 Introduction*

Nematodes of the genus *Trichinella* are parasites of wildlife (mammals, birds, and reptiles) of all the continents but Antarctica for which no information is available (Pozio and Murrell 2006). When humans fail in the proper management of domestic animals and wildlife, these nematodes are transmitted from the sylvatic environment to the domestic one, sometimes through synanthropic (intermediary between domestic and sylvatic) animals. In addition, some species can transfer in a reversible path from domestic animals to wildlife. For more than 100 years after its discovery in 1835, this parasite was considered to be transmitted only through the consumption of pork, and only in the last 50 years have different transmission patterns been documented (Campbell 1988). Humans acquire the infection by the ingestion of raw or undercooked meat and meat-derived products of different animal origins (e.g., pork, horse, game), which harbor *Trichinella* infective larvae in cells of striated muscles (Gottstein et al. 2009). Trichinellosis not only is a public health hazard by affecting human patients but also represents an economic problem in porcine animal production and food safety (Pozio 2014). The average yearly incidence of the disease in humans worldwide is several thousand cases with a mortality rate of about 0.2% (Murrell and Pozio 2011), and the global number of disability-adjusted life years was estimated to be 76 per billion per year (Devleesschauwer et al. 2015).

### *9.2.2 The Life Cycle*

The natural cycle of *Trichinella* spp. is identical in all hosts species irrespective if they belong to mammals (including humans), birds, or reptiles (Fig. 9.1). In the host body, these parasites develop two generations of worms. Adult worms are embedded in the intestinal mucosa. Female worms (1.26–3.35 mm in length; 29–39 μm in width) produce newborn larvae (NBL; 110 μm in length; 7 μm in width) that migrate into the lymphatic vessels and then enter the blood vessels to reach and penetrate striated muscle cells. In this niche, NBL develop to the infective L1 (0.65–1.1 mm in length; 25–40 μm in width) in 2 weeks (Despommier 1998). In the muscle cell, L1 belonging to the clade of non-encapsulated species appear to be free among muscle fibers (Fig. 9.2), whereas L1 belonging to the clade of encapsulated species are coiled and enclosed by a collagen capsule (350–450 μm in length; 180–300 μm in width) (Fig. 9.2). In this ecological niche, larvae can survive for many years (over 20 years in polar bears and up to 39 years in humans), waiting to be ingested by a new host (Gottstein et al. 2009). When a new host ingests infected muscle tissues, L1 are released in the stomach, reach the duodenum, penetrate into the villa, and undergo four molts within 2 days and then rapidly develop to the adult stage. Males and females copulate, and in 6–7 days post-infection, the females begin to produce

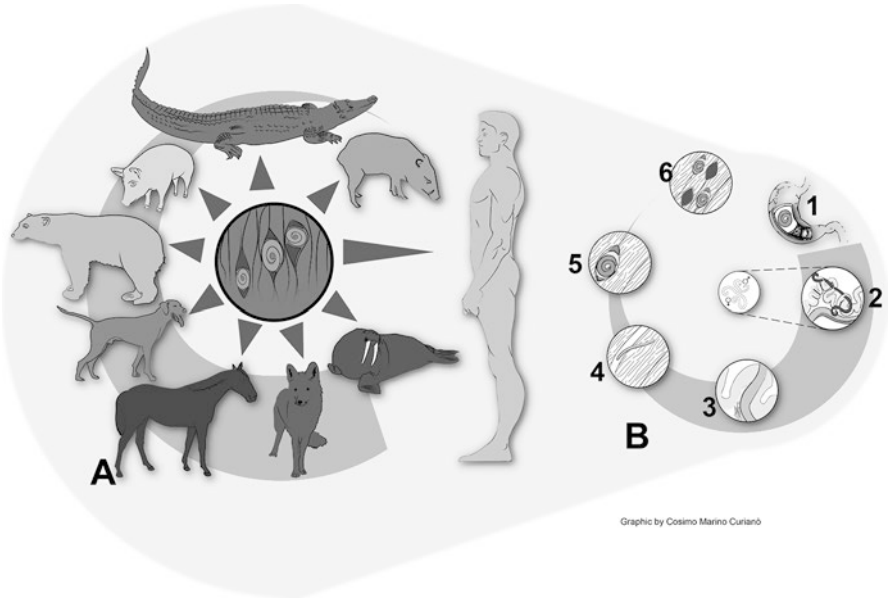


**Fig. 9.1** Life cycle of *Trichinella* spp. Panel A: main domestic and sylvatic sources for *Trichinella* spp. infection in humans. Panel B: developmental cycle which occurs within hosts: *1* Ingested muscle tissues are digested in the stomach, and first-stage larvae (L1) are released; *2* L1 penetrate the intestinal mucosa of the small intestine and develop to sexually active adult males and females within 48 h; *3* 5–6 days post-infection, female worms release newborn larvae (NBL) which are transported by lymphatic and blood vessels; *4* NBL exit capillaries and enter cells of striated muscles; *5* the larvae grow to infective L1 within the muscle cell (nurse cell) and depending on the species of *Trichinella* may be enclosed by a capsule (encapsulated); *6* if the infected muscle is not ingested by a new host to repeat the cycle, the L1 die after a period of time ranging from weeks to years

NBL for 1–2 weeks or longer as influenced by the host's immune response at the gut level, which results in the adult worm expulsion.

### *9.2.3 Taxonomy and Phylogeny*

Two clades have been recognized in the genus *Trichinella*, one that encompasses species (*T. spiralis*, *T. nativa*, *T. britovi*, *T. murrelli*, *T. nelsoni*, *T. patagoniensis*) and genotypes (*Trichinella* T6, T8, and T9) that encapsulate in host muscle tissues following muscle cell reprogramming and a second that does not encapsulate (Pozio et al. 2009; Korhonen et al. 2016). The species and genotypes of the first clade parasitize only mammals (Table 9.1). Among the three species that comprise the second clade, one infects mammals and birds (*T. pseudospiralis*) and two parasitize mammals and reptiles (*T. papuae* and *T. zimbabwensis*) (Table 9.1) (Pozio et al. 2009; Korhonen et al. 2016). Divergence time analysis based on the nematode

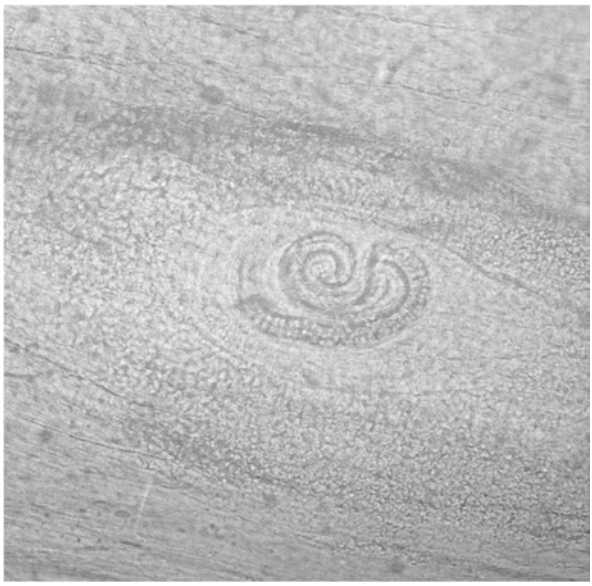

**Fig. 9.2** First stage larva of an encapsulated *Trichinella* species in a striated muscle

diversification estimate of 532–382 million years ago (mya) implied that *Trichinella* spp. and *Trichuris suis* had a most recent common ancestor (MRCA) ~281 mya and that the encapsulated and non-encapsulated *Trichinella* taxa shared an MRCA ~21 mya, coinciding with the transition from Oligocene to Miocene (Korhonen et al. 2016).

### *9.2.4 Epidemiology*

The etiological agents of human trichinellosis show virtually worldwide distribution in domestic and/or wild animals (Table 9.1). This global distribution of *Trichinella* nematodes and varying cultural eating habits represent the main factors favoring human infections in industrialized and non-industrialized countries. Human trichinellosis has been documented in 55 (27.8%) countries around the world. In several of these countries, however, trichinellosis affects only ethnic minorities and tourists because the native inhabitants do not consume uncooked meat or meat of some animal species. *Trichinella* sp. infection has been documented in domestic animals (mainly pigs) and in wildlife of 43 (21.9%) and 66 (33.3%) countries, respectively. Of the 198 countries of the world, approximately 40 (20%) are small islands far from the major continents or city-states where *Trichinella* sp. cannot circulate among animals for lack of local fauna (both domestic and wild) (Pozio 2007). Finally, information on the occurrence of *Trichinella* sp. infection in domestic and/or wildlife is still lacking for 92 countries (Pozio 2007). Most of the species and genotypes of the genus *Trichinella* show a well-defined distribution

**Table 9.1** Main features of *Trichinella* species

| *Trichinella* species | Distribution | Main hosts | Infectivity to swine | Infection in humans (deaths) |
|---|---|---|---|---|
| Encapsulated clade | | | | |
| *T. spiralis* | Cosmopolitan | Domestic pig, wild boar, carnivores, horse, rats | High | Yes (yes) |
| *T. nativa* | Arctic and subarctic regions of North America, Asia, and Europe | Carnivores | No | Yes (yes) |
| *Trichinella* T6 | Arctic and subarctic regions of North America, Rocky Mountains | Carnivores | No | Yes (no) |
| *T. britovi* | Temperate areas of the Palearctic region, North and West Africa, Middle East | Carnivores, seldom domestic pig and wild boar, horse | Low | Yes (no) |
| *Trichinella* T8 | South Africa, Namibia | Carnivores | No | No |
| *T. murrelli* | Temperate areas of the Nearctic region | Carnivores, horse | No | Yes (yes) |
| *Trichinella* T9 | Japan | Carnivores | No | No |
| *T. nelsoni* | Eastern Africa south of the Sahara up to South Africa | Carnivores, seldom warthog and bush pig | Low | Yes (yes) |
| *T. patagoniensis* | Argentina | Carnivores | No | No |
| Non-encapsulated clade | | | | |
| *T. pseudospiralis* | Cosmopolitan | Mammals (carnivores, marsupials, swine, rodents), carnivorous and omnivorous birds | Average | Yes (no) |
| *T. papuae* | Southeast Asia, Papua New Guinea, Australasia | Domestic and wild swine, crocodiles | Average | Yes (no) |
| *T. zimbabwensis* | Africa south of the Sahara | Crocodiles, monitor lizards, carnivore mammals | Average | No |

area, whereas *T. spiralis*, which originated in Eastern Asia (the current China), shows a cosmopolitan distribution due to the passive introduction by domestic pigs and *T. pseudospiralis* which shows a cosmopolitan distribution due to its spread by birds (Pozio and Zarlenga 2013). Not all species of the genus *Trichinella* play the same weight in the epidemiology of trichinellosis in humans. In the domestic habitat, the most dangerous species is *T. spiralis*. In Europe, a small percentage of pigs

**Table 9.2** Epidemiological patterns of foodborne nematodes

| Parasites | Distribution | Reservoir | Source of infection for humans | Estimated yearly incidence at the world level |
|---|---|---|---|---|
| *Trichinella* spp. | Cosmopolitan | Mammals, birds, reptiles | Meat and meat-derived products | 3000–4000 infections |
| *Ascaris* spp. | Cosmopolitan | Humans, pigs | Egg-contaminated vegetables and fomites | Hundreds of million infections |
| *Toxocara* spp. | Cosmopolitan | Dogs, cats, wild canids | Egg-contaminated soil and vegetables | Hundreds of million infections |
| *Baylisascaris procyonis* | North America, Central Europe | Raccoon dog | Egg-contaminated soil and vegetables | Some hundred infections |
| *Capillaria philippinensis* | Philippines, Japan, Indonesia, Iran, India, Korea, Lao PDR, People's Republic of China, Taiwan, Egypt | Fish-eating birds (final hosts), freshwater and brackish-water fish (intermediate hosts) | Freshwater and brackish-water fish | Some dozen infections |
| *Capillaria hepatica* | Cosmopolitan | Rodents | Eggs released into the environment by the decomposition of infected rodents | Unknown |
| *Gnathostoma* spp. | Cosmopolitan | Fish-eating mammals (final hosts), fish, birds, amphibians, reptiles, and mammals (intermediate hosts) | Consumption of intermediate hosts | Thousand infections |
| *Trichostrongylus* spp. | Cosmopolitan | Livestock | Egg-contaminated soil, fruits, vegetables | Thousand infections |

is also infected by *T. britovi* and very rarely by *T. pseudospiralis*. In the American continent, only *T. spiralis* has been detected in pigs (Burke et al. 2008; Ribicich et al. 2005), but one animal of Argentina was infected by *T. pseudospiralis* (Krivokapich et al. 2015). Other species infecting domestic and/or sylvatic swine are *T. nelsoni*, *T. papuae*, and *T. zimbabwensis* (Pozio 2014). In wildlife, *Trichinella*

parasites act as biological indicators of the food web. However, the sylvatic cycle may be influenced by human actions, which can increase or decrease the transmission events. For example, the common habit of hunters to leave animal carcasses in the field after skinning, or removing and discarding the entrails, increasing the probability of transmission to new hosts (Pozio and Murrell 2006). On the contrary, *Trichinella* parasites are seldom transmitted or are not transmitted at all, where their hosts have access to alternative food resources resulting from human activity (e.g., synanthropic and domestic animals, garbage) (Pozio 1998).

The most important source of human infection worldwide is the domestic pig (Murrell and Pozio 2011), but, e.g., the horsemeat in Europe and wild boar or other wild pigs have played a significant role during outbreaks within the past three decades (Holzbauer et al. 2014; Fichi et al. 2015; Pozio 2015). The meat of pigs at risk for these zoonotic pathogens unlikely reaches the international market but can reach humans as a gift from relatives or friends or in the course of social events (Pozio 2015). Since *Trichinella* spp. circulate in the domestic habitat only in poor and disadvantaged areas not served by veterinary services, most of free-ranging and backyard pigs are not tested for *Trichinella* in official abattoirs. It follows that the weight of *Trichinella* infections in the international meat trade is negligible since *Trichinella*-infected pigs do not reach the international market. From 1950 to the present, there have been 43 reports of importation of *Trichinella* spp.-infected animals or meat, most of which (60%, 26/43) have been related to live horses or their meat (Pozio 2015).

### *9.2.5 The Disease in Humans*

Trichinellosis (syn. Trichinosis) is the disease caused by parasites of the genus *Trichinella* in humans. The first signs of *Trichinella* sp. infection commonly include diarrhea and abdominal pain due to intestinal invasion. This is usually followed by a few days of fever and myalgia (a flu-like malaise) and then disappears in less than 1 week in mild infections. It has been observed that the shorter the duration between infection and the appearance of diarrhea and fever, the longer the duration of both fever and facial edema. In most persons, the acute stage begins with the sudden appearance of general discomfort and severe headaches, an increase in fever, chills, and excessive sweating. The major syndrome of the acute stage consists of persistent fever (39–40 °C for 8–10 days), periorbital or facial edema, myalgia, and severe asthenia (Table 9.3). These signs and symptoms are always associated with high eosinophilia, leukocytosis, and increased muscle enzymes (Kociecka 2000; Dupouy-Camet et al. 2002). Other signs of trichinellosis are conjunctival and subungual hemorrhagic lesions caused by vasculitis, the leading pathological process of trichinellosis. In addition, maculopapular rash (after the onset of muscular pain) and formication have been reported for a small proportion of persons. Complications usually develop within the first 2 weeks. They are observed mainly in severe cases, but they have also been reported in moderate cases, in persons who were improperly

**Table 9.3** Mean clinical signs and symptoms and laboratory features of foodborne nematodes in humans

| Parasites | Clinical signs and symptoms | Laboratory features |
|---|---|---|
| *Trichinella* spp. | Diarrhea, abdominal pain, fever, myalgia, periorbital or facial edema, general discomfort, headache, asthenia, conjunctival and subungual hemorrhagic lesions, maculopapular rash | Eosinophilia, leukocytosis, increased muscle enzymes |
| *Ascaris* spp. | Acute lung inflammation, difficulty in breathing, fever, abdominal distension, pain, nausea and diarrhea, appetite loss, lactose maldigestion, impaired weight gain, asthma, Loeffler's syndrome | Eosinophilia |
| *Toxocara* spp. | Visceral infection: hepatosplenomegaly, fever, respiratory signs, lymphadenopathy, pallor, convulsions, strabismus, myocarditis<br>Covert infection: weakness, abdominal pain, lymphadenopathy, skin lesions, pruritus, respiratory features, headache<br>Ocular infection: retinochoroiditis, peripheral retinitis | Eosinophilia<br>No eosinophilia |
| *Baylisascaris procyonis* | Visceral larva migrans: fever, lethargy, nausea, macular rash, pneumonia, hepatomegaly<br>Ocular larva migrans: subacute neuroretinitis syndrome | Eosinophilia |
| *Capillaria philippinensis* | Abdominal pain, diarrhea, borborygmi, weight loss, muscle wasting, weakness, edema, hypotension, hyporeflexia | Protein-losing enteropathy, malabsorption of fats and sugars, decreased excretion of xylose, low serum levels of potassium, sodium, calcium, carotene, and total protein |
| *Capillaria hepatica* | Abdominal pain, fatigue, anorexia, high morning fever, nausea, vomiting, enlargement of spleen and liver, diarrhea or constipation, abdominal distension, edema of extremities, pneumonia | Marked leukocytosis, eosinophilia, moderate hyperchromic anemia, cellular marrow with normoblastic erythropoiesis |
| *Gnathostoma* spp. | Gastrointestinal gnathostomosis: low-grade fever, anorexia, vomiting, nausea, pruritus, urticaria<br>Cutaneous gnathostomosis: migrating swelling, subcutaneous hemorrhages, cutaneous eruption or nodules mainly on the trunk | Leukocytosis with a marked eosinophilia |
| *Trichostrongylus* spp. | Diarrhea, abdominal pain, nausea, anemia, weight loss | Eosinophilia |

treated (including those for whom treatment was begun too late) and, particularly, in the elderly. A positive correlation has been reported between age and the frequency and severity of complications (Dupouy-Camet and Bruschi 2007). The most common complications are cardiovascular disturbances, myocarditis, which develops in 5–20% of all infected persons, thromboembolic disease, neurological complications such as encephalopathy and neuromuscular disturbances, ocular lesions, and respiratory and digestive complications (Dupouy-Camet and Bruschi 2007). In rare cases of severe infection, death may occur due to early cardiac insufficiency, encephalitis, or pneumonia. Sudden death resulting from the passage of NBL into the myocardium has also been described.

### *9.2.6 Diagnosis and Treatment*

The diagnosis of trichinellosis should be based on anamnesis (e.g., raw meat consumption), clinical signs and symptoms, and laboratory tests (immunodiagnosis or muscle biopsy) (Table 9.4). The diagnostic technique most used is the enzyme-linked immunosorbent assay (ELISA) with excretory/secretory antigens, which have the highest ratio between sensitivity and specificity and are best used in combination with Western blot to confirm ELISA-positive results (Gómez-Morales et al. 2012). Seroconversion occurs between 2 and 8 weeks post-infection (Pozio et al. 2003). To recover larvae for species identification or confirmation of infection,

**Table 9.4** Diagnosis of foodborne nematode infections in humans and animals

| Parasites | Diagnosis in humans | Diagnosis in animals |
|---|---|---|
| *Trichinella* spp. | Detection of larvae in a muscle biopsy by compressorium, digestion, histology; serology (ELISA + confirmatory Western blot) | Detection of larvae in preferential muscles by digestion according to ISO 18743 |
| *Ascaris* spp. | Detection of eggs in feces after concentration | Detection of eggs in feces after concentration |
| *Toxocara* spp. | Serology | Detection of eggs in feces after concentration |
| *Baylisascaris procyonis* | Serology | Detection of eggs in feces after concentration |
| *Capillaria philippinensis* | Detection of eggs, larvae, and/or adult worms in feces after concentration | – |
| *Capillaria hepatica* | Detection of eggs and adult worms in liver by needle biopsy | Detection of eggs in feces after concentration |
| *Gnathostoma* spp. | Recovery and identification of larvae from surgical specimens, urine, sputum, vaginal discharge<br>Serology (Western blot) | Detection of eggs in feces after concentration |
| *Trichostrongylus* spp. | Detection of eggs in feces after concentration | Detection of eggs in feces after concentration |

muscle biopsy (0.2–0.5 g of muscle tissue) should be collected preferentially from the deltoid muscle. *Trichinella* sp. larvae can be detected in the biopsy by compressorium, HCl-pepsin digestion, or histological analysis (Nöckler and Kapel 2007). The collection of the muscle biopsy is seldom used because it is an expensive, invasive, and painful method.

The two drugs of choice are mebendazole (25 mg/kg two to three times a day for 15 days) and albendazole (20 mg/kg two to three times a day for 15 days). Corticosteroids should be used for symptomatic treatment (e.g., prednisolone 30–60 mg/day) and always in combination with anthelmintic, but caution should be exercised due to the possibility of anaphylactic shock (Dupouy-Camet et al. 2002).

### *9.2.7 Infections in Animals*

Naturally infected animals are not known to show any clinical sign of the disease even when the worm burden has been very high (e.g., more than 8000 larvae/g in the diaphragm pillars of pigs) (Pozio 2014). In experimentally infected pigs, dyspnea, periorbital edema, and reduced weight gain have been observed in some animals (Ribicich et al. 2007); however, these clinical signs cannot easily be detected in pigs reared in herds kept without controlled management conditions, that is, the only pigs at risk for *Trichinella* sp. infections. Most of naturally infected animals harbor few larvae (less than 10 larvae/g) in the preferential muscles. The preferential muscles for infection can vary according to the host species, but the diaphragm pillar, masseter, and base of the tongue are the most infected muscles in swine. Tongue and anterior tibial muscles are the preferential muscles in carnivore mammals (Kapel et al. 2005) and tongue, masseter, or diaphragm in horses (Hill et al. 2007). To identify *Trichinella* spp.-infected animals, a muscle sample from the preferential muscles should be digested by HCl-pepsin solution according to ISO18743 (2015) (Table 9.4). The use of the compressorium (or trichinoscopy) method is strongly discouraged because it is a laborious and time-consuming method, which does not allow to detect slightly infected animals and animals harboring larvae of non-encapsulated species (Nöckler and Kapel 2007). Serological tests (e.g., ELISA) on serum or meat juice samples are highly sensitive, but they show a low specificity. It follows that a Western blot should be used to confirm ELISA-positive sera (Gomez Morales et al. 2012). Furthermore, to increase the specificity, excretory/secretory antigens should be used instead of crude antigens (Gamble et al. 2004). At the beginning of the infection and in the last phase of the infection, animals (e.g., pigs, horses) can harbor infective larvae in their muscles, but the serology can be negative (Hill et al. 2007). In conclusion, serology performed with validated tests can be used only for epidemiological surveys, but not for the detection of the infection in animals intended for human consumption. Commercial kits do not use high-quality antigens, have been not validated by an independent body, and are the source of false positive reactions; therefore, their use is discouraged.

### 9.2.8 Prevention and Control

To prevent human trichinellosis, all animals intended for human consumption should be tested by digestion (World Organization for Animal Health 2013). As an alternative, pig carcasses can be frozen under specific conditions (Table 9.5), but certain *Trichinella* species infecting game and horses are resistant when freezing is carried out using the recommended temperature and time combinations (Gottstein et al. 2009). Parasites of the genus *Trichinella* are highly sensitive to temperature

**Table 9.5** Prevention and control of foodborne nematodes

| Parasites | Prevention | Control |
|---|---|---|
| *Trichinella* spp. | Cooking meat at >70 °C per 1 min in the core of the meat product<br>Freezing meat at < −15–−30 °C in the core of the meat product for 6–30 days | Pigs kept under controlled management conditions<br>Testing all *Trichinella*-susceptible animals intended for human consumption by digestion |
| *Ascaris* spp. | Wash vegetables intended to be consumed raw | Encourage the use of latrines and not using untreated wastewater for watering vegetables; health education; and anthelminthic treatment |
| *Toxocara* spp. | Anthelminthic treatment of pet animals, pet owners should avoid allowing their animals to deposit waste in areas where children play; promoting good hand hygiene and teaching children not to eat soil; sand in sandboxes can be steam sterilized or replaced and sandboxes covered when not in use | Enactment of local community laws that enforce immediate pickup and disposal of dog and cat feces should be encouraged; education to pet owners; flame gun to destruct pet feces; bitches should be treated with fenbendazole 50 mg/kg/day, every day for 3 weeks before parturition until 3 weeks after parturition |
| *Baylisascaris procyonis* | Children should not be allowed to play in areas likely to be contaminated with raccoon feces | Control of raccoon populations; pet raccoons must be dewormed; identification and rapid elimination of raccoon defecation sites |
| *Capillaria philippinensis* | Does not consume raw fish; improvement in overall sanitation and education of fish consumers | Anthelmintic treatment |
| *Capillaria hepatica* | Education and improvement of hygiene | Rodent control and sanitary disposal of dead animals |
| *Gnathostoma* spp. | Does not consume raw or marinated meat and fish; drink only boiled or treated waters | Health education programs |
| *Trichostrongylus* spp. | Wash hands before preparing or eating meals; vegetables should be thoroughly washed or cooked before eating | Removal of livestock feces from the human settlements; pasture rotation |

higher than 70 °C for 1 min in the core of the meat. *Trichinella* larvae can survive in meat-derived products for weeks up to months if the curing condition does not allow the dehydration and salt concentration. At risk are meat products under vacuum, fat, and oil. The curing period of industrialized products such as salami and ham devitalizes the larvae in 1–2 weeks. On the contrary, the curing period of homemade meat products cannot be standardized, and there is the risk that meat from *Trichinella* sp.-infected pigs is used (Gottstein et al. 2009).

Since parasites of the genus *Trichinella* circulate mainly among wildlife, the main preventative measures for farmed animals are to (1) avoid access to wild animal carcasses, their scraps, and offal to domestic animals; (2) avoid the use of wild animal carcasses, their scraps, and offal for feeding domestic animals; (3) avoid access to pig carcasses, their scraps, and offal to domestic animals; and (4) avoid free-range of domestic pigs in the wild (Table 9.5) (World Organization for Animal Health 2013; Pozio 2014).

The global yearly pig production has reached one billion in 2015. Except for some regions with cultural and religious reservations regarding the consumption of pork, the pig production is characterized by an increasing dichotomy of production systems: traditional subsistence-driven small-scale production on the one side and specialized industrial farming on the other. Indoor farms are now responsible for more than half of the global pig production, but indoor farming alone does not prevent *Trichinella* spp. transmission if controlled management conditions are not in place (Pozio 2014). Because *Trichinella* spp. mainly infect wildlife, the eradication of these zoonotic agents cannot be achieved, but circulation among domestic pigs can be controlled by appropriate and continuous veterinary controls (Gamble et al. 2007).

## 9.3 *Ascaris* spp.

### *9.3.1 Introduction*

Ascariasis in humans and in pigs is caused by two of the most socioeconomically important nematodes: *Ascaris lumbricoides* Linnaeus, 1758, and *Ascaris suum* Goeze, 1782, respectively. Human ascariasis is a soil-transmitted helminthiasis, included in the WHO list of neglected tropical diseases, infecting more than one billion people (Dold and Holland 2011). Even if the majority of infections are asymptomatic, clinical manifestations of human ascariasis typically involve acute and chronic symptoms (lung inflammation and fever due to larval migration; abdominal pain, nausea, retarded growth in children, and intestinal obstruction due to the massive presence of adult worms) (Dold and Holland 2011). Ascariasis in pigs is frequent in both intensive and extensive breeding systems, being a source of substantial economic losses (Skallerup et al. 2012).

### 9.3.2 The Life Cycle

Hosts contract *Ascaris* spp. infection via the fecal-oral route. When infective eggs (55–75 μm × 40–50 μm) are ingested, L3 hatch in the small intestine and migrate to the caecum and proximal colon where they penetrate the mucosa (Crompton 2001). The larvae then migrate via the portal blood to the liver reaching the lungs on days 6–8 p.i. The larvae penetrate the alveolar space and move to the pharynx where they are swallowed, resulting in returning to the small intestine 8–10 days p.i. *Ascaris* spp. molt to L4 in the small intestine on day 10 p.i., where they develop to adult worms (male 15–25 cm × 2–4 mm; female 20–35 cm × 4–6 mm). The estimate of daily egg production per female is in the range of 200,000 eggs, but the number of eggs produced per female decreases with worm load. Unembryonated eggs enter the environment via the feces and can remain viable in the soil for up to 15 years, and during embryonation, larvae undergo two molts in the egg (Geenen et al. 1999). Even though both *A. lumbricoides* and *A. suum* display strong affinity for their conventional hosts (Nieberding et al. 2004), experimental cross-transmission studies have demonstrated that *A. lumbricoides* can infect pigs and vice versa (Barton 2001; Seehausen 2004). In *A. lumbricoides* non-endemic areas in North America, Denmark, and Italy, infected human hosts were found to harbor worms of pig origin (Jex et al. 2011; Zhu et al. 1999; Cavallero et al. 2013), indicating that pigs are a potential reservoir of infection for the human host population. However, molecular epidemiological studies in *Ascaris* spp.-endemic regions indicate that the level of cross infection between host species is low or absent and that gene flow is limited between/among different genotypes (Blouin 2002; Tamura et al. 2011; Librado and Rozas 2009).

### 9.3.3 Taxonomy

Morphologically indistinguishable, human and pig *Ascaris* have been shown to differ by only six (1.3%) nucleotides in the first internal transcribed spacer (Zhou et al. 2012) and by 3–4% in the mitochondrial genome sequence (Blouin 2002), indicating that the species are closely related at a phylogenetic level. Molecular characterization using a PCR-RFLP approach on a nuclear marker has confirmed that most pig nematodes displayed the typical *A. suum* pattern, while human nematodes from endemic regions show the typical *A. lumbricoides* pattern. Cross infection is confirmed in both hosts by instances of *A. suum* genotypes in humans and *A. lumbricoides* in pigs (Cavallero et al. 2013). Moreover, a significant percentage of nematodes displaying the "hybrid" pattern has been observed in both human and pig nematodes, strongly inferring the presence of gene flow between the two taxa (Cavallero et al. 2013). This combined evidence suggests that *A. suum* can function as a relevant agent of human infection. The zoonotic potential of *A. suum* needs to be considered in order to plan more efficient control programs.

### 9.3.4 Epidemiology

*Ascaris lumbricoides* and *Ascaris suum* occur and flourish where climatic conditions permit survival and development of their free-living stages and where poverty, inadequate water and sanitation facilities, and poor hygienic practices promote fecal contamination of the environment and fecal-oral contacts (Table 9.2). *Ascaris* spp. infections are prevalent in many less-developed countries today and historically were endemic in some developed countries. The DALYs lost due to ascariasis is 10.5 million (Chan 1997). The estimates of the global distribution and disease burden of *A. lumbricoides* and ascariasis have been based on informed approximations. In common with most parasitic infections, the transmission of *A. lumbricoides* is constrained by temperature and humidity. This is because the eggs excreted by female worms require a period of embryonation in the external environment prior to being infective to humans. Experimental studies suggest that maximum rates of embryonation of *Ascaris* ova occur at temperatures between 28 and 32 °C, with embryonation arresting below 5 °C and above 38 °C. Similarly, *Ascaris* ova do not embryonate at low humidity (atmospheres less than 80% saturation). These differing rates of development and survival will influence parasite establishment in the human host and hence observed limits of transmission, which can be observed at global and country levels. The spatial analysis and a range of environmental factors show that high and low land surface temperature and extremely arid environments limit *A. lumbricoides* transmission. In particular, the prevalence of *A. lumbricoides* is generally <4% in areas where maximum land surface temperature exceeds 35 °C and drops to <1% by 40 °C. Prevalence is also <2% in areas classified as arid and <0.1% in "hyperarid" areas. Within these biological limits, local endemicity will be mediated by settlement patterns and urbanization. Interestingly, the prevalence of *A. lumbricoides* is higher in urban and peri-urban than in rural settings, especially in Africa. Such differences may reflect variations in inadequate water and sanitation or higher population density in peri-urban and urban areas compared to rural areas.

Today, the prevalence of *A. lumbricoides* is rapidly decreasing in China especially in urban areas where urbanization has been accompanied by improvements in water and sanitation. Globally, country level analysis suggests that the prevalence of *A. lumbricoides* is negligible in those countries with a gross domestic product > US$20,000 in 2010. Based on the global limits of transmission, Pullan and Brooker (2012) estimated that 5.23 billion people (0.99 billion of school-going age) were living in areas of stable *A. lumbricoides* transmission worldwide in 2010 and hence at risk of infection. The vast majority (71%) of these individuals were living in Asia and Oceania, with only 18% living in Africa and the Middle East and 11% in Latin America and the Caribbean.

The distribution of *A. suum* in pigs is cosmopolitan and remarkably extensive, but exact estimates do not exist. Although the prevalence and intensity vary with geographical region and production system from intensive herds to backyard pigs, a large proportion of swine herds are infected worldwide. Among poor pig farmers in developing countries, *A. suum* in general ranks within the most important pathogen

worldwide, and even though industrialized indoor production with high levels of hygiene may have lower prevalence than more traditional systems, recently implemented changes in management to accommodate for better welfare, i.e., group housing of dry sows (non-lactating, most often pregnant sows) and sprinklers as a surrogate for wallowing, may increase the prevalence within these herds. High prevalence ranging from 17% to 35% has been reported from the Scandinavian countries by fecal analysis in fatteners and gilts (=pregnant pig before first farrowing) and more recently 22% in Danish sows/gilts. Adult worms were detected in 17% of finishers at slaughter in Canada. In Japan, 15% slaughtered pigs ($n$ = 129) were found positive by coproscopy. African studies, also based on fecal egg counts, revealed prevalence of 2% in growers on small-holder farms in Nigeria, 3–13% in growers in Ghana, and 14% and 40% with only small variation across age groups in Burkina Faso and Uganda. Some of these coprological studies undoubtedly overestimate the prevalence as low or moderate egg counts may be due to coprophagia and therefore represent false positive findings, especially if stocking rate is high. In contrast, infections with only one worm or all worms of the same sex will result in underestimation of prevalence as compared to postmortem-based studies. In the early 1990s, 60% of Danish farms were infected with *A. suum*, but farming practices have now changed to loose housing of sows in dynamic groups, where sows in different stages of gestation move in and out on a weekly basis, and recently 76% of the surveyed Danish farms were found by coproscopy to be infected. In smaller organic farms with traditional husbandry in Austria, *A. suum* was present in 30% of sow units and 59% of finishing units. More than 50% of pigs at slaughter had "milk spots" on the liver. In Germany, 7% of 144 breeding farms had *A. suum* infections. In a small Dutch survey including 36 farms, the prevalence of *A. suum*-positive farms (across age groups) was 50% for free-range, 73% for organic, and 11% for conventional farms. The highest prevalence was seen among finishing pigs with about half of the examined groups being infected on free-range and organic farms, while none were found to be positive on conventional farms. A marked difference between production systems was also confirmed with regard to prevalence of milk spots: 8% vs. 1% in pigs slaughtered from Danish organic and conventional farms, respectively, based on standard recordings of condemnations from the abattoir. In Denmark, all pigs from the organic farms would have stayed outside until at least weaning.

### *9.3.5 The Disease in Humans*

Ascariasis is the term used to describe the spectrum of disease symptoms observed in infected humans and pigs. Morbidity and mortality increase with worm burden (Pawlowski and Davis 1989), and those who harbor light infections tend to be asymptomatic. Aggregation leads to relatively few individuals harboring sufficient worms to precipitate life-threatening or severe morbidity (Anderson and May 1991). As *Ascaris* larvae develop, different stage-specific antigens are observed (Kennedy

and Qureshi 1986), and various tissues are invaded; therefore, the effects of infection differ over the course of larval migration and development. While the majority of infections are asymptomatic, an estimated 120–220 million cases with *A. lumbricoides* demonstrate associated morbidity (Albonico et al. 1999; Chan 1997). The manifestations of ascariasis can be broadly characterized into acute and chronic symptoms. Human hosts tend to experience acute lung inflammation, difficulty in breathing, and fever as a result of larval migration through the pulmonary tissue. Abdominal distension and pain, nausea, and diarrhea are also characteristic symptoms of adult worm infection and chronic ascariasis (Table 9.3) (Crompton 2001). Entangled adult worms have also been documented as leading to mechanical intestinal obstruction in 0.005–2 per 1000 infections per year (Pawlowski and Davis 1989). Infection in children is associated with appetite loss (Hadju et al. 1996), lactose maldigestion (Carrera et al. 1984), and impaired weight gain (Hadju et al. 1996; Stephenson et al. 1980). The majority of persons with *A. lumbricoides*/*A. suum* will exhibit no signs or symptoms. This is because pathology is strongly related to the number of worms present (the intensity of infection) and most individuals harbor only a few worms. In the minority (5%) of individuals who harbor large worm burdens, infection can result in clinical disease, including intestinal, biliary, and pancreatic obstructions. If left untreated, these complications can prove fatal. In addition to these acute manifestations, intervention studies have demonstrated that moderate worm burdens are associated with reversible growth deficits in children. Chronic ascariasis has also been implicated in some studies with reduced cognitive performance and school performance.

The pathology caused by infection corresponds to the life-cycle stage and the intensity of infection. Acute disease can arise due to larval migrations or adult worms causing intestinal obstruction, whereas chronic disease is due to the insidious effects of infection on nutrition and cognition and is most common among children, due to age-related intensity patterns. The main features of ascariasis include: (1) Pulmonary migration induces hypersensitivity, which can manifest as asthma. The reaction to migration can be severe resulting in cough, hypersecretion of mucus, and bronchiolar inflammation, which is usually subclinical; (2) The intestinal phase of infection is generally asymptomatic, but heavy infection can cause physiological abnormalities in the small intestine resulting in malabsorption of nutrients, vitamin A, and other micronutrients, nutritional deficiency, and growth failure, especially in children; (3) Moderate and heavy infection in children may also adversely affect cognitive development; (4) Heavy infection can cause serious complications, the most common of which is small bowel obstruction by a bolus of worm, leading to gastrointestinal discomfort, vomiting, and occasionally intussusception and death. *Ascaris*-related intestinal obstruction most commonly occurs in the ileum of young children; (5) Female *A. lumbricoides* worms can migrate up the common bile duct into the liver where they can cause bile duct obstruction leading to cholangitis or pancreatitis; (6) The worms can also die, releasing eggs. Granulomatous reactions around the dead worm can result in liver abscess and acute upper abdominal pain, sometimes with fever and jaundice. Histologically there is a granulomatous reaction round the dead worm with release of the eggs.

### 9.3.6 The Disease in Pigs

Porcine ascariasis is also known to interfere with the health and performance of pigs and is responsible for reduced feed to gain ratios resulting in considerable economic losses (Stewart and Hale 1988). Similarly, to human infection, porcine hosts display stunted growth and consume less food than uninfected controls (Stephenson et al. 1980; Forsum et al. 1981). In *A. suum* infections, white spots (WS) are white pathological lesions that are formed by the mechanical injury and inflammatory response induced by migrating larvae in the liver. WS formation over the superficial hepatic surface and within the liver tissue is characteristic of porcine infections in response to larval migration through the liver (Ronéus 1966). The majority of *A. suum* infections in pigs are subclinical. The penetration of cecum and proximal colon by newly hatched third-stage larvae (L3) results in petechial bleedings of the mucosa only, whereas the following liver migration is accompanied by severe pathology (Roepstorff and Nansen 1998). During the lung penetration phase 6–8 days p.i., pigs are subject to respiratory stress (pneumonitis) reflected in increased breathing rate, dyspnea, and dry coughing. The condition resembles Loeffler's syndrome in humans infected with *A. lumbricoides* and is associated with an increased number of blood eosinophils. Considering the large number of experimental infections performed in our and other laboratories, it is remarkable that barely any of these have reported such symptoms, which may indicate low incidence or only vague symptoms.

### 9.3.7 Diagnosis and Treatment

The detection of *Ascaris* eggs in fresh or fixed stool samples processed by a flotation or sedimentation method and examined by microscopy is the most reliable means of identifying cases of *Ascaris* spp. infection in both people and pigs (Table 9.4). Humans can be successfully treated by albendazole (400 mg single dose), mebendazole (500 mg single dose), pyrantel embonate (11 mg/kg single dose), or levamisole (2.5 mg/kg single dose). Deworming of fattening pigs is not entirely effective and does not reduce the occurrence of liver white spots due to the migratory larvae.

### 9.3.8 Prevention and Control

For the control of *Ascaris* spp., it is essential to reduce the parasite intensity by means of improvements in sanitation, health education, and anthelminthic treatment. The eradication of *Ascaris* spp. relies primarily on sanitation measures for the safe disposal of human feces in order to interrupt transmission, prevent reinfection, and gradually reduce and eliminate worm burdens (Table 9.5). Transmission of

*Ascaris* spp. among pig populations is dependent on housing systems, hygiene, management practices, and anthelminthic treatment. The most important risk factors for ascarid infection in pigs include large fatteners and gilts (the age group with the highest infection intensity), country, weaning age (late weaning is associated with higher prevalence of infection), and water supply (drinking facilities located in the lying area) (Roepstorff et al. 1998).

## 9.4 *Toxocara* spp.

### *9.4.1 Introduction*

*Toxocara canis* is the main etiological agent of visceral and ocular larva migrans, covert toxocariasis (synonym: toxocariasis), and neurological toxocariasis in humans (Despommier 2003). The dog and other canids (e.g., coyote, fox, jackal, wolf) act as hosts, whereas, every species of mammals including humans and birds can serve as paratenic hosts by harboring the second-stage larvae in their tissues. *T. canis* has a cosmopolitan distribution. Two other nematodes of the superfamily Ascaridoidea, *Toxocara cati* of cats and *Baylisascaris procyonis* of raccoons have occasionally been reported as agents of visceral and ocular larva migrans. Since the life cycle of *T. cati* is similar to that of *T. canis*, except that felids are definitive hosts, most of the information provided for *T. canis* is also applicable to *T. cati*.

### *9.4.2 The Life Cycle*

The life cycle of *T. canis* is complex. When adult dogs ingest larvated eggs, most of the larvae (150–180 μm) migrate to somatic tissues. In a proportion of dogs, some larvae undergo tracheal migration, return to the intestine, and develop to adult worms. Those larvae in the tissues of dogs remain dormant for several years, and if a bitch becomes pregnant, the somatic larvae become activated and migrate across the placenta to the fetus. The larvae then complete their migration to the intestine in the newborn pup where they develop to adult worms (male 4–6 cm in length, with a curved posterior end; female up to 15 cm) and produce eggs (95 μm × 70 μm). Adult worms in pups can also develop from larvae transferred from the bitch in her milk and from ingested eggs. Pups are often heavily infected. The lactating bitch can be reinfected with eggs from her pups, while she is grooming them. Larvae in *T. canis* eggs eaten by any species of mammal or bird (paratenic hosts) hatch, migrate into the tissues, and remain viable for prolonged periods of time (up to more than 1 year). Stray dogs, foxes, etc., that scavenge rodents, lagomorphs, and birds, and occasionally large animals, e.g., sheep, pigs, wild boar, and pets, which hunt or are fed with raw meat, ingest these larvae. The adults develop in the intestine without any further migration.

### 9.4.3 Epidemiology

Humans can become infected by ingesting *T. canis* eggs from dog feces by geophagia, poor hygiene, contact with dogs (mainly pups), contaminated food and water, and consumption of raw or undercooked meat of dogs and other animals harboring the larvae (Table 9.2). Pups can shed up to $10^5$ eggs per gram of feces, and a female worm can produce more than $10^5$ eggs/day. Therefore, a heavily infected bitch and her pups can disseminate more than $10^7$ eggs daily. The prevalence of infection in dogs varies widely. In most litters of puppies, the prevalence is considered about 100%. Prevalence and fecundity of worms decline considerably in adult dogs, and the overall prevalence has declined in pets in developed countries in the last decade. The prevalence in pets also varies between treated and untreated animals and between adults and puppies. For example, 30% and 1–4% of untreated and treated (4 times per year) dogs, respectively, were infected in the United Kingdom. In Poland, 58% of puppies and 2.5% of adult dogs were infected. The prevalence in pet dogs (both adults and pups) ranges from 0.4% in Australia, 3–5% in Canada, and 5% in the USA (Palmer et al. 2008; Mohamed et al. 2009; Jenkins et al. 2011). Urban foxes are another source of infection with *T. canis* for gardens. In the European Union, the prevalence of infection ranges from 40% to 73% of urban and rural foxes. Eggs begin to develop only at or above 10 °C and reach the L2 stage at 15–25 °C in 2–7 weeks, but they can remain viable at low temperature protected by snow or feces. Only heat and desiccation is known to kill the embryo within eggs in the environment. In favorable conditions, eggs remain viable in large numbers for 6–12 months. Eggs can be common in soil samples (e.g., 0.1–23 eggs/g of soil), although most surveys do not differentiate *T. canis* and *T. cati* eggs. Because the egg surface is sticky, it could be carried long distances attached to objects. A number of invertebrates (e.g., slugs, earthworms, cockroaches, beetles) can ingest the eggs which pass intact through their gut facilitating concentration and dissemination. Free-ranging farm animals and birds can ingest *Toxocara* spp. eggs. Pigs, wild boars, and birds have been infected with L2 when fed raw viscera. L2 of *T. canis* can persist in pigs for more than 1 month, but they have been described as dying relatively quickly in pigs. It has been reported that L2 persist for 7 months in lambs, 4.5 months in pigeons, and 3.5 years in chickens (Taira et al. 2004). Larvae can be detected in all host tissues, with highest numbers in the liver. The viability of larvae declines after slaughter, but infective larvae can still remain after 4 days at 4 °C.

### 9.4.4 The Disease in Humans

There are three well-described clinical presentations of *Toxocara* spp. infection in humans: 1. visceral toxocariasis (VT) or visceral larva migrans, when body organs, such as the liver or lungs, are affected; 2. ocular toxocariasis (OT) or ocular larva migrans, when the eye is affected; and 3. covert or common toxocariasis, when

symptoms remain mild and nonspecific (Table 9.3). The main clinical signs and symptoms of VT are high eosinophilia, hepatosplenomegaly, fever, respiratory signs, lymphadenopathy, pallor, neurological manifestations (e.g., convulsions, strabismus), and myocarditis. Most of the patients with VT are children 2–4 years of age. OT can occur at any age. The inflammatory response involves granuloma which can cause severe visual loss. Mild toxocariasis is characterized by highly variable signs and symptoms (e.g., weakness, abdominal pain, lymphadenopathy, skin lesions, pruritus, respiratory features, headache). Neurological signs and symptoms have also been documented. For example, a strong relationship between seropositivity and epilepsy has been documented in children with a diagnosis of ocular larva migrans (Quattrocchi et al. 2012).

### *9.4.5 Diagnosis and Treatment*

In humans, clinical signs and symptoms of toxocariasis are not pathognomonic. The diagnosis of choice is made by an ELISA using excretory/secretory larval antigens to detect circulating antibodies. However, this test has not been standardized or validated. The specificity and predictive values of this test are high in developed countries, but the values decrease in developing countries where cross-reactions with other nematode infections can occur (Fillaux and Magnaval 2013).

Serologic antibody tests are available on the market. The recommended test for toxocariasis is EIA using *Toxocara* excretory/secretory antigens to detect IgG against *Toxocara* larvae. In patients with a presumptive diagnosis of toxocariasis, sensitivity of *Toxocara* EIA is 78% and 73% for VT and OT, respectively, and the specificity of the test is >90% for VT and OT at a titer 1:32. EIA titers can remain elevated for years, making it difficult to determine if a positive serologic test result indicates past exposure versus active infection. Stool examination for ova and parasites is not useful in diagnosing *Toxocara* because the larvae do not mature into adult worms and therefore no eggs are shed. Eosinophilia, hypergammaglobulinemia, and increased isohemagglutinin A and B titers are laboratory findings associated with acute toxocariasis. Laboratory abnormalities are less likely to be found in OT patients than in VT patients. Albendazole (400 mg × 2 for 5 days) and mebendazole (200 mg × 2 for 5 days) are the recommended treatments for *Toxocara* infection in humans.

### *9.4.6 The Disease in Dogs*

In newborn pups, the migration of an overwhelming larva burden can cause hemorrhagic pneumonia. Adult worms induce hypertrophy of the muscular layer of the gut, villous atrophy, and malabsorption. A heavy adult worm burden can be the cause of poor growth, emaciation, diarrhea, and constipation in pups. Infections in

dogs are diagnosed by the recovery of worm eggs on fecal flotation techniques using high-specific gravity solutions. The test is sensitive for pups, whereas its sensitivity decreases to 50% for adult dogs due to the low levels of egg production.

### *9.4.7 Prevention and Control*

Public health and veterinary services should be aware of signs and symptoms of toxocariasis and educate patients and parents and pet animal owners on how to avoid *Toxocara* spp. infection and how to control the infection. Families who own dogs or cats should be encouraged to routinely take their animals to the veterinarian for deworming, because these pets will typically not have symptoms of illness despite shedding *Toxocara* spp. eggs (Table 9.5). This is especially important for families with new kittens or puppies, because younger animals have a higher likelihood of being infected with *Toxocara* spp. Animal owners should avoid allowing their pets to deposit waste in areas where children play, or they should promptly remove and properly dispose dog and cat feces that have been deposited in these places (Table 9.5). Enactment of local community laws that enforce immediate pickup and disposal of dog and cat feces should be encouraged. These actions ensure that if *Toxocara* spp. eggs are present, they do not have enough time to undergo embryonation and become infectious. Veterinarians should provide education to pet owners regarding the risks of *Toxocara* spp. infection. Even if families do not own animals, parents need to understand that their child can come into contact with soil contaminated with infectious *Toxocara* spp. eggs, especially in areas frequented by both pets and feral dogs and cats such as parks or playgrounds. Children diagnosed with pica should have underlying causes of the disease identified and treated by their pediatrician to decrease their risk for *Toxocara* spp. infection. Promoting good hand hygiene and teaching children not to eat soil will decrease their risk for ingesting. The egg shell confers considerable resistance from environmental conditions. On concrete exposed to sunlight, eggs will desiccate, but those in cracks will be protected. High concentrations of sodium hypochlorite solution (bleach) which can be a useful disinfectant for surfaces are usually neutralized by organic matter or do not have sufficient contact time to be effective. A flame gun can be used to ensure destruction of the eggs. In damp, shaded soil, eggs could survive for years. Only severe measures can ensure disinfection of soil in gardens. It has been suggested that surface soil which can be heavily contaminated with *T. canis* or *B. procyonis* should be thoroughly tilled and flamed several times or 10–20 cm of top soil removed and replaced (Kazacos 1991). Sand in sandboxes can be steam sterilized or replaced and sandboxes covered when not in use. Infection with *T. canis*, at least from pet dogs, can be prevented by treatment of the animals. Bitches should be treated with fenbendazole 50 mg/kg/day, every day for 3 weeks before parturition until 3 weeks after parturition. The treatment must be repeated for each pregnancy. Treatments at 1- or 2-month intervals for dogs and cats, respectively, should be appropriate. Treatment of stray dogs and foxes is not practical. Stray

populations should be excluded from gardens, play areas, and playgrounds by fencing. Removal of dirt from beneath fingernails and thorough hand washing are necessary. Vegetables eaten raw should be peeled or well washed, but as the eggs are sticky, vegetable gardens should be fenced to exclude all canids, although this will not exclude cats. While the importance of larvae in meat is undetermined, meat, particularly liver, should be well-cooked (Overgaauw and van Knapen 2013).

## 9.5 *Baylisascaris procyonis*

### *9.5.1 Introduction*

*Baylisascaris procyonis* is an intestinal nematode of raccoons and other procyonid species (e.g., kinkajous), which are the natural final hosts (Kazacos et al. 2011). Domestic dogs are also susceptible to this roundworm and can shed eggs (Miyashita 1993; Kazacos 2001; Bowman et al. 2005). This nematode is well documented as an important and frequent cause of visceral, ocular, or neural larva migrans in mammals including humans and in birds. Dogs, but not raccoons, can also develop neural larva migrans following *B. procyonis* infection (Rudmann et al. 1996). The number of documented infections in humans is limited, but the severity of the infection, which leads to death about one fourth of patients, makes this parasite of great importance for the public health (Kazacos et al. 2013).

### *9.5.2 Life Cycle*

Adult male worms reach lengths of 7–12 cm and females 14–28 cm. Unlike most ascarid species in which the L3 is the infective stage within the egg, L2 in the eggs of *Baylisascaris* spp. is the infective stage, with the second molt occurring in the infected host animal (Bauer 2013). L3 of *B. procyonis* are 1500–1900 μm long and 60–80 μm wide. Cross sections of the larvae show important diagnostic features such as prominent single lateral alae, a large compressed gut, and smaller triangular excretory columns (Kazacos 1997). Cubs acquire infection by ingesting embryonated eggs from the contaminated environment (Fig. 9.3). L2 penetrate the intestinal wall where they develop with the preadult stage returning to the intestinal lumen to mature. Adult raccoons may also become infected by ingesting L3 in prey intermediate hosts, after which further development to mature worms occurs in the intestinal lumen. When an intermediate host ingests embryonated eggs, L2 are released from hatched eggs, penetrate the intestinal wall, and migrate through the liver to the lungs and via blood to other tissues. A few larvae may invade the central nervous system and eyes (Bauer 2013). In the tissues of intermediate hosts, the larvae grow, molt, and remain as L3 (Kazacos 2001).

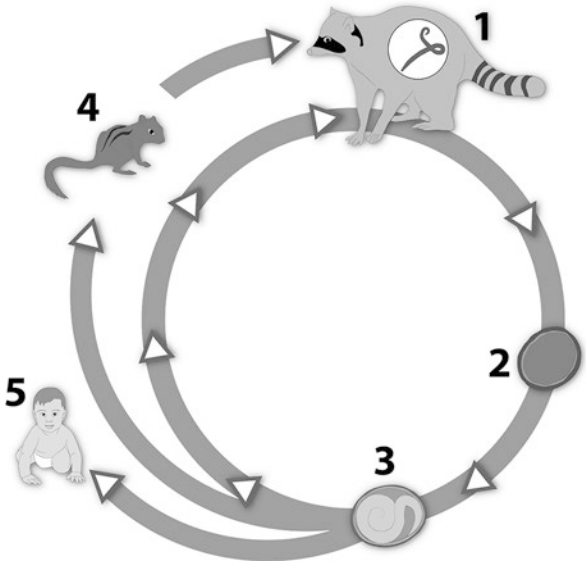


**Fig. 9.3** Life cycle of *Baylisascaris procyonis*. *1* Adult worms live in the gut of raccoons (*Procyon lotor*) and dogs following ingestion of embryonated eggs or intermediate hosts harboring third-stage larvae (L3); *2* unembryonated eggs are shed with the feces of infected hosts; *3* eggs embryonate in the environment and harbor second-stage larvae; *4* various small mammals can act as intermediate hosts after ingesting fully embryonated eggs which then develop to L3 in tissues; *5* humans can accidentally acquire the infection by ingesting embryonated eggs

### *9.5.3 Epidemiology*

Out of nine species in the genus *Baylisascaris*, only *B. procyonis* has been documented in humans. This zoonotic parasite is indigenous of North American raccoons (*Procyon lotor*). The prevalence of infection in raccoon populations varies by region according to the distribution of the host but may be as high as 60% or more in some areas (Kazacos 2001). *B. procyonis* infection has also been identified in central Europe and Japan, following the introduction of raccoons from North America (Table 9.2). Eggs remain infective for years and can survive freezing temperatures of −15 °C (Shafir et al. 2011). More than 100 animal species, both mammals including humans and birds, are known to act as intermediate hosts or "dead-end" hosts of *B. procyonis* and commonly develop clinical signs of larva migrans. Pigs, small ruminants, and cats appear to be less susceptible to this parasite. Free-ranging raccoons habitually defecate at preferred sites where *B. procyonis* eggs can accumulate. These sites are commonly associated with raccoon resting and sleeping places. The number of documented infections in humans is limited, but most of them have been diagnosed in the USA. The introduction of raccoons in other continents as pet resulted in the introduction of *B. procyonis* in central Europe.

### *9.5.4 The Disease in Humans*

Four clinical patterns of *B. procyonis* infection have been described in humans: neural, ocular, visceral larva migrans, and subclinical baylisascariasis (Table 9.3). The intravitam diagnosis of *B. procyonis* larva migrans is difficult and is based on clinical signs, history of exposure to raccoons, neuroimaging, laboratory findings, detection of specific serum antibodies, and detection of larvae in needle aspiration of brain biopsies (Gavin et al. 2005). A few cases of visceral larva migrans have been described in young children associated with nonspecific clinical signs (fever, lethargy, nausea, macular rash, pneumonia, and hepatomegaly). Clinical signs and internal pathology are caused by extensive extraneural migration of *B. procyonis* larvae (Gavin et al. 2005). In a German case-control study, two patients with previous contact to infected raccoons had antibodies to *B. procyonis,* detected using immunoblotting and elevated serum levels of immunoglobulin E and specific liver enzymes, consistent with visceral larva migrans (Conraths et al. 1996). Ocular larva migrans can occur with or without other clinical manifestations. Cases without other signs are assumed to be the result of infection with few *B. procyonis* larvae. The raccoon roundworm is considered the most common cause of the "diffuse unilateral subacute neuroretinitis syndrome" (Gavin et al. 2005). Progressive inflammatory and degenerative alterations in the retina and optic disk usually affect only one eye. The disease has been observed in both children and adults. Most cases have been reported from North America, but one case has been reported in Germany; the patient had kept a raccoon as pet indoors (Küchle et al. 1993). Baylisascariosis can also occur without any symptoms. In a *B. procyonis*-endemic region of the USA, 8% of 389 children screened by ELISA had antibodies to *B. procyonis* but no history of disease (Brinkman et al. 2003). However, since nematodes share a lot of antigens among them, the specificity of the ELISA is questionable.

In cases of ocular larva migrans, a single, motile larva may occasionally be detected in the retina by ophthalmoscopic examination. Postmortem diagnosis can be made by histopathological examination and detection of *B. procyonis* larvae or by PCR amplification of DNA in tissue samples. An ELISA and an immunoblot assay (Dangoudoubiyam and Kazacos 2009), which are based on excretory/secretory antigens from L2 of *B. procyonis*, have been used for serological testing (Table 9.4). A recombinant antigen from *B. procyonis* L3 has been also developed. Clinical cases of neural larva migrans have been diagnosed in humans from North America (Haider et al. 2012). Patients were mainly toddlers, young children, and individuals with mental or developmental impairment, and nearly all were males. A significant number of the human cases of neural larva migrans caused by *B. procyonis* have been fatal or resulted in moderate to severe neurological sequelae. There is only one report in the literature of full recovery; it was preceded by early treatment using albendazole and high weekly doses of corticosteroid daily (Pai et al. 2007).

### 9.5.5 Prevention and Control

Raccoon populations should be controlled, particularly in residential areas and public parks. If raccoons are kept as pet animals or for public display in zoological gardens and other facilities, preventive measurements such as quarantine and deworming are essential to reduce the risk of transmission of *B. procyonis* to other animals and humans (Table 9.5). The most effective means of preventing infection in people and animals is to avoid exposure to raccoons and their feces. Practical control measurements include identification and rapid elimination of raccoon defecation sites from backyards (including the soil) by heat treatment such as burning using a flame or by steaming with boiling water (Vantassel 2012). Heat is the most effective method of killing *B. procyonis* eggs, which become nonviable at temperatures above 62 °C (Shafir et al. 2011). Children should not be allowed to play in areas likely to be contaminated with raccoon feces.

## 9.6 *Capillaria philippinensis*

### 9.6.1 Introduction

*Capillaria philippinensis* was first documented more than 50 years ago when the first human case was discovered in Luzon, Philippines. This zoonotic nematode is the causative agent of intestinal capillariasis a severe disease that may lead to death unless patients are treated. Infection can result from the consumption of raw freshwater or brackish-water fish (Cross 1992).

### 9.6.2 The Life Cycle

The natural cycle of *C. philippinensis* occurs between fish-eating birds which act as final hosts and freshwater or brackish-water fish which act as intermediate hosts (Fig. 9.4), but adult worms develop also in the gut of experimentally infected gerbils and monkeys; it follows that the role of mammals in the natural cycle cannot be ruled out. In endemic areas, humans can play the role of final hosts by defecation in proximity to water resources. Adult worms develop in the gut mucosa of the final host (females 2.3–5.3 mm in length, 29–47 μm in width; males 1.5–3.9 mm in length and 23–28 μm in width) and release unembryonated eggs (36–48 μm × 18 μm) within 22–24 days. Eggs, which reach water, embryonate and can be ingested by fish in which they hatch in few hours. The larvae develop in the fish gut doubling in length in 3 weeks. When a fish-eating animal or human ingest the fish, the larvae develop at the adult stage in the host gut within 2 weeks. In humans and

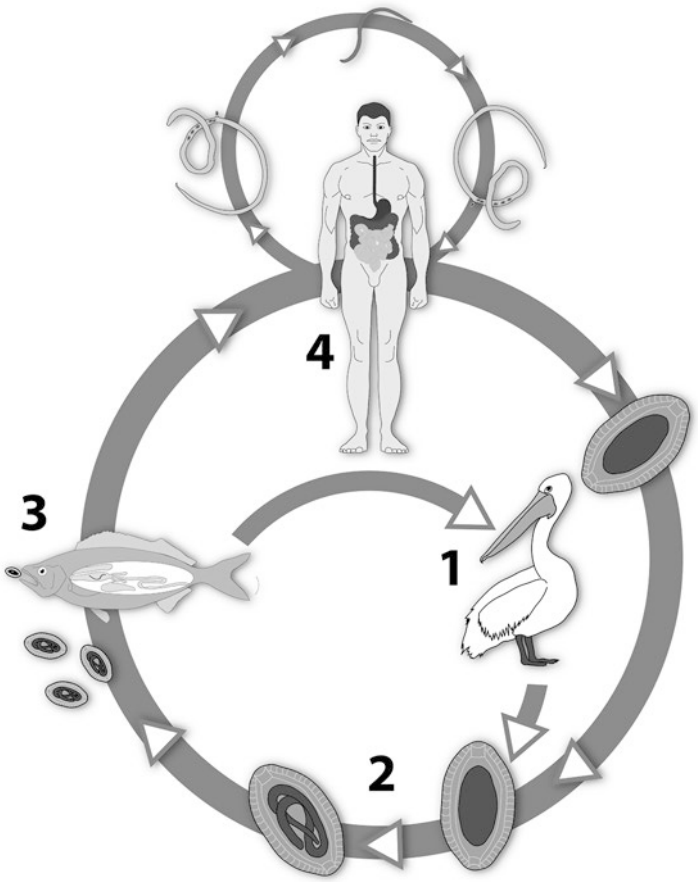


**Fig. 9.4** Life cycle of *Capillaria philippinensis*. *1* Adult worms develop in the gut mucosa of piscivorous birds and humans; *2* shed eggs embryonate in water; *3* fish are infected by ingesting mature eggs; *4* when humans and fish-eating birds ingest infected fish, the larvae develop to the adult in the gut. In humans, adult females can in addition to eggs produce larvae which develop to adult worms resulting in massive infections

in experimentally infected gerbils and monkeys, adult females can produce larvae instead of eggs which develop to the adult stage causing massive infections.

### *9.6.3 Epidemiology*

The most important focus of *C. philippinensis* infections in humans has been documented in the Philippines where about 2000 cases and hundreds of deaths occurred since 1964. Intestinal capillariasis in humans has also been documented in Egypt,

Japan, Indonesia, Iran, India, Korea, Lao PDR, People's Republic of China, and Taiwan (Table 9.2) and in travelers who acquire the infection in one of these endemic countries and develop the disease when they return home (Lu et al. 2006; Saichua et al. 2008; Soukhathammavong et al. 2008; Fan et al. 2012; Jung et al. 2012; Vasantha et al. 2012). In Thailand, 754 cases with 5 deaths were documented from 1976 to 2006 (Saichua et al. 2008). Even if the fish species acting as intermediate hosts of *C. philippinensis* have been not deeply investigated, experimental infections and sporadic reports in naturally infected fish suggest that *Cyprinus carpio*, *Puntius gonionotus*, *Rasbora boraperensis*, *Eleotris melanosoma*, *Ambassis commersoni*, *Apagon* sp., and *Hypseleotris bipartita* act as intermediate hosts.

### *9.6.4 The Disease in Humans*

Capillariasis is one of the few nematode diseases which can cause severe illness and death in untreated people. After an incubation period of about 3 weeks, the first symptoms of abdominal pain, diarrhea, and borborygmus appear. Patients with advanced intestinal capillariasis usually present with watery diarrhea, weight loss, abdominal pain, borborygmi, muscle wasting, weakness, and edema (Table 9.3). Within weeks, diarrhea increases, with 8–10 voluminous stools passed each day. Patients lose a great deal of body weight and suffer from malaise, anorexia, and, more rarely, vomiting. In addition, patients experience muscle wasting and weakness, distant heart sounds, hypotension, edema, gallop rhythm, pulsus alternans, abdominal distention and tenderness, and hyporeflexia. Laboratory findings include protein-losing enteropathy, malabsorption of fats and sugars, decreased excretion of xylose, and low serum levels of potassium, sodium, calcium, carotene, and total protein. In *C. philippinensis* infection, crypts have atrophied, flattened villi, and leukocyte cell infiltration, which are considered signs of intestinal cell injury (Tesana et al. 1983; Sangchan et al. 2007; Wongsawasdi et al. 2002). Therefore, the destruction of intestinal wall cells may interrupt nutrient absorption, causing weight loss. Low levels of IgG, IgM, and lgA with elevated levels of IgE present at the time of illness revert to normal on follow-up several months later. If treatment is not initiated soon enough, patients die because of the irreversible effects of the electrolyte loss, heart failure, or from septicemia (Whalen et al. 1969).

### *9.6.5 Diagnosis and Treatment*

The detection of *C. philippinensis* is based on the recovery of eggs, larvae (250–300 μm), and/or adult worms in the stool of the patients after concentration by a sedimentation technique (Table 9.4). Multiple stool examinations may be necessary, or small intestinal biopsy or intestinal aspiration also could reveal the parasites or eggs. An inexperienced laboratory technician may confuse *C. philippillensis* with

*Trichuris trichiura* eggs which have prominent mucoid bipolar plugs. The eggs of *C. philippinensis* are excreted sporadically in feces, and this may lead to a delay in the diagnoses of intestinal capillariasis. Early diagnosis is necessary in the treatment of intestinal capillariasis patients. In this regard improvement in the training of laboratory workers and the collection of multiple stool examinations are essential. In endemic areas, a clinical diagnosis can be made in patients presenting with abdominal pain, diarrhea, and gurgling stomach. Individuals with chronic infections experience weight loss, wasting, and an intractable diarrhea. Serology (ELISA) using heterologous crude larval antigens from *Trichinella spiralis* can identify intestinal capillariasis cases in endemic areas where coproscopy has been negative for eggs, larvae, and adult stages (Intapan et al. 2010); however, the risk of cross-reactions with other helminthic antigens cannot be ruled out. Albendazole is the drug of choice at 400 mg/day in two equal doses for 10 days. Insufficient duration of treatment results in a recurrence of infection, as determined by eggs and parasites in stool samples within 3–4 weeks.

### *9.6.6 Prevention and Control*

There is a reservoir host in birds and an intermediate host in fish, with the relationship of the naturally occurring cycle of bird-fish consumption. Ingestion by humans of infected fish will lead to infection in humans, resulting in intestinal capillariasis and, potentially, death. A primary strategy to fight the disease consists of avoidance of eating raw fish (Table 9.5). Because humans may transmit the infection back to fish, additional efforts include improvement in overall sanitation and education about the risk of defecation in proximity to water sources, such as lakes, streams, and rivers. Intestinal capillariasis can be prevented by educating high-risk populations on the hazards of eating small freshwater fish whole and uncooked. Cooking for a short period of time >65 °C in the core of the fish product for at least 5 min is considered sufficient to kill larvae in the fish intestine. Human-to-human transmission most likely occurs during the outbreaks in endemic regions such as the Philippines and Thailand, since indiscriminate disposal of feces is common in these countries. In such cases, improvement of sanitary conditions would be beneficial. Treatment of infected persons with albendazole should be included in a control program. Since *C. philippinensis* is a parasite infecting wild birds and fish, this zoonotic aspect of the pathogen impedes realistic control measures and eradication of the parasite. The health education campaign aims to avoid the consumption of raw fish and to avoid defecation near or into water resources in order to control *C. philippinensis* infection.

## 9.7 *Capillaria hepatica*

*Capillaria hepatica* is a cosmopolitan nematode parasitizing the liver of rodents, the primary host, and numerous other mammals including humans (Table 9.2). Adult female worms of *C. hepatica* lay eggs into the liver parenchyma. It is the causative agent of hepatic capillariasis and spurious infections in humans. The eggs are released into the environment by the death and decomposition of its host or by spurious release in the feces of a predator of the infected rodent. In humans, 72 cases of hepatic capillariasis have been documented worldwide. Humans as well as animals acquire hepatic infections by ingesting embryonated eggs in food or drink. The eggs occur in the soil or on vegetation in areas especially where there is an abundance of rodents. Children more often than adults acquire the infection because of geophagy and placing contaminated objects in the mouth. Human hepatic capillariasis has been reported in Europe (Germany, Switzerland, Italy, England, Greece, former Czechoslovakia, former Yugoslavia, Turkey), North and South America (USA, Canada, Mexico, Brazil), Asia (India, Korea, Japan, Thailand), Africa (South Africa, Ivory Coast, Nigeria), and Australasia (New Zealand). Enlargement of the liver is first noticed by the patient. There may also be abdominal pain, fatigue, anorexia, high morning fever, nausea, and vomiting (Table 9.3). Enlargement of the spleen, diarrhea or constipation, abdominal distension, edema of the extremities, and sometimes pneumonia are reported. Laboratory findings show marked leukocytosis with eosinophilia and moderate hyperchromic anemia, and bone marrow examination may reveal a cellular marrow with normoblastic erythropoiesis and a marked proliferation of the eosinophilic series of leucocytes (Table 9.4). Liver function tests and serum proteins may be abnormal. The prognosis is not good for hepatic capillariasis; most infections are fatal. The prognosis can be favorable if the diagnosis is made early and treatment initiated (Nithikathkul et al. 2011). Rodent control and sanitary disposal of dead animals, education, and improvement of hygiene are measures for the prevention and control of this zoonotic parasite (Table 9.5) (Fuehrer et al. 2011).

## 9.8 *Gnathostoma* spp.

### *9.8.1 Introduction*

Gnathostomosis is a disease caused by infection with the larval stage of nematodes of the genus *Gnathostoma*, order Spirurida. The final hosts are carnivore mammals (e.g., felids and canids), whereas, freshwater fish, amphibians, reptiles, rodents, and swine act as intermediate or paratenic hosts (Ando et al. 1992). Humans acquire the infection primarily from consuming raw fish, but infections can also arise from eating raw frogs, snakes, and wild boar and from drinking infected copepods in water. Skin penetration by L3 in food handlers and prenatal infections have been

documented (Rusnak and Lucey 1993). Human gnathostomosis has been documented in Africa, Asia, and Central America. Larval and immature *Gnathostoma* sp. adults cause migratory, often transitory, subcutaneous swellings in humans. The worms occasionally enter the internal organs and central nervous system, but they rarely reach sexual maturity in humans.

### *9.8.2 The Life Cycle*

Adult worms of *Gnathostoma* spp. live in tumors in the stomach wall of fish-eating mammals (Fig. 9.5). Females produce brownish ovoid eggs (56–79 μm by 35–43 μm) with a mucoid plug at one end. The eggs pass in the animal feces, reach water, and embryonate in 7–10 days. L1 hatches from the egg and is eaten by a freshwater copepod, in which it develops into a L2. When the infected copepod is eaten by a second intermediate host, including fish, birds, amphibians, reptiles, and mammals, the parasite enters the tissue and grows to a L3. Paratenic hosts may eat a second intermediate host, and the infective larva becomes encapsulated in the tissue and does not develop further. When a second intermediate host, or paratenic host, is eaten by a definitive host, the parasite is digested from the tissue, penetrates the stomach wall, and migrates to the liver and then to other organs, eventually returning to the peritoneal cavity and penetrating the stomach to form a tumor-like mass. The worms reach maturity and produce eggs in approximately 6 months. Felines and canines are definitive hosts for *G. spinigerum,* and domestic and wild pigs are natural hosts for G. *hispidum.* Infections are acquired by eating any of the many intermediate hosts. The most common species detected in humans is *G. spinigerum*, a short, stout nematode with a subglobose head armed with 7–9 transverse rows of hooklets. Spines also extend halfway down the body. Males measure 11–25 mm and females 25–54 mm in length. In addition to the morphological identification, the five zoonotic species of *Gnathostoma* can be identified among them by the molecular analysis of the cytochrome C oxidase subunit 1 of mitochondrial DNA and the internal transcriber spacer region 2 (Ando et al. 2006).

### *9.8.3 Epidemiology*

Gnathostomosis is acquired by eating one of the many intermediate hosts. The main source of human infection is raw or poorly cooked meat of catfish, eels, frogs, chickens, ducks, and snakes (Table 9.2). Infections occur in all age groups and both sexes. Gnathostomosis has been reported prevalently in Japan and Southeast Asia (Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Thailand, and Vietnam) (Herman and Chiodini 2009; Sieu et al. 2009). Cases have also been documented in China, Korea, India, Sri Lanka, and Australia (Rusnak and Lucey 1993; Jeremiah et al. 2011; Kim et al. 2013). In the last several years, it has become an

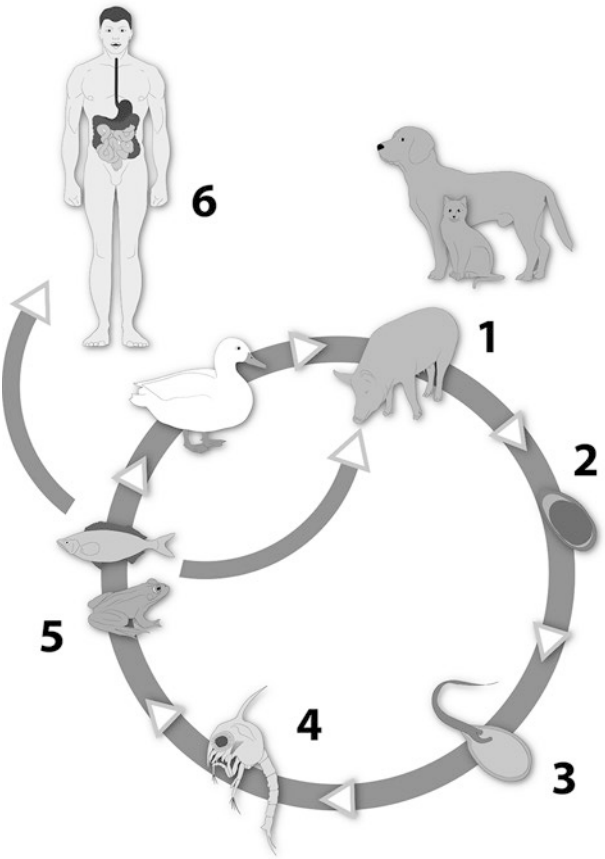


**Fig. 9.5** Life cycle of *Gnathostoma* spp. *1* Adult worms of *Gnathostoma* spp. live in stomach tumors of fish-eating mammals; *2* female worms produce eggs which are excreted by feces; *3* eggs embryonate and hatch releasing first-stage larvae (L1); *4* L1 are ingested by freshwater copepods and develop to L2; *5* infected copepods are ingested by a second intermediate host (e.g., fish, amphibians), and the parasites enter the tissue where they become L3. Paratenic or transport hosts (e.g., birds) acquire the parasite by feeding on second intermediate hosts. The infective-stage larva becomes encapsulated in the host tissue and does not develop further; and *6* Humans can acquire the infection by consuming one of the many intermediate hosts

increasing problem due to the consumption of raw fish marinated in lime (ceviche) in Brazil, Ecuador, Guatemala, Mexico, and Peru (Vargas et al. 2012). In Africa, the infection has been reported in Botswana, Namibia, Zambia, and Zimbabwe (Smith and Kok 2006; Herman and Chiodini 2009; Mukarati et al. 2013). In Europe, gnathostomosis has been diagnosed in two persons of Spain (Table 9.2). In addition, gnathostomosis has been reported in travelers who acquired the infection in one of these endemic countries and developed the disease when they returned to the home country or in persons who acquired the infection consuming raw fish imported from endemic countries. Changes in dietary habits are the main cause of expansion of the

geographical range of the disease. However, as observed for more of foodborne helminthic infections, it seems that the important factor is where the sushi is eaten rather than simply the consumption itself. Cases tend to occur as a result of consumption of food from local restaurants in countries where the disease is endemic and where few regulations if any govern the sourcing or storage of fish for consumption (Nawa et al. 2005). Indeed, these restaurants tend to use cheaper local freshwater or brackish-water fish, in contrast to sushi bars and restaurants in the industrialized countries, which primarily use more expensive saltwater fish which are free of *Gnathostoma* spp. and harbor relatively few potentially transmissible parasites. Of the more than 12 species recognized by morphology in the genus *Gnathostoma*, six species have been detected in humans: *G. spinigerum* (in Asia and Africa), *G. hispidum* (in Asia, Australia, Europe), *G. doloresi* (in Asia), *G. nipponicum* (in Asia), and *G. binucleatum* (in America).

### *9.8.4 The Disease in Humans*

Usually, only a single larva is involved in human infections. Transient gastrointestinal symptoms (low-grade fever, anorexia, vomiting, nausea, pruritus, and urticaria) may occur within 24–48 h for 2–3 weeks from the larva in the intestinal wall or liver. Cutaneous gnathostomosis develops from a week to 5–12 months later and manifests primarily as episodes of migrating swelling, possibly with subcutaneous hemorrhages, cutaneous eruption, or nodules mainly on the trunk but also involving the upper limbs, head, and throat (Table 9.3). Although intermittent, these signs can persist for years. There is a leukocytosis with a marked eosinophilia. Occasionally an abscess develops. Systemic infection varies with organ, i.e., liver, lung, gut, etc. Neurological migration produces intracranial necrotic tracks and subarachnoid hemorrhage, severe radicular pain and/or headache, and paralysis and has long-term side effects and 8–25% mortality.

### *9.8.5 Diagnosis and Treatment*

Diagnosis of *Gnathostoma* infection in humans is made by the recovery and identification of larvae from surgical specimens, urine, sputum, or vaginal discharge (Table 9.4). An ocular larva is visible. Larvae are 2–15 mm long, reddish white, with a characteristic head bulb bearing usually 3–4 rows of hooklets and rows of small cuticular spines. Larger parasites and their damage and migrating lesions might be visible on imaging. A presumptive diagnosis in endemic areas is based on history, symptoms, or serology. Specific antigens are available for immunodiagnosis. On Western blot, 24 and 21 kDa antigens of crude extract have been considered specific (Laummaunwai et al. 2007). More recently, a recombinant antigen has been successfully used for the serodiagnosis (Janwan et al. 2013). In differential diagnosis,

other parasitic infections such as hookworms, cutaneous and visceral larval migrans, myosis, sparganosis, cutaneous paragonosis, loaosis, and meningitis caused by *Angiostrongylus cantonensis* should be considered. Spontaneous recovery from cutaneous gnathostomosis is possible, but cutaneous migration could lead to complications of the CNS or ocular. The larva can be removed surgically from the eye. In cerebral gnathostomosis, albendazole is effective at the dose of 400 mg twice daily for 3 weeks and ivermectin at the dose of 200 μg/kg for 2 days (Nontasut et al. 2005). Repeat treatment is advised as relapses occur in 20–50% of patients.

### *9.8.6 Prevention and Control*

Health education programs would be beneficial for control in endemic areas. Infections could be prevented by eating only well-cooked intermediate or paratenic hosts (e.g., fish, eels, snakes, frogs, and poultry) and drinking only boiled or treated waters that are potentially copepod infested (Table 9.5). *Gnathostoma* spp. larvae are killed by freezing infected meat to −20 ° C for 3–5 days. Marinating infected meat in various substances generally is not effective. Vinegar appears to kill the organism in approximately 6 h and soy sauce in 12 h; lime juice is not effective after 5 days at room temperature or after 30 days at +4 °C.

## 9.9 *Trichostrongylus* spp.

Members of the genus *Trichostrongylus* are common host-specific nematodes of the stomach or gut of ruminants worldwide, and human infections have been widely documented (Table 9.2). The infection is acquired while eating uncooked plants contaminated by infective L3. The most common source of infection is domestic animals which share living areas with humans. Furthermore, the use of animal feces as fuel has been implicated in the infection of people who gather and prepare the feces for this purpose (Ghadirian and Arfaa 1975). The common source of infection in non-endemic areas is most likely the accidental ingestion of larvae acquired from livestock pasture or the ingestion of unwashed fruits and vegetables grown using animal manure fertilizer. A manure spreader has been implicated in the infection of people in Italy (Cancrini et al. 1982). The most common species detected in humans is *Trichostrongylus orientalis*, but other species such as *T. axei*, *T. capricola*, *T. colubriformis*, and *T. vitrinus* have been also identified (Nolan 2011). In areas where *T. orientalis* is prevalent, uncooked vegetables are contaminated by human feces. In humans, the symptoms and their severity depend on the number of worms present. Most infections are asymptomatic, but in heavy infections (over 100 adult worms), diarrhea (sometimes tinged with blood), abdominal pain, nausea, mild anemia, weight loss, and a transient or persistent eosinophilia have been reported (Table 9.3) (Ralph et al. 2006). In endemic areas where species of *Trichostrongylus*

are found, hands should be washed before preparing or eating meals, and vegetables should be thoroughly washed or cooked before eating. Animal manure can be composted to a high-enough temperature to kill *Trichostrongylus* eggs and larvae before being used as a fertilizer (Table 9.5). *T. orientalis* can be controlled by proper sanitation, removal of animal feces from areas occupied by humans, and the sterilization of feces before its use as a fertilizer. Infections with *Trichostrongylus* spp. of animal origin can be controlled by periodic treatment of domestic livestock with anthelmintics (Table 9.5). The time between treatments should depend on the local conditions. Since well-nourished livestock are better able to reduce their parasite burdens, animals should be kept well fed and their diet supplemented with minerals. Particular care should be taken with animals under a year of age as they are still developing an immune response to the worms and therefore may have higher worm burdens than older animals. Since in warm weather larvae can survive on pasture for about 1 month, pasture rotation, with a period of greater than 1 month, will help to reduce worm burdens in livestock.

## 9.10 Conclusions

The epidemiology of these parasites is affected by the human behavior. The social and cultural aspects of human behavior determine the types and preparation of food, the species of animals with which we interact, and which foods are consumed. The exposures from environmental conditions, food processing, and infrastructure are also a reflection of the socioeconomic status of a region. Cultural practices of consumption of undercooked meat and fish and the changing dietary patterns of ready-to-eat food products, together with the rapid expansion of the human population and migration, promote improper sanitation and hygiene standards especially in developing countries. The spillover of parasitic infections from companion animals, livestock, and wildlife species has emerged as the dynamics of society and culture, and the epidemiology and control of foodborne parasites have changed. Educating how to properly cook foods, avoiding risky sources of food, and applying hygiene practices and sanitation standards are required if reductions in many parasitic species are to be achieved.

*Trichinella* and the other foodborne nematodes covered in this chapter occur prevalently in underdeveloped areas and/or among wildlife. Only *T. spiralis*, *Ascaris* spp., *Toxocara* spp., and *Trichostrongylus* spp. are circulating also among domestic animals. The improvement of the animal breeding conditions, the development of controlled farming conditions and veterinary services, and the education of consumers, farmers, fisherman, and hunters represent the main efforts to prevent these infections reducing the transmission event to humans.

There are emerging patterns of movements of migrants from less-developed parts of the world to developed countries in search of better opportunities. This

mobility implies also importing different cultures, health beliefs, food preferences, and hence risk factors for foodborne transmission of parasites. For this reason, natural barriers for human infection with parasites, which was considered to be geographically limited because of parasites' adaptations to specific definitive and intermediate hosts and particular environmental conditions, are slowly being breached (Orlandi et al. 2002).

Increasing number of tourists traveling from developed to endemic areas and the testing of locally produced and/or imported food are likely to occur over the next several years. There is also an increasing amount of meat and fish products illegally translocated around the world by travelers, hunters, immigrants, and entrepreneurs (Pozio 2015). At the same time, developed countries are experiencing a reduction of physicians with knowledge of the parasitic diseases. There is low interest in industry to invest resources for the development of effective diagnostic tests, because of the lack of large and rich markets.

Because *Trichinella* spp., *C. philippinensis*, *C. hepatica*, *Gnathostoma* spp., and *B. procyonis* mainly infect wildlife, the eradication of these zoonotic agents cannot be achieved, but circulation among domestic animals can be controlled by appropriate and continuous veterinary controls (Gamble et al. 2007). However, factors such as armed conflicts or a change in a social–political system can result in severe economic and demographic changes, including high inflation and external economic sanctions. These circumstances can have wide-ranging effects on management of food production: (i) replacement of experienced veterinary control officers with fewer and less experienced personnel, (ii) reduction in the number of large slaughterhouses and an increase in small abattoirs that are unable to afford full-time in-house inspection, and (iii) an increase in small-holder farms with reduced government oversight to ensure high standards in rearing practices (infection risk management). In Eastern Europe, the breakdown of the Soviet Union and its satellite countries and the resultant socioeconomic factors gave rise to a large increase of *Trichinella* infections in the pigs of Croatia, Bulgaria, Romania, and Serbia (Marinculic et al. 2001; Cuperlovic et al. 2005; Blaga et al. 2007; Sofronic-Milosavljevic et al. 2013). The 1998–2002 economic crisis in Argentina also led to an increase of *Trichinella* spp. infections in pigs (Ribicich et al. 2005). These examples underline the importance of maintaining veterinary services with specific training in dealing with foodborne parasites.

In comparison to other classes of foodborne pathogens, particularly bacteria, the health impact of parasites is difficult to assess primarily due to the lack of uniform standards for monitoring the incidence of foodborne illness directly attributed to parasitic infections. Therefore, important efforts should be addressed for the standardization, implementation, and documentation of control measures. Integration of veterinary and public health efforts in "One Health" is needed to monitor these foodborne parasites and to develop a comprehensive food safety program for these diseases, most of which are zoonotic.

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# Chapter 10
# Taeniasis and Cysticercosis

**Jo Henderson-Frost and Robert H. Gilman**

## 10.1 Overview

Cestodes, or tapeworms, belong to the class Cestoidea of the phylum Platyhelminthes. Members of this family vary greatly in size and behavior but have similar morphology consisting of a head, called a scolex, a neck, and a worm body, called a strobila, which is formed by body segments called proglottids. Each proglottid contains both male and female reproductive organs; self-fertilization is possible but intersegmental mating more common. The oldest proglottids are farthest from the scolex. When gravid, each proglottid contains thousands of eggs and is termed mature. Cestodes do not have a digestive or circulatory system. They absorb nutrients from the lumen of the definitive host's small intestine through microvilli, which cover the surface of each proglottid and excrete waste through a pair of excretory tubules. Cestodes have a rudimentary nervous system consisting of ganglia in the scolex and nerves in the proglottids.

Within the Cyclophyllidea order, three of 45 *Taenia* species are capable of infecting humans: *T. saginata*, *T. solium*, and *T. asiatica*. The life cycles of these *Taenia* species are similar (Fig. 10.1). As definitive host, humans acquire intestinal infection with these cestodes by ingestion of undercooked meat—pork in the case of *T. solium* and *T. asiatica* and beef in the case of *T. saginata*—containing encysted larvae termed cysticercus. This infection is called taeniasis. Maturation to the adult tapeworm occurs in the small intestine after ingestion of cysticerci by a definitive host, at which point egg production and release into the environment permit the life

J. Henderson-Frost
Albert Einstein College of Medicine, Bronx, NY 10461, USA

R.H. Gilman (✉)
Bloomberg School of Public Health, Department of International Health, Johns Hopkins University, Johns Hopkins School of Public Health,
615 N. Wolfe St. Room 5515, Baltimore, MD 21205, USA
e-mail: gilmanbob@gmail.com



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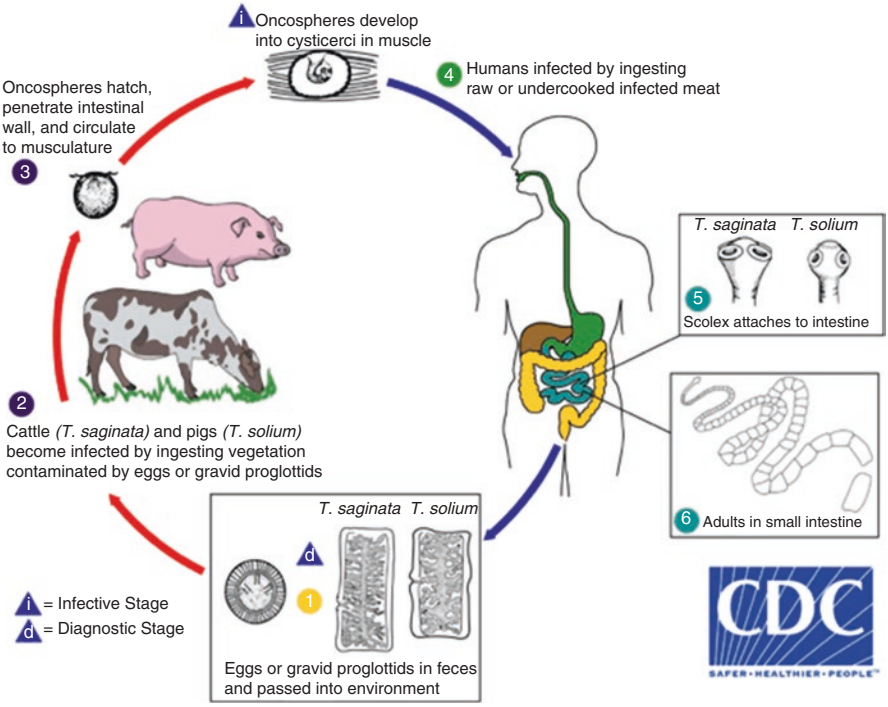


**Fig. 10.1** Life cycle of *T. saginata* and *T. solium* (Reprinted from the Centers for Disease Control and Prevention (CDC) https://www.cdc.gov/dpdx/taeniasis/index.html)

cycle to continue. As intermediate host, humans acquire tissue infection of cysticerci by ingestion of *T. solium* eggs. After ingestion, the eggs mature into metacestodes, which are infective larvae capable of encysting in tissues. This infection is called cysticercosis. *T. solium* is the only species known to cause significant human pathology; most commonly larvae encyst in the central nervous system (CNS), which is called neurocysticercosis. *T. saginata* and *T. asiatica* are similar morphologically and closely related genetically. They cause limited pathology in humans but significant economic losses in the livestock industry.

Species identification may rely on examining the scolex, if obtainable. The scolex of *T. solium* has a rostellum with a double row of hooklets in addition to the suckers, which the three species share. Eggs of all three species are identical and therefore do not permit species identification by confocal or electron microscopy or Ziehl-Neelsen stain. The eggs are spherical, typically 31–43 μm in diameter, covered by a durable embryophore composed of keratin-like blocks (Fig. 10.2a) (Jimenez et al. 2010). When an egg is ingested by an intermediate host the intestinal enzymes allow the embryonated larva, or oncosphere, to be released (Fig. 10.2b).

The adult tapeworm of *T. solium*, *T. saginata*, and *T. asiatica* consists of a scolex characterized by four suckers, followed by a short neck and a strobila of varying

length. Detection of morphologic characteristics by microscopy is the most reliable method for species identification of adult tapeworms. Injection of India ink into the uterus of the gravid proglottid allows for visualization of uterine branches: *T. saginata* has 15–20 uterine branches per side, *T. asiatica* has 12–30, and *T. solium* has 7–13. Other notable morphologic species differences detectable by microscopy include the presence (*T. saginata*, *T. asiatica*) or absence (*T. solium*) of the vaginal sphincter muscle and the trilobed (*T. solium*) or bilobed (*T. saginata*, *T. asiatica*) ovary (Table 10.1).

Adult tapeworms may not be obtainable to perform species identification, and thus DNA-based methods are necessary for reliably differentiating *Taenia* infections. Early techniques included polymerase chain reaction (PCR) with restriction fragment length polymorphism (RFLP) (Gasser and Chilton 1995) or with restriction enzyme analysis (PCR-REA) (Mayta et al. 2000). More recently, several multiplex PCR techniques have been developed to increase throughput (Yamasaki et al. 2004; Jeon et al. 2009). Multiplex PCR is able to differentiate between species and

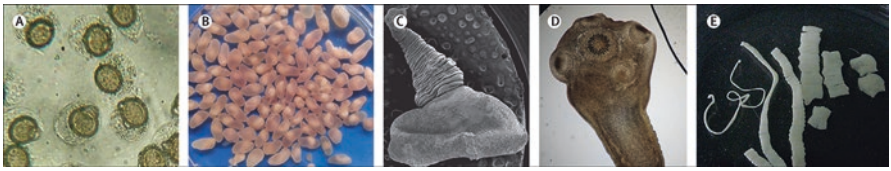


**Fig. 10.2** Growth stages of *Taenia solium*: infective *T solium* egg (**a**), larva or cysticercus (**b**), evaginating cysticercus (**c**), tapeworm scolex (**d**), and tapeworm strobila (**e**) (Reprinted from Garcia et al. (2014b), with permission from Elsevier)

**Table 10.1** Morphological differences between *Taenia solium*, *Taenia saginata*, and *Taenia asiatica*

| | *T. solium* | *T. saginata* | *T. asiatica* |
|---|---|---|---|
| Scolex | | | |
| Size | 0.6–1.0 cm | 1.5–2.0 cm | 0.2–2.0 cm |
| Rostrellum | Present | Absent | Absent or sunken |
| Hooks | 22–32 in double row | Absent | Rudimentary |
| Suckers | 4 | 4 | 4 |
| Strobila | ≤1000 proglottids | >1000 proglottids | <1000 proglottids |
| Mature proglottid | | | |
| Uterine branches | 7–13 per side | 15–20 per side | 12–30 per side |
| Vaginal sphincter | Absent | Present | Present |
| Ovary | 3 lobes | 2 lobes | 2 lobes |
| Testes (number) | 350–600 | 800–1200 | 300–1200 |
| Metacestode cysts | 5–15 mm | 7–10 mm | 2 mm |

Adapted from WHO/FAO/OIE Guidelines for the surveillance, prevention and control of taeniasis/cysticercosis

also identify asymptomatic carriers (Yamasaki et al. 2004), which is likely necessary to break the transmission cycle. Most recently, loop-mediated isothermal amplification (LAMP) (Nkouawa et al. 2010) and pyrosequencing (Thanchomnang et al. 2014) approaches have been described.

Overlapping geographic distributions and morphologic similarities make *Taenia* speciation a challenging endeavor. Nonetheless, species identification is important for clinical and investigational purposes. *T. solium* may cause cysticercosis and neurocysticercosis in humans, unlike *T. saginata* and *T. asiatica*. *T. solium* taeniasis is associated with an increased risk of neurocysticercosis, and therefore speciation may be useful clinically. Concurrent CNS involvement should be considered in cases of taeniasis, especially if antiparasitic therapy is to be administered.

## 10.2 *Taenia solium*

### *10.2.1 Biology*

The larvae of *T. solium* are cysts called cysticerci; each cysticercus contains one scolex. When the definitive host (human) ingests meat infected with cysticerci, the scolex evaginates and attaches to the wall of the host's small intestine, causing mild inflammation at the attachment site (Fig. 10.2c). The *T. solium* adult scolex has four suckers and a rostellum armed with a double row of hooklets (Fig. 10.2d). It lives for several years in the upper jejunum, absorbing food through its tegument. Maturation there to the reproductive adult stage tapeworm requires 10–12 weeks. A mature tapeworm can reach 2–4 m in length and contain up to 1000 hermaphroditic proglottids (Fig. 10.2e). Approximately 2 months after infection, proglottids are shed during defecation, usually 3–5 at a time. Each proglottid can contain 50,000 eggs, which are released with passage of the proglottid and can survive for several months in the external environment. After ingestion by an intermediate host (pig or human), eggs hatch in the host's gastrointestinal tract, thereby releasing larval oncospheres. The larvae penetrate the intestinal walls and migrate through the bloodstream to encyst in various tissues, particularly striated muscle in the neck, tongue, and trunk of pigs, and the central nervous system (CNS) and skeletal muscle of humans. The encysted larva, also called cysticercus, is now in the metacestode stage; over 60–90 days, it matures to reach a size of approximately 1 cm. It consists of a scolex and larval body. The cysticercus can exist for many years in the host tissue. It suppresses the host's immune response, and thus there may be little or no inflammation, while the cyst remains alive. Once the cyst begins to degenerate, however, there is typically a localized cellular immune response that may result in edema, scarring, and increased symptomatology for the host.

Cysts progress from viable to mineralized through a series of well-characterized involution stages (Del Brutto 2014b). Upon entry to the CNS, the cyst is in the viable or vesicular stage. A scolex may be appreciated on imaging. If the cyst lodges in parenchymal tissue, typically it induces minimal inflammation in the viable stage. As the cyst starts to die, it involutes, and the cystic fluid becomes turbid. The scolex begins to undergo hyaline degeneration. This stage is referred to as colloidal or colloidal vesicular. The host immune response increases, marked by astrocytic gliosis, collagen deposition, and edema. The cystic wall continues to thicken, and the scolex granularizes in the following stage, referred to as granular or granular nodular. Typically there is less frank edema during the granular stage but rather an increase in astrocytic changes and the formation of multinucleated giant cells (Del Brutto 2014b). The final stage is the calcified or calcified nodular in which the cyst fully mineralized to a calcification. The time course of progression is poorly characterized and likely depends on host immune factors. Cysts may persist in the viable stage for months to years (Del Brutto 2014b). It is also unknown why some cysts calcify and persist permanently, while others are resorbed entirely, bypassing the calcified stage.

### *10.2.2 Transmission*

The human is the only definitive host for *T. solium.* Taeniasis, human gastrointestinal infection by the adult worm, occurs when people eat undercooked pork that contains live cysticerci (Fig. 10.3). The intermediate host is usually the pig, but humans can also act as intermediate hosts. Other species such as dogs, cats, and sheep may occasionally be infected, but they rarely develop significant disease. Infection in the intermediate host, known as cysticercosis, manifests with encysted metacestode larvae in skeletal muscle or other tissue. Humans may develop single or numerous cysts in muscle or in the central nervous system (CNS); in the CNS, the disease is known as neurocysticercosis.

The cestode life cycle is propagated via foodborne and fecal-oral transmission. Pigs can acquire cysticercosis through consumption of sewage containing human waste and cestode eggs. *T. solium* infection in pigs almost never occurs in industrialized countries, where livestock are raised commercially with regulation to ensure minimal contact with human feces. Human-to-human spread can occur by fecal-oral transmission if poor hygiene practices occur during food preparation. Infections are often clustered in households, a phenomenon likely related to food preparation practices. Approximately 15% of patients with neurocysticercosis harbor a tapeworm at the time of diagnosis; these patients tend to have high cyst burden (Gilman et al. 2000; Garcia and Del Brutto 1999). Autoinfection can occur by personal fecal-oral contamination or when, in humans, gravid proglottids of an intestinal worm rupture. Hatching oncospheres invade the intestinal wall and spread hematogenously or lymphatically to muscle and nervous tissues. The

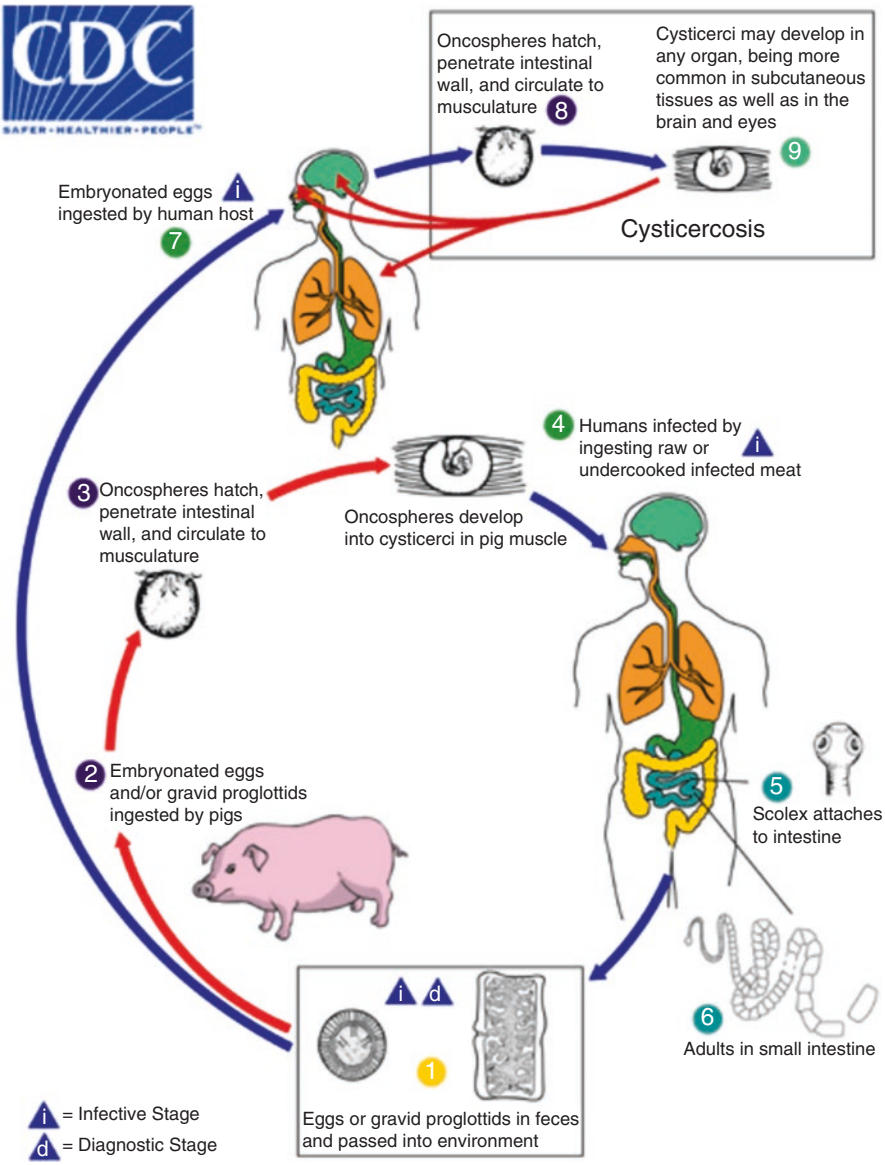


**Fig. 10.3** Stages of infection in cysticercosis (Reprinted from the Centers for Disease Control and Prevention (CDC) https://www.cdc.gov/dpdx/cysticercosis/index.html)

incidence of autoinfection is unknown (Schantz 1996). The prevalence of antibodies to oncospheres suggests that this may occur more frequently than previously thought. Infection by the oncosphere does not always result in cysticercosis because the host immune system is often capable of resolving light infections in the early stages.

### *10.2.3 Epidemiology*

*T. solium* has a worldwide distribution but is most common in South America, Africa, India, and Southeast Asia. Prevalence is highest in rural areas where humans and pigs live in close proximity and where good hygiene and sanitation practices are lacking. In many non-Muslim, low-income countries, where pork is a common food source, *T. solium* is the most common helminthic infection (Carangelo et al. 2001; Garcia-Noval et al. 2001). *T. solium* is no longer endemic in high-income countries, but the prevalence has been increasing due to immigration. Molecular diagnostic techniques have improved in recent decades, and in endemic regions of the world, access to imaging modalities for clinical diagnosis has also improved. Although there has been a recent increase in prevalence studies, it is difficult to estimate the global burden of disease. These studies use a range of diagnostic techniques and examine a combination of porcine cysticercosis, human cysticercosis, and human taeniasis. The World Health Organization has classified cysticercosis as a "major neglected disease" (Savioli and Daumerie 2010; Coyle et al. 2012), and The Lancet has estimated 50 million are infected worldwide (The Lancet Infectious 2014).

Whereas *Taenia solium* transmission is uncommon in the United States, the prevalence has been increasing in recent years most likely due to emigration from endemic areas (Coyle et al. 2012). The mean annual incidence of *T. solium* infection is estimated at 5.8 cases per 100,000 in the Hispanic population and 0.5 cases per 100,000 in the total population based on population-based active surveillance (O'Neal et al. 2011). Using hospitalization data from the 1990s, it was estimated that 16% of Latinos diagnosed with seizures in Houston have neurocysticercosis (del la Garza et al. 2005). Ten percent of the LA cases are estimated to have been locally acquired (Sorvillo et al. 1992). The rate of US hospitalizations due to a primary diagnosis of neurocysticercosis is estimated to be 8.03 per one million (O'Keefe et al. 2015). Testing sera from refugees in the United States from Burma, Lao People's Democratic Republic, Bhutan, and Burundi yielded seropositivity by Western blot of 18.3–25.8% (O'Neal et al. 2012). Outbreaks have even occurred in populations with no exposure to endemic regions by travel or immigration. One such example is a series of cases from a community of orthodox Jews in New York City who contracted *T. solium* cysticercosis from hired cooks who emigrated from tapeworm-endemic parts of Latin America. Food prepared by infected employees was contaminated with eggs shed in employee's stool (Schantz et al. 1992).

Seroprevalence of *T. solium* taeniasis in endemic regions has historically been difficult to obtain: stool studies have diagnostic and logistical limitations, and

serology may be persistently positive after clearance of the infection. (For more on diagnostics, see Sect. 10.2.7.) It has been estimated that up to 6% of people in endemic regions may harbor an intestinal worms at a given time and serve as a source of infectious eggs that cause cysticercosis (Allan et al. 1996; Cruz et al. 1989). Between 10% and 25% of people may be seropositive, and of these, 10–18% have abnormal CT findings suggestive of cysticercosis (Schantz et al. 1994; Cruz et al. 1999; Garcia-Noval et al. 2001; Sanchez et al. 1999). However, these studies may be overestimating overall prevalence because they are typically conducted in the most endemic regions with increased risk factors for transmission.

In areas where *T. solium* infection is endemic, neurocysticercosis is a major cause of epilepsy; in many areas, it is the leading cause of epilepsy. The prevalence of epilepsy in these countries is three to six times higher than in industrialized nations (Nicoletti et al. 2002; de Bittencourt et al. 1996; Savioli and Daumerie 2010), and neurocysticercosis is estimated to cause epilepsy in greater than 1% of the world population from endemic regions (Coyle et al. 2012). A large proportion of the patients admitted to neurology wards in developing countries suffer from neurocysticercosis (Garcia et al. 2003). A study in 1999 estimated that 400,000 people in Latin America have symptomatic neurocysticercosis. One-quarter to one-half of adult seizure disorders in tapeworm-endemic areas can be attributed to *T. solium* neurocysticercosis (Bern et al. 1999). More recently Coyle et al. have estimated the prevalence of symptomatic and asymptomatic neurocysticercosis in Latin American countries to be between 15% and 38% (Coyle et al. 2012). In Peru, seroprevalence is as high as 24% with an additional 13% of the population calculated to have calcifications due to neurocysticercosis and negative serology (Montano et al. 2005). In one community study from India, *T. solium* infection was confirmed by serology in 15.9% of patients presenting with epilepsy, and greater than 28.4% had imaging suggestive of neurocysticercosis. These rates suggest that over 1 million people in India have epilepsy due to neurocysticercosis (Rajshekhar et al. 2006). Similarly, between 30% and 50% of people presenting with epilepsy in sub-Saharan Africa are estimated to have neurocysticercosis (Winkler et al. 2009). In China, 3–6 million people are estimated to be infected (Savioli and Daumerie 2010; Coyle et al. 2012).

Contamination of livestock feed with human feces due to open sewers and improper disposal is the major risk factor for transmission from humans to pigs. Other risk factors include poor education, unsanitary food preparation, unrecognized infected pork, insufficient inspection of pork, and poverty necessitating sale and/or consumption of contaminated meat. Since food contamination is a predominant feature of transmission dynamics, any socioeconomic class may be affected. However, an association between socioeconomic status and seroprevalence has been recognized. A general association with age and sex has also been noted. More specifically, Garcia et al. find single enhancing cysts to be more common in children and younger adults (<30 years of age) and inflammation to be a more prominent feature in women and children (Garcia et al. 2014b).

### *10.2.4 Cost*

Because neurocysticercosis tends to affect older children and young adults, it has a disproportionate economic effect due to lost wages. The DALYs lost from neurocysticercosis complicated by epilepsy and headache in Mexico are estimated to be 0.25 per 1000 persons per year (Bhattarai et al. 2012). In Cameroon, the losses have been estimated to be much greater: 9.0 per 1000 persons per year (Praet et al. 2009). Estimates of treatment costs range widely depending on the disease characteristics, treatment center, healthcare system, and wage economy: between $2000 and $3000 per patient in Mexico (Bhattarai et al. 2015) and around $1000 in Peru (Rajkotia et al. 2007). In South Africa, the monetary burden of neurocysticercosis was calculated to be between 18.6 and 34.2 million dollars per year (Carabin et al. 2006). Between 2003 and 2012, hospital charges for inpatient stays due to neurocysticercosis totaled more than $908 million USD (O'Neal and Flecker 2015).

### *10.2.5 Control*

As with other foodborne diseases, hygiene practices and sanitation measures are paramount to limit new *T. solium* infections. Neurocysticercosis prevention should target human taeniasis or porcine cysticercosis. Measures that decrease transmission from pigs to humans include regulation and effective screening in slaughterhouses, proper housing and care of pigs in peri-domestic areas, and preparation of pork by salt pickling, freezing at −20 °C for at least 10 days, or cooking above 80 °C (Murrell 2005). Implementation of these measures may be difficult if a country's pork industry is poorly regulated and/or pigs are raised and slaughtered in private rather than a slaughterhouse. In Peru, for example, some pigs enter the market illegally, and many may be infected with *T. solium.* Pork sellers may disguise infected pork or mix it with uninfected meat (1993). Proper management of human waste disposal decreases human-pig and human-human transmission. Coprophagy is common among free range pigs, and human feces are a cheap source of food. Restraining pigs has been shown to be effective in reducing transmission. Incidence increases when there is limited access to latrines and free ranging pigs (Murrell 2005).

Public education is a critical aspect of infection control. In poor rural populations, economic incentives like compensation increase the efficacy of educational interventions (Gonzalez et al. 2003). The impact of educational-based interventions have been studied: in Tanzania an education program led to decreased consumption of infected pork and decreased incidence of porcine cysticercosis (Ngowi et al. 2008), and in Mexico, an education program led to decreased prevalence of porcine cysticercosis at 1-year follow-up due to decreased access of pigs to roaming (Sarti et al. 1997). Mass treatment of pigs with oxfendazole (30 mg/kg, one dose) is also effective in reducing porcine cysticercosis (Gonzalez et al. 1997) and confers protection from new infection for at least 3 months (Gonzalez et al. 2001). Medical interventions in humans also play an important role in prevention. Treatment of intestinal infections

with niclosamide or praziquantel reduces cysticercosis, by alleviating the risk of autoinfection and reducing the contamination of livestock feed and human food with infected feces. Mass treatment of the population with praziquantel has been shown in one study to reduce the prevalence of intestinal infection by 53%, seizures by 70%, and the prevalence of antibodies in both pigs and humans (Sarti et al. 2000).

Vaccine development has been a prominent area of *Taenia* research in the last several decades. Researchers have shown that recombinant antigens, purified from oncospheres, confer immunogenicity in pigs. However, difficulty with large-scale propagation of cestodes in vivo or in vitro thwarted vaccine development efforts prior to the introduction of recombinant DNA technology. More recent advances have led to the successful development of recombinant vaccines for *Taenia* cestodes in their intermediate hosts. The most common vaccine is TSOL18 (recombinant protein produced by *Escherichia coli*), which has been developed for use in pigs and has been shown to eliminate transmission. Another control method that has garnered recent attention is pharmacologic treatment of pigs. After treatment with oxfendazole, pigs are protected from new cysticercosis infection for at least 3 months (Gonzalez et al. 2001), yet mass treatment campaigns of both humans and pigs have been shown to be likely insufficient for elimination of disease (Garcia et al. 2006). If combined with oxfendazole treatment, vaccination would cure previously infected pigs and immunize against reinfection (Assana et al. 2010). The most efficacious control strategy is likely to combine mass treatment and vaccination of pigs as well as mass treatment of humans. New infection in pigs is a model of environmental contamination. The results of a large scale phase III trial regarding elimination of cysticercosis demonstrated that porcine infection was nearly completely interrupted in endemic villages suggesting that elimination is possible in endemic areas (Garcia et al. 2016). The economic feasibility of widespread vaccine use or treatment campaigns is unknown.

### *10.2.6 Clinical Aspects*

Taeniasis, the infection of the human gut with the adult *T. solium*, is usually asymptomatic but may occasionally cause epigastric pain, nausea, diarrhea, increased hunger, or weight loss. In contrast to the mobile proglottids of *T. saginata*, immobile *T. solium* proglottids can be visible in the person's stool but usually pass unnoticed. Infection with larval stage of the pork tapeworm, *T. solium* cysticercosis, can occur in almost any part of the body. In humans, cysticerci are most commonly found in skeletal muscles and in the brain. They are also found in the skin, subcutaneous tissue, eye, and heart and may cause subcutaneous nodules or muscular pseudohypertrophy in cases of high cyst burden in skeletal muscles. Cysticerci in the tissues are rarely symptomatic unless they encyst in the eye or in neural tissue or meninges, a condition known as neurocysticercosis.

Neurocysticercosis is classified according to disease location in the central nervous system: disease is either parenchymal or extraparenchymal or both. Extraparenchymal disease is divided into intraventricular and subarachnoid

subtypes. Clinical manifestations depend on cysticerci location, inflammation intensity, and parasite load. Signs and symptoms are usually due to inflammation but may also be due to mass effect if a cyst in the cerebrospinal fluid system or parenchyma is exceptionally large. In the brain parenchyma, cysts are commonly located at the gray-white junction. When cysticerci are found in the ventricular systems, they most commonly reside in the fourth ventricle, followed by the third ventricle. In subarachnoid disease, also called racemose disease, the cysts are frequently lobulated, large, and translucent without a scolex, located at the base of the brain (basilar cisterns) and/or the Sylvian fissures (Fig. 10.4). The appearance may be described as a "grape-like cluster." Racemose disease carries a high mortality rate due to the risk of hydrocephalus.

Parenchymal cysts are rarely fatal, but they can cause significant morbidity. Seizures are more common in parenchymal disease than intraventricular or subarachnoid disease; they are estimated to be the predominant symptomatology in about 70% of cases (Del Brutto 2014b). As a result, neurocysticercosis is the most common cause of epilepsy in the developing world. New onset of seizures during young or middle adulthood in endemic areas is strongly suggestive of neurocysti-

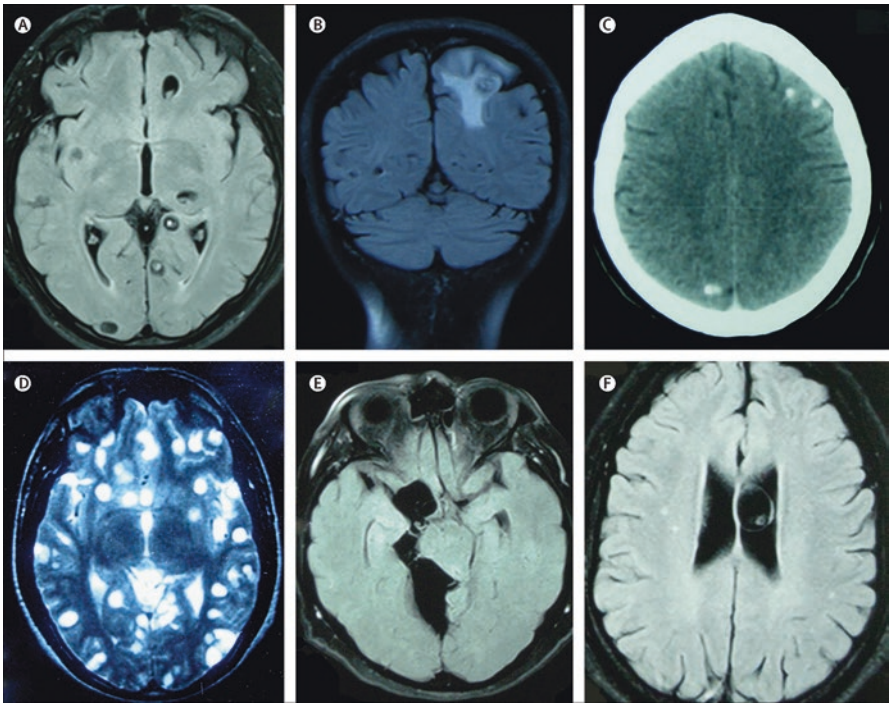


**Fig. 10.4** MRI imaging of human neurocysticercosis: contrast used was gadoterate meglumine. Viable cysts in structural MRI (**a**); and enhancing nodule (**b**); many brain calcifications visible (**c**); massive parenchymal neurocysticercosis (**d**); basal subarachnoid neurocysticercosis (**e**); and intraventricular cysticercosis (**f**) (Reprinted from Garcia et al. (2014b), with permission from Elsevier)

cercosis. The epidemiology of seizure type is poorly characterized in the literature. Seizures can be partial or general, and EEG will often reveal focal slowing in the region of the lesion. The most common type is likely partial seizure with secondary generalization (Del Brutto 2014b).

Classical seizures were thought to occur as a result of cyst degeneration and subsequent inflammation (White 2000). However, it has also been observed that many patients who present with seizures have viable parenchymal cysts (Del Brutto 2014b). Another etiology of seizure is related to calcification: perilesional edema around a calcified lesion is increasingly recognized as epileptogenic in an otherwise asymptomatic patient with calcified parenchymal disease. In a 5-year prospective cohort of 110 patients with calcified disease, 36% experienced seizure (Nash et al. 2008). The pathophysiology is not completely understood, but the predominant theory is that the transient inflammation is induced by parasite antigen leakage from the calcification (Nash 2012).

Cysticerci have been reported to cause psychiatric symptoms including psychosis (Verma and Kumar 2013; Singh et al. 2004), more commonly depression (Forlenza et al. 1997). There are also case reports of mutism (Agarwal et al. 2001) and binge-eating disorder (Fernandez-Aranda et al. 2001). Recently, calcified parenchymal disease has been found to be associated with hippocampal atrophy (Del Brutto et al. 2015), which may explain behavioral changes. Although poorly understood, it is recognized that neurocysticercosis may be associated with a range of cognitive impairments from subclinical decline to dementia. One study estimates cognitive impairment to be 12.5% among treatment-naive patients (Ciampi de Andrade et al. 2010). Viable parenchymal disease is more likely to be associated with cognitive decline than calcified disease (Rodrigues et al. 2012).

In contrast to parenchymal disease, intraventricular presentation can be fatal if the cysts or inflammation obstructs the ventricular system and causes hydrocephalus. Hydrocephalus is actually a common presentation of intraventricular neurocysticercosis. If the cyst attaches to the ventricular wall, ependymal cells react by inducing influx of inflammatory cells resulting in edema that may block CSF communication. Approximately 30% of patients develop increased intracranial pressure or hydrocephalus due to CSF outflow impedance (Sinha and Sharma 2012), causing headache, nausea or vomiting, dizziness, visual problems, ataxia, confusion, or papilledema. This likely represents a biased sample of those who seek medical attention and are found to have intraventricular disease. Bruns syndrome, a described phenomenon of acute and episodic hydrocephalus as a result of positional change, is also possible in fourth ventricular neurocysticercosis as loose cysts may roll to block CSF outflow tracks (Shahani et al. 2015).

Subarachnoid neurocysticercosis is the most severe form of the disease and results in significant morbidity. Meningeal involvement causes significant inflammation and abnormal exudative thickening of the meninges that may disseminate and can lead to obstructive hydrocephalus. Cysticerci in the subarachnoid space may cause stroke or transient ischemic attacks of a vasculitic etiology. Inflammation reaction of nearby small arteries can lead to occlusion and thereby precipitate cerebral infarction. Neurocysticercosis can also cause encephalitis (especially in

younger adults or children and in females), meningitis, or arachnoiditis, which would typically cause focal neurological deficits and intracranial hypertension. Severe cysticercal meningitis has been defined as subarachnoid disease with >1000 cells/mm$^3$ cellular inflammation of the CSF with negative fungal and bacterial cultures. This condition is seldom recognized and is thought to be underreported. Although less common, it is important to include cysticercal meningitis on the differential of bacterial meningitis in endemic countries (Cardenas et al. 2010).

The more rare forms of cysticercosis include spinal, muscular, and ocular disease. It is estimated that between 1% and 5% of patients with neurocysticercosis have spinal disease and is more commonly associated with subarachnoid disease (Callacondo et al. 2012): approximately 60% of patients with subarachnoid disease in the brain have concurrent spinal disease (Garcia et al. 2014b). Spinal disease is more commonly leptomeningeal (extramedullary or subarachnoid) rather than intramedullary (Abdel Razek et al. 2011). It may be clinically silent but may also cause motor (e.g., weakness) and sensory changes below the level of the cyst and/or radicular pain. Muscular disease is rare and usually found in cases of concurrent CNS involvement; if seen, the cyst burden is often high causing pseudohypertrophy. In the eye, the cysticercus may invade the anterior chamber, the vitreous humor, or the retina, obscuring vision. Subretinal cysticerci can cause retinal detachment and vision loss; typically the vision loss is uniocular (Wender et al. 2011). Fundoscopic examination often reveals the intraocular cyst. Rarely, cysts form in the optic nerve or extraocular muscles, but they may cause extraocular muscle paralysis (Del Brutto 2014b).

### *10.2.7 Diagnosis*

Diagnosis of taeniasis can be difficult because stool microscopy is not very sensitive and the eggs of *T. solium* are identical to those of other *Taenia* and *Echinococcus* species. However, eggs from different species can be distinguished with PCR, enzyme electrophoresis, or immunological tests. Diagnosis becomes easier if proglottids or the scolex is recovered. Use of a purgative before and after administration of treatment improves the chances of recovering the worm's scolex and strobila. The proglottids of *T. solium* and *T. saginata* can be differentiated by the anatomy of the female reproductive organs: *T. solium* has 5–10 uterine branches on each side, no vaginal sphincter muscle, and only one ovarian lobe while *T. saginata* has at least 15 uterine branches, a vaginal sphincter muscle, and two ovarian lobes. Sensitivity for the detection of eggs in stool may be improved by using concentration techniques. Sedimentation, as opposed to flotation, techniques for the isolation of eggs are required due to the density of *Taenia* eggs. Of note, it is important to fix eggs or proglottids before examination to prevent infection of laboratory personnel.

Diagnosis of *T. solium* cysticercosis is not straightforward and must take into account clinical history, physical examination, epidemiological context, radiological studies, and testing. Del Brutto et al. have designed diagnostic criteria using absolute, major, minor, and epidemiological diagnostic criteria (Table 10.2). A definitive

**Table 10.2** Criteria for diagnosis of neurocysticercosis (Del Brutto 2001)

| | |
|---|---|
| Absolute criteria | Histological demonstration of the parasite from biopsy of a brain or spinal cord lesion<br>Cystic lesions showing the scolex on CT or MRI<br>Direct visualization of subretinal parasites by fundoscopic examination |
| Major criteria | Lesions highly suggestive of neurocysticercosis on neuroimaging studies<br>Positive serum EITB for the detection of anticysticercal antibodies<br>Resolution of intracranial cystic lesions after therapy with albendazole or praziquantel<br>Spontaneous resolution of small single enhancing lesions |
| Minor criteria | Lesions compatible with neurocysticercosis on neuroimaging studies<br>Clinical manifestations suggestive of neurocysticercosis<br>Positive CSF ELISA for detection of anticysticercal antibodies of cysticercal antigens<br>Cysticercosis outside the CNS |
| Epidemiological criteria | Evidence of a household contact with *Taenia solium* infection<br>Individuals coming from or living in an area where cysticercosis is endemic<br>History of frequent travel to disease-endemic areas |

diagnosis of neurocysticercosis is established by the presence of one absolute criterion or the presence two major, one minor, and one epidemiologic criterion. A probable diagnosis is established by one of three combinations of criteria: (1) one major plus two minor criteria, (2) one major plus one minor and one epidemiologic criterion, or (3) three minor plus one epidemiologic criterion (Del Brutto et al. 2001).

In approximately one-half of cases, examination of cerebrospinal fluid (CSF) will be abnormal. Most commonly neurocysticercosis is associated with a moderate pleocytosis (~300 cell/mm$^3$) with a predominance of lymphocytes, neutrophils, or eosinophils; normal glucose moderately increased protein (50–300 mg/dL). In less than 20% of cases, low glucose is seen (Cardenas et al. 2010). Eosinophils in the CSF are suggestive of neurocysticercosis, but they are found in only about 40% of cases. Abnormal CSF findings are most likely to be found in patients with subarachnoid disease. CSF findings may normalize several months after treatment.

Neuroimaging studies are some of the most important elements of diagnosis to identify number, size, and location of cysts. Computerized tomography (CT) is sensitive and specific for neurocysticercosis. Cysticerci are hypodense lesions on CT with well-defined margins. The scolex of a viable cyst may be visible, which is pathognomonic for neurocysticercosis (Fig. 10.4a). As the cyst degenerates, local inflammation causes contrast leakage into the lesion, and it enhances. Once the cyst is fully degenerated, the calcification usually does not enhance and appears hyperdense on CT (Fig. 10.4c). However, in cases of perilesional edema, magnetic resonance imaging (Preechawai et al. 2008) at the time of the seizure presentation is likely to show T2-FLAIR enhancement surrounding one or more calcification. In the literature, this condition is termed "perilesional edema" and refers to calcified disease (Nash and Patronas 1999).

While CT is the only imaging modality that reliably identifies calcified lesions, MRI is preferred to stage cystic lesions and visualize intraventricular and subarachnoid cysts and meningeal inflammation. Contrast-enhanced MRI is the preferred imaging modality to stage lesions, e.g., colloidal cysts will demonstrate increased signal intensity of cystic fluid and enhancement of cystic wall on T1-weighted images (Chang and Han 1998) (Fig. 10.4b). MRI is also used to evaluate arachnoiditis and track disease progression in subarachnoid cases where this may have enhancement at the base of the brain (Garcia et al. 2014b) and/or significant distortion in the Sylvian fissures or basilar cisterns (Fig. 10.4e). It is often more difficult to visualize intraventricular cysts because cystic fluid is isodense with cerebrospinal fluid (Fig. 10.4f). There may be evidence of a cyst based on the morphology of the ventricle, e.g., a trapped fourth ventricle due to a mechanical obstruction (Venna et al. 2012). Periventricular enhancement on MRI can distinguish neurocysticercosis from lymphoma or other infections and assist in determining if the cyst is a candidate for removal. FLAIR sequences yield the most information for intraventricular and subarachnoid disease, as the extent of inflammation dictates medical management and in particular the steroid course. Diffusion-weighted MRI and B-scan ultrasonography are also used for the diagnosis of ocular cysticercosis.

Immunological testing is also useful for diagnosis of neurocysticercosis, though antibodies can disappear over time; this time course is not well elucidated. Antibodies may persist for months to years after the cyst degenerates even with treatment. Antibody detection by enzyme-linked immunoelectrotransfer blot assay (EITB) is the current standard, with 98–99.4% sensitivity and 100% specificity (Del Brutto 2014b; Gekeler et al. 2002). However, this test is less sensitive for cases of single parenchymal lesions as well as calcified disease (Del Brutto 2014b; Singh et al. 2010). Nonetheless, EITB is useful for screening and the current test of choice in clinical settings. The first EITBs for neurocysticercosis used semi-purified parasite membrane antigens, and now recombinant antigen has been shown to have comparable results (Noh et al. 2014). Enzyme-linked immunosorbent assay (ELISA) tests have also been developed; one promising ELISA uses a purified protein with cathepsin L-like protease activity (Zimic et al. 2009). These proteases are thought to be integral to oncospheral invasion of intestinal mucosa (Zimic et al. 2012). Yet, no commercial ELISA is present that can be used with sufficient sensitivity or specificity to be used for diagnosing cysticercosis in man.

Although serological tests may predict active infection, Garcia et al. argue that positive serology in the absence of imaging should not change treatment decisions because those must be tailored to the location of the cyst (Garcia et al. 2012). Antiparasitic treatment without knowledge of the location of the disease may precipitate acute inflammatory reaction, which can exacerbate hydrocephalus and risk herniation. Therefore, appropriate diagnosis and treatment in the absence of imaging is difficult. Ag-ELISA is useful for detecting viable cysts when MRI modality is not available and patient has calcifications on CT scan (Zea-Vera et al. 2013). This antigen test can also be useful in monitoring response to therapy, particularly in

cases of severe disease complicated by hydrocephalus (Fleury et al. 2007). The antigen used is called HP-10.

For diagnosis of porcine cysticercosis, many of the same techniques are used. However, positive serology is poorly correlated with active infection, and pigs may be seropositive yet cyst-free at necropsy. EITB is a test of exposure rather than cyst burden, but an increase in number of bands has been correlated with cyst burden (Gavidia et al. 2013). In one study, >=3 bands on EITB were significantly associated with >100 cysts on necropsy (Jayashi et al. 2014).

### *10.2.8 Treatment*

Treatment of taeniasis is straightforward: niclosamide and praziquantel are used to treat intestinal *T. solium* infection in a single dose—2 g of niclosamide or 5–10 mg/kg of praziquantel.

Niclosamide is a halogenated salicylanilide; it is safe and efficacious (85%) yet more expensive than praziquantel. It is not absorbed from the gastrointestinal tract and therefore does not induce a systemic inflammatory response to the cysticidal action (Murrell 2005). A saline purgative may be used following niclosamide treatment for taeniasis; test of cure is confirmed by examination of the scolex. This method also allows for differentiation between *T. solium* and *T. saginata* taeniasis as the scoleces are unique (Rajshekhar 2004). Preniclosamid and postniclosamide electrolyte-polyethyleneglycol salt compare favorably to castor oil in terms of scolex recovery for identification (Jeri et al. 2004). Praziquantel, an acylated isoquinoline-pyrazine, is also considered safe and efficacious (95%). In contrast to niclosamide, the cysticidal nature of praziquantel may induce an inflammatory response if there is concomitant neurocysticercosis or ocular cysticercosis (Murrell 2005). Concern has been raised that deworming campaigns, although thought to be effective, may need to exercise caution when administering albendazole to children for the same reason. Those with undiagnosed neurocysticercosis may experience seizures or other symptomatology as a result of the treatment (Garcia et al. 2007; Gilman et al. 2012).

The therapeutic approach to neurocysticercosis is more complicated than the approach to taeniasis and may be dictated by cyst location and stage and host symptomatology. Treatment may be difficult and must be individualized. There is a paucity of randomized controlled trials assessing the efficacy of treatment and long-term outcomes. A plan to updated international treatment guidelines was announced in 2015; the current guidelines are from 2002 (Garcia et al. 2002).

Symptomatic treatment of seizures and increased intracranial pressure in neurocysticercosis are crucial. All patients with seizures should receive antiepileptic drugs. Anticonvulsants usually control seizures and can be discontinued after 2 years if the patient remains seizure-free. The decision to discontinue may be individualized and based on the absence of intracranial parenchymal calcifications,

which may serve as epileptic foci. However, there is insufficient evidence to suggest that AEDs should be used prophylactically in calcified disease (Garcia et al. 2014b). Current guidelines recommend seizures in neurocysticercosis to be managed in the same manner as other secondary seizure conditions (Garcia et al. 2002).

Surgical intervention, e.g., neuroendoscopic excision, is recommended for intraventricular cases where possible; alternatives include possible open surgery for fourth ventricular disease and ventriculoperitoneal shunt. Hydrocephalus due to neurocysticercosis should be treated using standard surgical techniques such as ventriculoperitoneal shunt or removal of the cysticercus by craniotomy or ventriculoscopy if necessary. In these patients, antiparasitic treatment should be avoided until intracranial pressure has decreased to avoid clinical worsening due to drug-induced inflammation. Some argue that surgical management is also warranted in cases of calcified parenchymal disease in the event of recurrent perilesional edema and seizures (Carpio and Romo 2014). Surgery may be indicated in subarachnoid disease, yet control of intracranial hypertension and inflammation is the priority. Ocular cysticerci and spinal medullary lesions usually require surgical management as rupture and local inflammation may cause irreversible damage (Sabrosa and Zajdenweber 2002). All patients should undergo eye exam prior to beginning antiparasitic therapy.

Although it is now generally accepted that antiparasitic therapy warranted in most cases except those with calcified lesions, the role of this therapy has been controversial. It has been posited that treatment may increase inflammation as the cyst dies and that increased inflammation can exacerbate dangerous symptomatology. Thus, high-dose steroids are frequently coadministered with antiparasitic therapy and then tapered in an individualized manner. Otherwise patient may experience worsening headaches and vomiting secondary to edema (Garcia et al. 2002; Del Brutto 2014a). The steroid course may be quite long in patients with refractory inflammation, and methotrexate has been shown to be an effective steroid-sparing agent (Mitre 2007; Mitre et al. 2007). Albendazole is a first-line treatment for neurocysticercosis in the United States; praziquantel may also be used. Both albendazole (15 mg/kg/day for 8–14 days) and praziquantel (50–60 mg/kg/day for 15 days) kill cysticerci, though albendazole appears to penetrate the brain more effectively (Murrell 2005). The newest research shows that combined praziquantel and albendazole treatment is the most efficacious; the combination treatment did not increase adverse effects and increase cyst burden resolution at 6-month follow-up (Garcia et al. 2014a).

Certain groups of patients have definite benefit from antiparasitic therapy, like those with giant cysts, subarachnoid cysts, or cysts increasing in size. Most cysts will resolve without cysticidal medication; however, there is some evidence that treatment results in less scarring as lesions heal (Garcia et al. 2004). In a García et al. placebo-controlled trial, treatment with 800 mg of albendazole daily, with adjuvant steroid therapy, reduced the frequency of subsequent generalized seizures. In the first month after treatment, the albendazole group had more seizures than the placebo group, but this relationship reversed at 2 months and at 30 months. Intracranial lesions also resolved more quickly in the albendazole group (Garcia et al. 2004).

## 10.3 *Taenia saginata*

### *10.3.1 Biology*

The *T. saginata* scolex has four suckers and a unique, retracted, unarmed rostellum. Fully grown proglottids are called "mature" and when filled with eggs "gravid." Mature proglottids range in size from 0.21 to 45 cm, and gravid proglottids range from 0.3 to 2.2 cm. Proglottids have a large central genital pore, and each hermaphroditic proglottid is capable of producing thousands of eggs per day. A gravid proglottid contains between 50,000 and 100,000 eggs. The eggs of *T. saginata*, like the eggs of *T. solium*, are capable of surviving in the environment for up to several weeks or months if there is sufficient moisture. On average *T. saginata* grows to a length of 4–10 m and can have more than 1000 proglottids. The intact proglottids are mobile and can occasionally be seen moving in the stool. They may be expelled without defecation, which leads to easy contamination of bedding and clothing.

The life cycle of *T. saginata* is essentially identical to that of *T. solium*, with two notable exceptions: cows, not pigs, are the intermediate hosts of *T. saginata*, and humans, the definitive hosts, never act as an intermediate host of *T. saginata.* The life cycle of *T. saginata* most commonly occurs in cattle and human beings, although llamas, buffalo, and giraffes occasionally act as intermediate hosts (Fig. 10.1). Cattle are infected when they ingest eggs on local vegetation that has been contaminated by human feces. The eggs encyst in skeletal and cardiac muscle; cysticerci may remain viable in muscle for several months before eventual degeneration or calcification. Humans may develop taeniasis after ingestion of undercooked beef with cysticerci.

### *10.3.2 Epidemiology*

*T. saginata* is a ubiquitous parasite, and human taeniasis occurs in all countries where raw or undercooked beef is consumed. Millions of people are infected with taeniasis worldwide, but exact prevalence estimates do not exist. The *T. saginata* tapeworm is most prevalent in sub-Saharan Africa and the Middle East; other regions with high prevalence of *T. saginata* (defined as greater than 10% of the population) include Central Asia, the Near East, and Central and Eastern Africa. Areas of low prevalence (defined as less than 1% of the population) include Europe, the United States, Southeast Asia, and Central and South America (Murrell 2005).

Bovine cysticercosis resulting from *T. saginata* infection is a global problem occurring in cattle rearing regions of the world and resulting in significant financial loss. From carcass inspections, it is estimated that 1.23% of French cattle are infected (Dupuy et al. 2014). From Zimbabwe slaughterhouses, there are similar estimates from 2006 to 2007 (Sungirai et al. 2014). In Swedish dairy cows, the estimate is much higher (15.6%) using serological results from ELISA in addition to

inspection data (Eichenberger et al. 2013). Bovine cysticercosis renders beef unmarketable and is globally responsible for over two billion dollars in yearly economic losses (Hoberg 2002).

### *10.3.3 Transmission*

*T. saginata* transmission and propagation are closely tied to both food consumption and sanitary habits. Human taeniasis results from the consumption of contaminated beef that has not been frozen or thoroughly cooked. Perpetuation of bovine and human disease in an agricultural setting can occur in several ways (Hoberg 2002), including direct transmission through fecal contamination of pastureland by agricultural workers, application of untreated human sewage onto pastureland, and indirect contamination of the cattle food or water supply.

### *10.3.4 Control*

There is a *T. saginata* recombinant vaccine developed based upon the identification of homologues to host protective antigens of *T. ovis*. The vaccination of cattle using a combination of the *T. saginata* proteins TSA-9 and TSA-18 has resulted in 94–99.8% protection against parasite infection (Lightowlers 2003; Lightowlers et al. 1996). Research to develop a more practical and affordable vaccine is ongoing.

At the present, disease prevention depends on public health efforts to raise awareness of disease transmission and to improve sanitary practices. Cysticerci in beef can be inactivated by cooking meat at least 56 °C, freezing meat at −20 °C for at least 10 days, salting and pickling for days, or boiling at high pressures (Murrell 2005). Reliable meat inspection to remove infected meat from the market and proper disposal of human feces to interrupt transmission to cattle are also important preventive measures.

### *10.3.5 Clinical Aspects and Diagnosis*

Human taeniasis caused by *T. saginata* is typically asymptomatic. Patients may become aware of infection upon the passage of proglottids, or even several feet of strobila, in the stool. Motile proglottids often cause discomfort with discharge. The observation of a mobile proglottid is a proxy for *T. saginata* diagnosis. A small percentage of patients complain of colicky abdominal pain, nausea, changes in appetite, weakness, weight loss, constipation or diarrhea, anal pruritus, and general malaise. Abdominal discomfort and nausea are the most common complaints and

are often relieved with the ingestion of food. In infants, increased irritability may be the only sign of infection. Serious complications from *T. saginata* infection due to the motile nature of proglottids are possible but uncommon. Migrating proglottids may cause biliary tree obstruction and intestinal obstruction or perforation (Karanikas et al. 2007). There is also a case report of acute cholangitis (Uygur-Bayramicli et al. 2012) and several reports of surprise findings during abdominal or endoscopic surgeries.

Taeniasis may be associated with an elevated serum IgE and a mild eosinophilia in a minority of patients. Definitive diagnosis of taeniasis is made by direct visualization of eggs or proglottids in the stool or by cellophane tape swab of the perianal region. *T. saginata* tends to release proglottids on a daily basis, but large sections of strobila can break off in a day, without subsequent release of gravid proglottids for several days thereafter. For this reason, collection of multiple stool samples is recommended. An ELISA using anti-*T. saginata*-IgG1 antibody has been developed recently. In field testing, the assay was 92.9% sensitive for detecting *T. saginata* infection in cattle (Ogunremi and Benjamin 2010).

### *10.3.6 Treatment*

Treatment of *T. saginata* taeniasis is the same as an infection with *T. solium* (see Sect. 10.2.8): A single 5–10 mg/kg dose of praziquantel is highly effective for cestode infections. Alternatively, a single dose of niclosamide is also effective. Dosing of niclosamide is 2 g for adults, 1.5 g for children greater than 34 kg, and 1 g for children 11–34 kg. Both praziquantel and niclosamide are class B drugs, although treatment can and should be delayed until after pregnancy unless clinically indicated. Since praziquantel is released in breast milk and safety in children under 4 years of age has not been investigated, it is recommended that breastfeeding be avoided for 72 h after treatment. Treatment failures with praziquantel and niclosamide have been reported; nitazoxanide has been shown to be an effective and inexpensive alternative in the event of resistance to first-line agents (Lateef et al. 2008).

## 10.4 *Taenia asiatica*

### *10.4.1 Biology*

*Taenia asiatica* has recently been described as a species that closely resembles *T. saginata* both morphologically and genetically. At first, the species was considered a subspecies of *T. saginata* (known as *T. saginata asiatica*), but after more extensive phylogenetic studies, it has recently been recognized as a distinct species (Hoberg 2006). Genetic studies of ribosomal genes and the mitochondrial cytochrome C

oxidase I (COI) gene identified a close relationship between *T. saginata* and *T. asiatica* with a nucleotide difference of 4.6% (Jeon and Eom 2006); however, the life cycle of *T. asiatica* more closely resembles *T. solium* (Galan-Puchades and Fuentes 2000) where there is a nucleotide difference of 12.0% (Ale et al. 2014).

The scolex of *T. asiatica* has four suckers and a rostellum armed with rudimentary hooklets referred to as wartlike formations (Flisser et al. 2004). The strobila typically is composed of fewer than 1000 hermaphroditic proglottid segments. Like *T. saginata*, a vaginal sphincter muscle is present in mature proglottids, and gravid proglottids have a central uterus with 12–30 uterine branches per side. In comparison to other *Taenia* species, *T. asiatica* has more than 57 uterine twigs per side, with a larger ratio of uterine twig to uterine branches. The prominent protuberance on posterior aspect of *T. asiatica* gravid proglottids is also unique. The metacestode cysts contain rudimentary hooklets and typically measure 0.2 cm, compared with the 0.7–1 cm cysts of *T. saginata* and 0.5–1.5 cm cysts of *T. solium* (Murrell 2005).

The life cycle of *T. asiatica* is similar to that of *T. solium* (Fig. 10.1) though humans probably cannot act as intermediate hosts for *T. asiatica* (Galan-Puchades and Fuentes 2004). Pigs are the primary intermediate host, but others include cattle, goat, monkey, and wild boar. Although *T. asiatica* cysticercosis can occur in many organs, the larva seems to have a special tropism for the liver. Maturation from cysticercus to adult worm occurs in the small intestine of humans after ingestion of contaminated meat from an intermediate host.

### *10.4.2 Epidemiology*

*T. asiatica* was first postulated to be distinct more than 50 years ago when *T. saginata*-like tapeworms were identified in the Southeast Asian communities who did not consume beef. To date, cases of *T. asiatica* have been confirmed in Taiwan, Indonesia, Thailand, Korea, China, Malaysia, Vietnam, Japan, Nepal, and the Philippines. Taeniasis is thought to be common in these regions; however, *T. asiatica* prevalence estimates are lacking (Ale et al. 2014).

### *10.4.3 Transmission*

One of the major risk factors for *T. asiatica* infection is consumption of raw pork liver, but the species has also been found in communities where that practice is not commonplace. Pork is a staple part of the diet in many regions in Southeast Asia. As with *T. solium*, proximity of human feces to porcine feed is another major risk factor for transmission and life cycle propagation of *T. asiatica*.

The life cycle of *T. asiatica* may be shorter than its counterparts *T. saginata* and *T. solium*. Four weeks after ingestion of eggs, cysts can be identified in tissue in the intermediate host. Within the liver, parenchymal cysts are more common than portal

or capsular. Cysts may also be found in surrounding tissues such as omentum, lungs, and mesentery. Two to four weeks after ingestion of viable cysts, the tapeworm reaches maturity in the intestines and begins to shed proglottids. The lifespan of the mature tapeworm is unknown: there are case reports of proglottid shedding for decades, but this may represent reinfection (Ale et al. 2014).

### *10.4.4 Clinical Aspects and Diagnosis*

It was postulated that *T. asiatica* may cause human cysticercosis because pigs are the primary intermediate hosts as is the case for *T. solium.* Since *T. asiatica* cysticerci demonstrate liver tropism in porcine models, it has been suggested that human *T. asiatica* may not cause neurocysticercosis but rather hepatic disease. The potential for *T. asiatica* to cause human cysticercosis needs further investigation (Galan-Puchades and Fuentes 2000). If *T. asiatica* infection causes subclinical cysticerci in the liver, a routine liver imaging technique in addition to serological studies would be necessary to confirm this hypothesis (Galan-Puchades and Fuentes 2013).

*T. asiatica* causes intestinal taeniasis. Definitive diagnosis of *T. asiatica* taeniasis is by microscopic examination of stool. Observations of expelled scolex or proglottid morphology can be confirmed by PCR analysis of DNA. Coproantigen testing can also be performed on stool samples, but this methodology is only specific to the level of genus. Serum EITB may be used as well, but most EITB assays for *T. solium* have unknown cross-reactivity with *T. asiatica*. One EITB with a specific band for *T. asiatica* has been developed (Jeon and Eom 2009).

### *10.4.5 Treatment*

Effective treatment for *T. asiatica* taeniasis includes single doses of praziquantel or niclosamide, using the same dosage for taeniasis as for other *Taenia* species (see Sect. 10.2.8).

### *10.4.6 Control*

The prevention strategies for *T. solium* (see Sect. 10.2.5) apply to *T. asiatica* as well. Efforts should focus on improving hygiene practices to limit intermediate host infection and decreasing raw pork consumption and/or improving meat inspection to limit definitive host infection. Although yet to be demonstrated, it is hoped that the recombinant vaccine for *T. saginata* will be equally successful for *T. asiatica*, given their genetic and morphologic similarities.

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# Chapter 11
# *Echinococcus*

**Armando Gonzalez and Luis A. Gomez-Puerta**

Cystic hydatid disease is a zoonotic disease caused by infection with the larval stage (hydatid cyst) of the tapeworm *Echinococcus granulosus*. The genus *Echinococcus* (Family: *Taeniidae*) are zoonotic helminth parasites (phylum Platyhelminthes, class *Cestoda*) with a worldwide distribution. The adult worm lives in the small intestine of suitable canids, felids, and hyenids laying eggs that are excreted with the feces of the animal and contaminating the environment. Susceptible intermediate host species that accidentally ingest infective eggs will develop the parasite's larval stage or metacestode (Carmena and Cardona 2014). The life cycle of *E. granulosus* is similar to that of many other tapeworms that are transmitted between predator and prey, dogs, or other canids. Host animals are usually herbivores and ungulates. Humans may also harbor the intermediate form. Infection of an intermediate host is due to accidental ingestion of tapeworm eggs passed into the environment with feces from definitive hosts (Craig et al. 2015). Nevertheless, high environmental dog contamination index does not necessarily correspond to high prevalence in humans as transmission is strongly linked to human behavior and hygiene (Chaabane-Banaoues et al. 2015), the global public health impact of human CE is significant and is caused primarily by the G1 genotype (Budke et al. 2006).

A. Gonzalez (✉)
Center for Global Health Tumbes, Universidad Peruana Cayetano Heredia, Tumbes, Peru
e-mail: agonza41@jhu.edu

L.A. Gomez-Puerta
Department of Veterinary Epidemiology and Economics, School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Lima, Peru

Center for Global Health Tumbes, Universidad Peruana Cayetano Heredia, Tumbes, Peru



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## 11.1 Taxonomy

*Echinococcus* species are small cestodes (<1.0 cm), taxonomically included within the family *Taeniidae* (López-Neyra and Soler Planas 1943). *Echinococcus* species/genotypes have marked differences in host infectivity and specificity, geographical distributions, zoonotic potential, development, and pathogenicity (McManus 2013). Since infection susceptibilities to specific genotypes are different, knowing which genotype is prevalent allows a better perusal of infection risk (Craig et al. 2015). Different strains of *E. granulosus* were identified to precisely portray their specificity for intermediate (Nakao et al. 2013a). Knowing the prevalent genotype in an endemic location allows tailoring the surveillance, control and prevention programs.

*Echinococcus* have a heteroxenous life cycle, adult stages of these tapeworms develop in the small intestine of carnivorous mammals (definitive host), and their metacestodes (larval stages) develop in different organs, mainly the liver and lung, of herbivorous or omnivorous mammals (intermediate host) (Carmena and Cardona 2014; McManus 2013). Metacestodes of some *Echinococcus* species may incidentally infect humans causing echinococcosis (Alvarez Rojas et al. 2014). The genus *Echinococcus* has the following species, *Echinococcus granulosus* (sensu stricto), *E. felidis*, *E. equinus*, *E. ortleppi*, *E. canadensis*, *E. multilocularis*, *E. shiquicus*, *E. oligarthra*, and *E. vogeli* (McManus 2013; Nakao et al. 2013b).

### *11.1.1* Echinococcus granulosus *(sensu stricto)*

The name *Echinococcus granulosus* (sensu stricto) is used for three strains, sheep strain, Tasmanian sheep strain, and Buffalo strain, corresponding to genotypes G1, G2, and G3, respectively (McManus 2013; Nakao et al. 2013b; Romig et al. 2015). This specie uses sheep, cattle, buffaloes, pigs, camels, goats, and macropods as intermediate hosts (McManus 2013; Romig et al. 2015). Dogs, foxes, dingoes, jackals, and hyenas are considered as definite hosts (McManus 2013; Romig et al. 2015). Also, this specie can infect humans if they ingest eggs, producing cystic echinococcosis (Alvarez Rojas et al. 2014). Sheep and dogs are considered the principal hosts worldwide (Romig et al. 2015). *E. granulosus* sensu stricto is the most frequent agent of human cystic echinococcosis worldwide: 88% of1661 genotyped human isolates belonged to this species (Alvarez Rojas et al. 2014).

### *11.1.2* Echinococcus canadensis

This species includes the camel, pig, and cervid strains, which correspond to the genotypes G6, G7 and G9, and G8 and G10, respectively (McManus 2013; Nakao et al. 2013b). *Echinococcus canadensis* use dogs and wolves as definitive hosts, the

dog being the main definitive host (McManus 2013; Nakao et al. 2013b). Mammals considered intermediate hosts for *E. canadensis* are camels, goats, and sheep for genotype G6, wild boars and beavers for genotype G7, cattle for genotypes G6 and G7, pigs for genotypes G7 and G9, and cervids for genotypes G8 and G10 (McManus 2013; Romig et al. 2015). *E. canadensis* can infect humans by ingest of eggs, producing cystic echinococcosis (Oksanen and Lavikainen 2015). A single hydatid cyst from a CE patient inhabiting Heilongjiang Province was identified as G10 genotype of *E. canadensis*. This is the first report of G10 genotype of *E. canadensis* in a human in China. Adult worms and larvae of G10 genotype of *E. canadensis* were identified in wolves and dogs as definitive hosts and three deer species (moose, elk, and reindeers) as intermediate hosts, respectively (Yang et al. 2015).

### *11.1.3* Echinococcus felidis

Specie described by Ortlepp in 1937, parasitizing the lion (*Panthera leo*) in South Africa (Ortlepp 1937). The lion is considered the only definitive host for this cestode. Some wild ungulates act as intermediate hosts; these include warthogs, zebra, wildebeest, bushpigs, buffaloes, and antelopes. Likewise, *E. felidis* isolated from Africa has been suggested as a sister taxon of *E. granulosus s.s.* in recent molecular studies of mitochondrial genes (Huttner et al. 2008; Nakao et al. 2013b). Currently, genotypes or strains for this parasite are unknown (McManus 2013). On the other hand, there are no valid information on the pathogenicity of *E. felidis* to humans and livestock (Romig et al. 2015).

### *11.1.4* Echinococcus equinus

*Echinococcus equinus* represents the strain horse (genotype G4). This species uses horses, mule, donkeys, and other equines as intermediate host and dogs as definitive host (Carmena and Cardona 2014; Lahmar et al. 2014; McManus 2013; Simsek and Cevik 2014). However, *E. equinus* was recently molecularly confirmed from a prosimian host (lemur, *V. rubra*) (Boufana et al. 2015b; Boufana et al. 2012). *E. equinus* have not been shown to be infective to humans (McManus 2013).

### *11.1.5* Echinococcus ortleppi

*Echinococcus ortleppi* was described for the first time in dogs from South Africa (López-Neyra and Soler Planas 1943). This cestode uses dogs as definitive host and cattle, buffaloes, goats and sheep as intermediate hosts (Alvarez Rojas et al. 2014; McManus 2013). It is reported to have low pathogenicity for humans (Alvarez

Rojas et al. 2014; Grenouillet et al. 2014). This species is prevalent in South America but has been reported only sporadically on other continents (Balbinotti et al. 2012).

### *11.1.6* Echinococcus multilocularis

This parasite is distributed in many regions of Europe, Asia, and North America. *Echinococcus multilocularis* uses a variety of small mammals, mainly rodents and lagomorphs, as intermediate host, as well as pigs, dogs, and monkeys (McManus 2013; McManus and Thompson 2003). Likewise, foxes, dogs, cats, wolves, raccoon dogs, and coyotes are considered as definitive hosts for this parasite. *E. multilocularis* is responsible for causing alveolar echinococcosis in humans.

### *11.1.7* Echinococcus shiquicus

This species was reported for the first time in China by Xiao et al. (2005). Foxes and pikas are natural definitive and intermediate hosts for *E. shiquicus* (Xiao et al. 2005). However, previous study showed that *E. shiquicus* could also infect dogs (Boufana et al. 2013). *E. shiquicus* produces cysts similar to cystic echinococcosis or polycystic echinococcosis, but of unknown zoonotic status (Ma et al. 2015). Currently, genotypes or strains for this cestode are unknown (McManus 2013).

### *11.1.8* Echinococcus vogeli

*Echinococcus vogeli* is endemic in neotropical region of the Americas. The life cycle of *E. vogeli* involves neotropical rodents (agoutis, pacas, spiny rats) and armadillos as intermediate host and bush dogs (*Speothos venaticus*) and domestic dogs as definitive hosts. *Echinococcus vogeli* causes the polycystic echinococcosis in humans (Mayor et al. 2015). Currently there are more of 200 cases in humans from 12 countries in Central and South America (D'Alessandro and Rausch 2008; Vizcaychipi et al. 2013). Most confirmed cases were in Colombia and Brazil (D'Alessandro and Rausch 2008).

### *11.1.9* Echinococcus oligarthrus

The life cycle of *Echinococcus oligarthrus* uses small neotropical rodents (pacas, agoutis) and wild felids (jaguars, pumas, cougars, ocelots, and jaguarondis) as intermediate and definitive hosts, respectively. The distribution of *E. oligarthrus* is

similar to *E. vogeli*, in neotropical region of the Americas. Humans rarely play the role of accidental host to *Echinococcus oligarthrus*, this is because *E. oligarthrus* has a wild life cycle. Only few cases have been reported in humans.

## 11.2 Cystic Hydatid Disease

Different species of *Echinococcus* cause different diseases in humans. Cystic echinococcosis (CE) is caused by *E. granulosus* sensu stricto, *E. equinus*, *E. ortleppi*, and *E. canadensis*. Alveolar echinococcosis is caused by *E. multilocularis* and polycystic echinococcosis by *E. vogeli* and *E. oligarthrus* (Agudelo Higuita et al. 2016). Signs and symptoms of hydatid disease depend on the organ involved and the size of the cyst. The onset of symptoms varies from a few months to several years and eventually presents as a result of the pressure exerted on structures surrounding the growing cyst. Naturally, the potential risk to an infected patient is related to the site involved. The minimum time for development of protoscolices is unknown, but, on the basis of animal studies, it is estimated to be 10 months or longer after infection (Agudelo Higuita et al. 2016). Cysts occur more frequently in the liver (52–77%), followed by the lungs (9–44%) and other locations (13–19%) (Moro et al. 1999). The reported mortality rate for cystic hydatid disease is 1–4% after one operation and may increase to 20% if subsequent surgeries must be performed. Albeit rare, sudden death due to unrecognized hydatid cysts can occur (Hosseini et al. 2014; Marashi et al. 2014). In addition to the usual high cost of surgery for removal of the cyst, adult patients must usually suffer the additional expense of lost wages during their recovering (Schantz et al. 1982).

### *11.2.1 Clinical Symptoms*

The initial phase of infection is asymptomatic, small cysts not inducing major disease may remain asymptomatic for many years, if not permanently (McManus et al. 2003). Diagnosis of cystic echinococcosis is based on clinical findings, imaging techniques (McManus et al. 2003), and serology (Brunetti et al. 2010). The incubation period of cystic echinococcosis is unclear but probably lasts for many months to years (McManus et al. 2003). The rates of growth of cysts are variable, ranging from 1 to 5 cm in diameter per year. The slow rate is usually tolerated until its size produces dysfunction (Moro and Schantz 2009). The liver is the most involved organ by the echinococcal cysts (65–70%), followed by the lungs (25–30%). Cystic echinococcosis is found less frequently in the spleen, kidneys, heart, brain, and bones (Lianos et al. 2015).

Clinical symptoms Echinococcus:cystic hydatid disease: depend on the site of localization of the cysts, their size, and their condition determines the particular manifestations (Moro and Schantz 2009). Pain in the upper quadrant or the epigas-

trium was the most common symptom and hepatomegaly and a palpable mass are the most common signs. Nonspecific symptoms such as fatigue, fever, nausea, or dyspepsia may also be present. Patients with complicated hepatic hydatid disease may present with fever, jaundice, or anaphylactic symptoms, depending on the complication (Sayek et al. 2004). The mortality rate is estimated to be 0.2 per 100,000 population, with a case fatality rate of 2.2% (McManus et al. 2003).

In alveolar hydatid disease, the embryo of *E. multilocularis* seems to localize invariably in the liver of the intermediate host. The hepatic parenchyma is gradually invaded and replaced by fibrous tissue in which great numbers of vesicles are lodged (Garcia et al. 2007).Alveolar echinococcosis typically presents later than the cystic form. Cases of alveolar echinococcosis are characterized by an initial asymptomatic incubation period of 5–15 years, and a subsequent chronic course. Untreated or inadequately managed cases have high fatality Echinococcus:cystic hydatid disease: rates (McManus et al. 2003).

### *11.2.2 Clinical Criteria (Brunetti et al. 2010)*

At least one of the following three:

1. A slowly growing or static cystic mass(es) (signs and symptoms vary with cyst location, size, type, and number) diagnosed by imaging techniques
2. Anaphylactic reactions due to ruptured or leaking cysts
3. Incidental finding of a cyst by imaging techniques in asymptomatic carriers or detected by screening strategies

## 11.3 Epidemiology

### *11.3.1 Cystic Echinococcosis (CE)*

Cystic echinococcosis (CE) has a worldwide distribution with endemic areas in all inhabited continents. Domestic transmission of *E. granulosus* eggs from pet, stray, and working dogs is particularly important in areas with inadequate educational standards and veterinary control. The number of owned dogs and the frequency of contact with dogs were identified as risk factors for human AE in studies from China (Craig et al. 2015), while in a Spanish study, cohabitation with dogs and feeding of uncooked viscera were defined as risk factors for CE (Campos-Bueno et al. 2000). As home slaughter of sheep in parts of Southern Europe and of pigs in parts of Poland and the Baltic states is still widespread, local family dogs may be infected by feeding of infected offal. The highest prevalence of this disease has been found in temperate areas, including the Mediterranean countries, south and central Russia, central Asia and China, and some regions of Australia and America, especially Latin America (Arminanzas et al. 2015).

*Echinococcus granulosus* s.s. is the etiological agent of most human cases worldwide being G1 the most frequently identified genotype (72.9%), followed by *E. canadensis* (G6 NS G7 genotypes (Cucher et al. 2016). A total of 139 hydatid cysts were sampled from the liver and lungs of 97 adult cattle and 32 old culled camels and from the liver of 8 goats of various ages and 2 pigs in Ethiopia. The study reports the predominance of *E. granulosus* s.s. (G1) and *E. intermedius* (G6/7) in Ethiopian livestock, and shows the existence of *E. ortleppi* (G5) in the southwest and center of the country (Tigre et al. 2016). Likewise, a total of 78 cystic echinococcosis resulting from 78 patients collected between 2005 and 2012 at the laboratory of parasitology of the Mustapha hospital center of Algiers. The results obtained show that the surgical frequency of hydatidosis is significant young people and children. The epidemiological context associated at the disease is the conjointly presence of a dog and herbivores (Zait et al. 2014).

#### 11.3.1.1 Europe

In Europe, the endemic area of *E. granulosus* sensu stricto covers Southern and Southeastern Europe; *E. canadensis* G7 is prevalent in the Baltic countries, Poland, and southward to Romania. For *E. granulosus* sensu *lato*, most prevalence data are based on slaughterhouse investigations of intermediate hosts, while prevalence data concerning definitive hosts are scarce, especially for pet dogs. Prevalence rates of 0–31% are reported from farm and shepherd dogs in Italy and Spain and 14.2% from farm village dogs in Lithuania (Bruzinskaite et al. 2009; Carmena and Cardona 2013). CE is one of the five most frequently diagnosed zoonoses in the Mediterranean region and is reemerging in Southeastern Europe (Jenkins et al. 2005). Incidence rates for CE of 1.1–3.3/100,000 were recorded in Spain, up to 3.5 in Sardinia in Italy and 3.3 in Greece, Bulgaria, and Romania (Torgerson et al. 2011). Economic loss attributable to human CE was estimated for Spain at €133 million (Benner et al. 2010).

#### 11.3.1.2 Africa

Livers and lungs from 2040 donkeys slaughtered for animal or human consumption were examined in Tunisia. Hydatid cysts were found in 173 animals out of the 2040 donkeys examined (8.48%). Molecular analysis of 35 *Echinococcus* cysts confirmed that donkeys were infected with *E. granulosus* (G1) or *E. equinus* (G4). *E. equinus* and *E. granulosus* G1 genotype was identified from donkey isolates. Both species were found to affect the liver and lungs and each donkey was infected with only one genotype of *Echinococcus* (Lahmar et al. 2014).

The use of portable abdominal ultrasonography and serological testing for the diagnosis of hydatid infection demonstrated 1.6% of a rural community in Libya had hydatid cysts (Shambesh et al. 1999). These figures for hydatid disease are similar to those found in northwestern Kenyan nomadic populations, which had previously been considered to be the highest human prevalence previously known, also at 5.7% (MacPherson 1987).

#### 11.3.1.3 Asia

A significant difference was observed between females (25%) and male goats (6%). The findings of this study demonstrated that cystic hydatidosis is common and widely distributed in local goats, and they might play an important role in the life cycle and transmission of this zoonosis in Oman (Al-Kitani et al. 2014). *Echinococcus granulosus* is widely distributed in Kazakhstan, with human cases of CE and animal infection, in both dogs and livestock, being widespread. The reported human incidence of echinococcosis appears to have stabilized at between 800 and 1000 cases per year following a rapid increase in incidence in the last decade of the twentieth century.

The situation with regard to AE is less clear. Infection in wild animal hosts has long been known; however, the increasing infection of dogs represents a potentially greater risk to humans, because of the closer contact between dogs and humans. The evidence in this review does suggest that there are significant numbers of cases of human AE in Kazakhstan. The estimate of 130 cases per year, based on hospital records and the ratio of AE:CE cases, could overestimate the number of cases, as the referral center tends to deal with more complex cases of echinococcosis, and AE has more severe sequelae than CE (Abdybekova et al. 2015).

A study was designed to determine the seroprevalence of hydatid infection in Kashmir Valley and to find out association of risk factors for acquisition of this infection (Fomda et al. 2015). Out of 1429 samples, 72 (5.03%) were IgG positive by enzyme-linked immunosorbent assay, and seropositive samples were analyzed further by Western blotting. The percentage occurrence of the highly immunoreactive antigenic fractions in IgG ELISA positive samples was 57 kDa (72.2%) followed by 70 kDa (66.7%) and 39 kDa (58.3%) by immunoblotting. Samples with other parasitic infections were reactive with the cluster of 54–59 kDa antigenic fractions. Age < 15 years, male gender, contact with dog, and rural residence were the most significant factors associated with the seropositivity.

#### 11.3.1.4 South America

In South America, five countries have cystic echinococcosis cases identified in natural intermediate and definitive hosts for which sequencing data are available: Argentina, Brazil, Chile, Peru, and Uruguay. A total of six genotypes have been found in the region: G1, G2, G3, G5, G6, and G7. The types of livestock affected are sheep, cattle, pig, goat, and alpaca (Fig. 11.1). The majority of the cases are caused by *E. granulosus* s.s. (genotypes G1/G2/G3). The G1 genotype shows the widest distribution and is the most frequently found in the species of livestock analyzed as well as in the domestic definitive host (Cucher et al. 2016). *E. granulosus* (*s.s.*) G1 genotype from definitive hosts originating from the Falkland Islands was also confirmed by molecular analysis (Boufana et al. 2015a).

Molecular methods were used to examine *Echinococcus spp*. and genotypes infecting intermediate and definitive hosts as well as humans from the UK. Samples were derived from livestock intermediate (sheep, cattle, horses) and definitive hosts

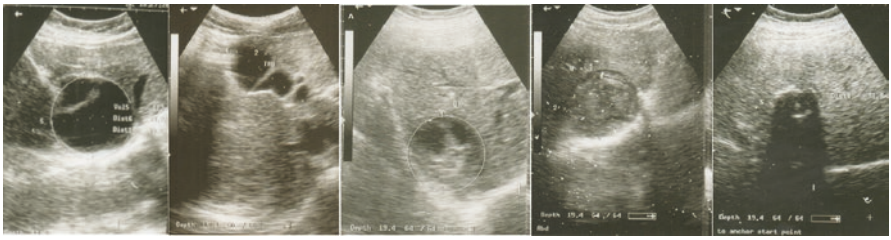

**Fig. 11.1** Ultrasound images of liver cystic lesions according to the WHO Classification: *A*: CE3a Type:6.5 × 4.5 cm. *B*: CE2 Type:5.4 × 2.8 cm. *C*: C3b Type:6.5 × 7.9 cm *D* CE4 Type:5.5 × 5.2 cm *D* CE5 Type:3.8 × 2.7 cm (Courtesy of Dr. Saul Santivañez, Center for Global Health, Universidad Peruana Cayetano Heredia, Lima-Peru)

(farm dogs, foxhounds) from various parts of the UK. All UK horse isolates and captive mammals had 100% sequence identity with *E. equinus*. Isolates from sheep and cattle all belonged to *E. granulosus s.s*. The four human CE isolates were confirmed as *E. granulosus s.s*. DNA extracted from feces of Welsh farm dogs and foxhounds confirmed these canids were infected with either *E. equinus* or *E. granulosus s.s*. (Boufana et al. 2015b).

### *11.3.2 Alveolar Echinococcosis (AE)*

Human AE is one of the most pathogenic helminthic zoonoses and causes a high burden of disease in Europe (Torgerson et al. 2008). Recent studies support the hypothesis that the infection pressure caused by *E. multilocularis* eggs has increased across certain large European regions. In Switzerland, a representative endemic area for central Europe, the annual incidence rates of new human AE cases varied between 0.10 and 0.16/100,000 individuals over a 45-year period, suggesting a high degree of epidemiological stability. However, approximately 10–15 years (corresponding to the incubation time of AE) after a distinct increase in the fox populations (with *E. multilocularis* prevalences of 30–60%), a higher incidence rate of 0.25/100,000 was recorded (Deplazes et al. 2011). Similar trends of increasing incidence have been observed in Austria, France, and Lithuania. The overall incidence of AE is variable (0.03–0.26) in Central Europe, but estimated to be 200 new cases per year (Deplazes, personal communication).

*E. multilocularis* occurs in the northern hemisphere, with large endemic areas in Europe including parts of the western continent (e.g., France, Benelux States) and all countries of central Europe including Northern Italy, Slovenia, Romania, and the Baltic States. Furthermore, foci also exist in Denmark, Sweden, and on Svalbard Island. Several studies have investigated the prevalence of *E. multilocularis* in pet dog populations. Low prevalence rates of <0.5% were recorded in the privately owned dog populations in France, Germany, Switzerland, and Denmark, but a higher prevalence (3–8%) was found in dogs with predatory habits and those able to

roam more widely (Deplazes et al. 2011). In Switzerland, 0.3% of randomly selected privately owned dogs were found to be infected with this tapeworm. Based on this prevalence, the individual probability of being infected at least once during 10 years can be estimated at 8.7%. Large population studies in Germany revealed that 0.13% of dogs in northern and 0.35% in southern Germany excreted *E. multilocularis* eggs in their feces. Considering the total dog population in Germany (approximately 5.4 × 106 dogs), around 13,000 are estimated to be infected.

The prevalence of *E. multilocularis* in cat populations, as determined at necropsy examination, ranged between 0% and 5.5% in various endemic areas. Cat infections are characterized by low worm burdens and strongly reduced worm development, resulting in lower egg production compared with foxes or dogs. Therefore, the epidemiological role of the cat in spreading this infection is estimated to be low (Hegglin and Deplazes 2013).

## 11.4 Diagnosis of Echinococcosis

### *11.4.1 Cestode Identification*

The diagnosis of *Echinococcus* in dogs or other definitive hosts requires the verification of adult stages and complete parasite in the feces, specific coproantigen, and DNA of the parasite. The diagnosis in the intermediate hosts is detecting the presence of larval cysts or metacestode in different organs, mainly the liver and lung.

### *11.4.2 Diagnosis of Echinococcus Eggs*

The diagnosis of *Echinococcus* eggs in fecal or environmental samples is very complicated, this because eggs of *Echinococcus* and *Taenia* species have the same morphological characteristics (Abuladze 1964). The concentration method had been most used, 0.5–2.0 grams of samples is mixed with water in test tubes of 10–15 ml. Then, the test tubes are centrifuged at 1000 ***g*** for 10 min, remove the supernatant and repeat it until the supernatant is clear. Sediment is mixed with a sucrose solution and centrifuge at 1000 ***g*** for 5–10 min (Ito 1980). A cover-glass is put in the top of the tube and then it is examined under microscopy (10x) (Health 2008).

### *11.4.3 Clinical Diagnosis*

Clinical findings such as a space-occupying lesion and residence in an endemic region are suggestive of hydatid disease. Abdominal ultrasonography is an important aid in the diagnosis of abdominal cysts (Singh et al. 1999). Portable ultrasonography machines have been developed and are being utilized with good results in

field surveys (Arminanzas et al. 2015; Singh et al. 1999; Das et al. 1995). Chest-X-radiography is useful for diagnosis of lung cysts (Mahdhaoui et al. 2003). CT scanning is also a great aid in diagnosis especially for non-typical lesions.

### *11.4.4 Serological Diagnosis*

A variety of serological methods have been developed and used for immunodiagnosis of CE in recent years, including indirect hemagglutination (IHA), immunoblotting, enzyme-linked immunosorbent assay (ELISA), indirect fluorescent-antibody (IFA), latex agglutination test, and immunochromatography test (Sarkari and Rezaei 2015). A western blot assay based on the identification of three specific antigens of 8, 16, and 21 kDa is currently used (Sarkari and Rezaei 2015). Major drawbacks in serological diagnosis are low sensitivity for detection of lung hydatid cysts and cross-reactivity with sera of patients with *Taenia solium* cysticercosis. Serological tests should be used in combination with imaging techniques when field surveys are conducted in order to detect most cases of hydatid disease. Available immunodiagnostic tests give a relatively high rate of false-negativity. False-negative results in immunodiagnostic tests for CE may be seen in patients with small cysts, intact cysts, cysts in extrahepatic locations, heavily calcified cysts (e.g., nonviable), or cyst in privilege sites (the brain or eye) (Sarkari and Rezaei 2015).

### *11.4.5 Necropsy of Intermediate Host*

After the necropsy, specimens should be preserved in 4% formalin or kept cool at 4 °C and deep-frozen at −20 °C for subsequent examination. It is necessary to save all information possible of the intermediate host: sex, age, locality, etc. (Health 2008). Metacestodes or larval cysts can be observed in many organs, sometimes is necessary to do palpation or incision in large animals. In wild animals, such as ruminants and rodents, several metacestodes from different cestode should be considered for differential diagnosis (Health 2008). Conventional histological techniques can be used in tissue fixed in formalin; the tissue can be stained with hematoxylin-eosin (HE) and periodic acid-Schiff (PAS) stain (Das et al. 1995; Singh et al. 1999). The presence of protoscoleces into the cysts is also diagnostic for the genus. Tissue samples preserved in refrigeration o congelation can be also preserved in absolute ethanol for molecular studies (Health 2008).

### *11.4.6 Necropsy of Definitive Host*

Regarding definitive hosts, necropsy is practiced mainly in experimental studies. If necropsy is used in epidemiological studies in wildlife or street dogs, it requires the approval of animal ethics (IACUC) and it is necessary to show that the animals will

be euthanized in the right way (Association 2013; Health 2008). After the euthanasia of the dog, the small intestine is removed and tied at both ends. The intestine is separated into several sections and immersed in 0.9% saline at 37 °C for examination. Cestodes adhering to the intestinal wall can be observed and counted by means of a hand lens or stereoscopic microscope. At the end, all material is boiled and washed by sieving to eliminate most of the particulate material and to make it non-infectious (Health 2008).

### *11.4.7 Arecoline Purging*

Arecoline is a drug used via oral administration to perform surveys of tapeworm infections in dog populations (Batham 1946). Arecoline is a parasympathomimetic agent; its use produces sweating, and stimulation of salivary, lacrimal, gastric, pancreatic, and intestinal glands in the dog (Schantz 1973). Likewise, it increases intestinal tonus and the mobility of smooth muscle. Also, arecoline has an effect on the cestode, by causing paralysis, but not death, causing it to relax and can be easily removed by the intestinal lumen. Arecoline has side effects on old, sick, and pregnant animals. Therefore, these animals should be excluded from arecoline purgation (Gemmell 1973; Health 2008; Schantz 1973). The dose used is 4 mg/kg; the result of purgation occurs in less than 30 min. After the purge, dogs may produce two fecal expulsions; the first is formed by feces and the second is formed by mucus; in the latter the *Echinococcus* is found. This mucus sample (about 5 ml) is diluted with 100 ml of distilled water. Then, parasite count is done with the help of a magnifying glass or stereo microscope (Gemmell 1973).

### *11.4.8 Coproantigen Tests*

Many protocols of enzyme-linked immunosorbent assay (ELISA) for detection of coproantigens of *Echinococcus granulosus* in dogs (Ahmad and Nizami 1998; Allan and Craig 2006; Casas et al. 2013; Craig et al. 1995; el-Shehabi et al. 2000; Lopera et al. 2003; Staebler et al. 2006) and *E. multilocularis* in foxes, dogs, and cats (Al-Sabi et al. 2007; Deplazes and Eckert 1996; Eckert 2003; el-Shehabi et al. 2000; Morishima et al. 1999) have been performed to date. The sensitivity and specificity of these tests have been estimated at 70% and 98%, respectively (Allan and Craig 2006; Buishi et al. 2005; Deplazes et al. 1999; Deplazes and Eckert 1996). Coproantigens can be detected prior to elimination of eggs by *Echinococcus* cestodes and therefore are not related to egg antigen (Deplazes et al. 1992; Health 2008; Rosenzvit et al. 1999). This has the advantage in the detection of prepatent infections. In addition, coproantigen levels return to baseline preinfection within 5 days of anthelminthic treatment of infected dogs (Health 2008; Rosenzvit et al. 1999).

### *11.4.9 Molecular Diagnosis*

The use of molecular techniques is used mainly for differential diagnosis of *E. granulosus* and *E. multilocularis* infections in definitive hosts (Bretagne et al. 1993; Mathis et al. 1996). Also, molecular methods are used to determine different genotypes in *E. granulosus* (G1-G10) (Alvarez Rojas et al. 2014) and in identifying *Echinococcus* species in definitive and intermediate hosts (Bart et al. 2006; McManus 2006). Recently, a robust molecular phylogeny of *Echinococcus* spp. was reconstructed from their complete mitochondrial genomes (Nakao et al. 2013b).

## 11.5 Treatment

### *11.5.1 Surgery*

Surgery has been the mainstay of therapy for large cysts, those that are superficial and likely to rupture, infected cysts, and those in vital anatomical locations or exerting substantial mass effect (McManus et al. 2003). Surgery should be carefully evaluated against other options before choosing this treatment. It is the first choice for complicated cysts. In the liver, it is indicated for (1) removal of large CE2-CE3b cysts with multiple daughter vesicles; (2) single liver cysts, situated superficially, that may rupture spontaneously or as a result trauma when PTs are not available; (3) infected cysts, again, when PTs are not available; (4) cysts communicating with the biliary tree (as alternative to PT); and (5) cysts exerting pressure on adjacent vital organs (Brunetti et al. 2010).

### *11.5.2 Chemotherapy*

The benzimidazole compounds—albendazole and mebendazole—have been the cornerstone of chemotherapy for cystic echinococcosis. Treatment with albendazole (10 mg/kg in divided doses—usually 400 mg—twice daily) results in the disappearance of up to 48% of cysts and a substantial reduction in size of a further 24% (McManus et al. 2003). Recent experimental studies in animals have shown that another benzimidazole compound, oxfendazole, may have very high parasiticidal effects. The use of oxfendazole in naturally hydatid infected sheep using daily, weekly, and monthly regimens of the drug resulted in 100, 97, and 78% cure or improved status, compared to 35% cured or improved animals in the control group. Further studies will seek to determine the potential effect of oxfendazole in the treatment of hydatid disease in humans (Gavidia et al. 2010). Oxfendazole showed strong cysticidal effect against *Taenia solium* cysts in pigs with cysticercosis (Gonzalez et al. 2012).

### *11.5.3 PAIR (Percutaneous Aspiration, Injection, Respiration)*

PAIR consists in percutaneous puncture using sonographic guidance, aspiration of substantial amounts of the cyst fluid, and injection of a protoscolicidal agent, usually hypertonic saline for at least 15 min, followed by reaspiration (Filice and Brunetti 1997). With the advent of drug therapy effective for CE, PAIR (puncture, aspiration, injection, and reaspiration) plus preoperative and postoperative chemotherapy is now advocated in health care centers worldwide. PAIR is particularly useful in uniloculated cysts, stages CE1 or CE2. A comprehensive analysis of published cases from 1990 to 2014 was performed using PubMed database. A total 298 publications on the PAIR treatment were analyzed and showed that clinical and parasitological cure occurred in 98.7% of patients undergoing laparoscopic intervention and in 97.5% of patients receiving PAIR plus chemotherapy (Chen et al. 2015).

## 11.6 Control Programs

The earliest successful program against echinococcosis was performed in Iceland, and it was based on a health educational campaign that eradicated the parasite (Gemmell 1987). Control programs have been aimed at preventing dogs from having access to infected offal by health education of dog owners. This approach includes periodic treatment of sheepdogs with praziquantel (every 45 days), reduction in the dog population, and close veterinary inspection of slaughterhouse facilities for the presence of dogs and cremation of infected offal (Larrieu and Zanini 2012). Control programs are currently being implemented in Argentina, Chile, and Uruguay. Partial success has been achieved in the first two countries; however, hydatid disease remains a serious problem in Uruguay. Control programs in New Zealand and Tasmania have effectively reduced the number of infected animals and the incidence of human infection (Gemmell 1987).

Except for the sylvatic animal echinococcosis, human infection with *E. granulosus* declines with regional development (Gemmel and Schantz 1997). The process of modernization, bringing with it better living standards, sanitary education, and environmental hygiene, has been most effective in controlling transmission of the parasite. However, as regional development represents a far-distant goal, there is presently a clearly defined need to implement active control programs against *E. granulosus* in order to effectively control the parasite by development. Past efforts that have been successful in controlling other diseases have demonstrated that control strategies must be able to be realistically sustained within health budgets, which are never large enough to meet the many needs of the community. Our simulation model can help policy makers plan for the costs of the program. The duration of intervention also represents a major obstacle in hydatid control, as it necessitates obtaining funding for a long period of time (Gemmel and Schantz 1997; Nonnemaker and Schantz 1997).

While some eradication programs against *E. granulosus* have been successful in reducing prevalence, eradication has proven difficult to achieve (Moro et al. 1994). There are two strategies for controlling CHD. The first one is the horizontal approach, emphasizing long-term primary health care. This approach includes education, sanitation, meat inspection, and improved public health. However, these measures are not promising approaches for effectively eliminating parasitic transmission in either the short or midterm, if ever. Historically, the driving force in changing attitudes is education and motivation, both of which diminish after the first years. Thus, it seems that a successful control program must be targeted at the parasite, and this includes the second, vertical approach. The vertical approach includes monitoring and treating dogs in a regular basis (Gemmel and Schantz 1997). However, apart from political instability, the main eradication difficulty in the past appears to have been the failure to provide and maintain regular monthly or six-weekly treatment of all dogs for up to 20 years. Eradication time may be accelerated through other methods that can shorten the duration of the program to 10 or 12 years (Dungal 1957; Beard 1973).

## 11.7 Resistance to Environment and Susceptibility to Disinfectants

*Echinococcus* eggs can survive for long periods in the environment if sufficient moisture is present, and they are resistant to cold temperatures. Nevertheless, eggs exposed to 24 h of ultraviolet radiation have a marked reduction in infectivity (Williams and Colli 1972). *Echinococcus granulosus* can survive for 24 h with no detectable changes, and some eggs are still infective after being exposed to −50 °C for 24 h. They are no longer infective after being exposed to −70 °C (Colli and Williams 1972). *Echinococcus multilocularis* eggs exposed to increasing temperatures were more resistant to heat if suspended in water as compared to eggs exposed on a filter paper at 70% relative humidity. *Echinococcus multilocularis* eggs were infectious after heat exposure at 65 °C for up to 120 min; however, no echinococcosis developed after treatment of the eggs at 65 °C for 180 min or at 70, 75, and 80 °C for 7.5, 15, or 30 min (Federer et al. 2015).

Significant reductions in egg infectivity were observed in eggs treated with Lugol's iodine, 0.015% ammonium dodecylbenzenesulfonate, 70% ethanol, or 5% or 10% glutaraldehyde (Perez-Esandi et al. 1974). Nevertheless, any disinfectant which does not have absolute ovicidal activity cannot be considered safe (Perez-Esandi et al. 1974). *Echinococcus* eggs can be killed by exposure to 33% of a commercial sodium hypochlorite at 3.75%, and this solution kills the oncospheres by 1–5 min. Seventy percent ethanol had no visible effects on *Echinococcus* eggs (Craig and MacPherson 1988; Laws 1967). Likewise, no cysts developed in 10 mice that received doses of 1000 eggs treated with 5% glutaraldehyde, and only 1 of 10 mice developed cysts after exposure to eggs treated with 10% glutaraldehyde.

## 11.8 Vaccines

The life cycle of *Echinococcus* can be prevented using a vaccine to prevent infection to intermediate and the definitive hosts.

### *11.8.1 Vaccination of Intermediate Hosts*

There have been several efforts to prevent infection with the larval form using a vaccine (Heath et al. 2003); however, few have successfully protected against *Echinococcus* eggs under experimental or field conditions. EG95 is a protein of the oncosphere of *Echinococcus granulosus* that could be associated with oncospheres adherence to the intestinal mucosa (Heath and Lawrence 1996; Lightowlers et al. 1996). Immunization studies have shown that the protein provides 96–98% protection in experimental trials with sheep (Lightowlers et al. 2000; Lightowlers et al. 1999; Lightowlers et al. 1996). This vaccine successfully controlled transmission under field conditions (Larrieu et al. 2013).

### *11.8.2 Vaccination of Dogs Against* E. granulosus *Infection*

Obviously new approaches to transmission control are required for a suitable eradication plan. A new program must increase feasibility, reduce the timeline and, in so doing, preserve the original enthusiasm. Immunological approaches may serve as a valuable adjunct and may even prove indispensable to achievable eradication. An alternative approach to control CHD involves the use of a vaccine targeted against the acquisition of the cystic stage of the life cycle in the intermediate host. The implementation of such a vaccination program would require only a small fraction of the logistic and financial efforts involved in current eradication programs. Sheep vaccination programs are impractical economically and have left a canine vaccination as the only plausible option. However, the concept of hydatidosis control by dog immunization has received little attention in the past because lumen-dwelling intestinal cestodes have erroneously been regarded as immunologically inert (Weimann 1970). Although the immune response of intermediary hosts of *E. granulosus* has been comparatively well studied, the immune response in the dog has received only limited attention.

Protective immunity has been reported in dogs after numerous sequential infections (Gemmel et al. 1986), but protective antigens have not yet been described. The use of inadequate immunization regimes or inappropriate immunogens can be attributed to this failure. Immunization of dogs using worm secretory antigens (SE) was described by Herd et al. (1975). SE substances originate from the scolex/rostellar antigens on the surface of the protoscolex and directly interact with areas of the

mucosa where host cells make contact. Host cells then take up and process antigenic components of the parasite (Smyth 1962, 1967). A significant reduction in the number of proglottids and eggs was observed in dogs immunized with SE antigens through systemic immunization (Desplazes, et al. 1994). Importantly, Carol et al. (1997) pointed out the relevance of the route of immunization to the outcome of the immune responses. They analyzed the immunogenicity of immuno-stimulating complexes (ISCOMs) containing surface molecules of *E. granulosus* protoscoleces in mice when administered by mucosal route. The same group later succeeded in inducing an immune response in dogs using ISCOMs from tegumental antigens from protoscoleces delivered intranasally (Carol and Nieto 1998). However, this group did not challenge the dogs. ISCOMs have been used with a variety of antigens as means of adjuvant preparation (Morein, et al. 1984; Morein and Äkerblom 1992). This approach has shown to significantly enhance both humoral and cellular immunity when delivered by either oral (Mowat et al. 1991; Mowat and Donachie 1991) or intranasal routes (Jones et al. 1988). This project will evaluate the potential for using secretory/excretory ISCOMs as a novel approach in vaccinating dogs against *E. granulosus*.

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# Chapter 12
# Other Cestoda of Public Health Relevance

**Hector H. Garcia and Miguel M. Cabada**

## 12.1 Introduction

Cestode parasites of human relevance are included in the phylum Platyhelminthes, subphylum Neodermata, class Cestoda, subclass Eucestoda, and order Cyclophyllidea and Pseudophyllidea. In this chapter we will discuss three families that commonly infect human and may be food-borne, namely, Taeniidae, Hymenolepididae, and Diphyllobothriidae.

The adult stage of all cestode parasites has a segmented body composed of the scolex, the neck, and the strobila which is formed by numerous segments called proglottids. These live in the lumen of the small intestine of the definitive host attached to the mucosa by the scolex. The scolex bears the attachment organs which could be hooks, suckers, or shallow grooves called bothria. The tapeworm lacks a gastrointestinal tract and absorbs nutrients through a specialized tegument in the scolex and proglottids. There are four species of tapeworms that commonly infect the human intestine: *Taenia solium*, *Hymenolepis nana*, *Diphyllobothrium latum,* and *Taenia saginata*.

Human disease may be caused by infection with cestode larvae after the ingestion of the tapeworm eggs. This type of transmission is most often via person-to-person. After ingestion by the exposed person, the oncospheres contained in the eggs are released in the intestinal lumen and start the invasion. The cestode larval forms may invade a specific location forming a cystic structure like in cysticercosis

H.H. Garcia, MD, PhD (✉)
Center for Global Health – Tumbes and Department of Microbiology, Universidad Peruana Cayetano Heredia, Lima, Peru

Cysticercosis Unit, Instituto Nacional de Ciencias Neurologicas, Lima, Peru

M.M. Cabada, MD, Msc
Division of Infectious Disease, Department of Internal Medicine, University of Texas Medical Branch, Galveston, TX, USA

Collaborative Research Center – Cusco, Universidad Peruana Cayetano Heredia, Cusco, Peru



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or migrate through the tissue forming subcutaneous nodules like in sparganosis. Important human cestode larvae with potential food-borne transmission are *Taenia solium* cysticercosis, cystic echinococcosis (*Echinococcus granulosus*), and sparganosis (*Spirometra mansonoides*). Cestode infections, especially in their larval forms, cause significant morbidity and mortality among human in underdeveloped countries. They are also emerging as important causes of disease in industrialized countries, particularly in rural areas and among immigrants.

## 12.2 *Taenia solium*

*T. solium* taeniasis is a food-borne infection with an extensive geographic distribution. The infection of the human brain with the larval form of *T. solium* (neurocysticercosis) is the main complication of this form of taeniasis and has a large impact on human health due to the associated seizure disorders.

### *12.2.1 Description of the Life Cycle*

The adult *T. solium* tapeworm measures between 2 and 4 m and lives attached to the human intestine by the scolex that has four suckers and two crowns of hooks. A gravid proglottid may contain up to $5 \times 10^4$ eggs that are infective upon excretion in the stool. The eggs are spherical with a thick brown radiated shell and a diameter between 30 and 40 μm. Each egg has one oncosphere with six hooks that is released in the intestine after ingestion by the secondary host, which is most often a pig. The oncosphere invades the wall of the intestine, gains access to the circulation, and spreads to distant sites causing cysticercosis. Cysticerci, the larval stage of the *T. solium*, are fluid-filled bladders measuring about 10 mm in diameter, containing one invaginated scolex. These are often found in the muscle (Fig. 12.1) and brain

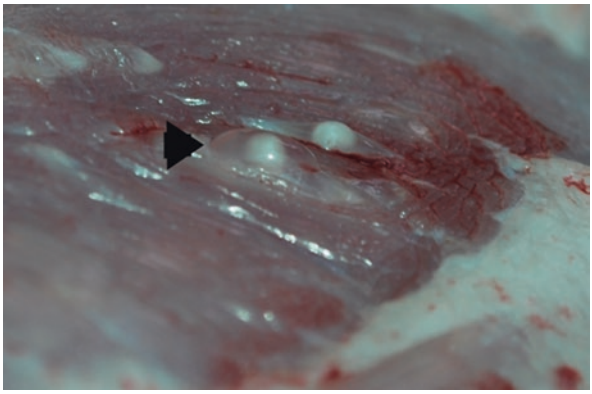

**Fig. 12.1** Cysticerci in pork muscle

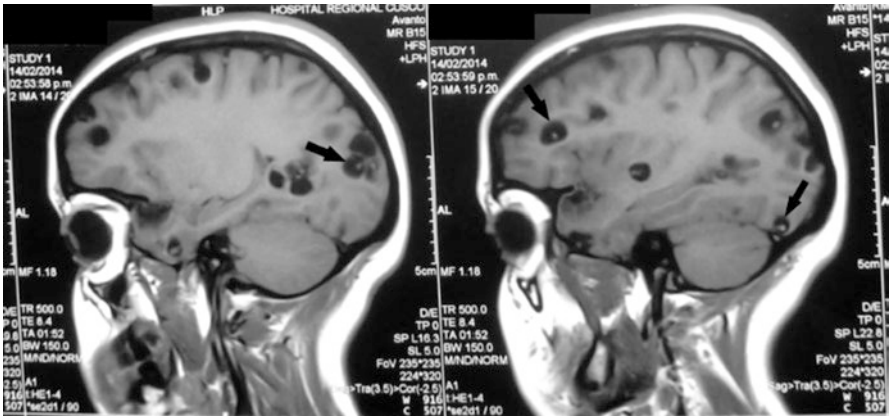

**Fig. 12.2** Magnetic resonance imaging of an individual with neurocysticercosis showing multiple cysticerci. *Arrows*, cysticercus scolex

tissue (Fig. 12.2), but may involve any organ. Humans become the secondary host after ingesting eggs and may develop neurocysticercosis subsequently. The cycle is completed when humans ingest viable tissue cysticerci from swine that hatch in the intestine producing an adult tapeworm.

### *12.2.2 Transmission*

Humans are the only definitive host for *T. solium* taeniasis, which is caused by the ingestion of raw, undercooked, or inadequately frozen swine products infected with the larval stage of the parasite. After ingestion, the scolex contained in the cysticercus attaches to the intestinal mucosa and matures into an egg-producing adult *Taenia* in about 12 weeks.

Transmission of cysticercosis among humans is associated with close contact with a *Taenia* carrier (Lescano et al. 2009). Cases of cysticercosis cluster around tapeworm carriers which suggest that transmission is likely due to poor hygienic practices and person-to-person transmission. However, there is a potential for food-borne transmission among humans if *Taenia* carriers are also food handlers or the environment is heavily infected.

The interaction between human and swine is crucial for the transmission cycle of the infection. Pigs acquire cysticercosis through ingestion of human feces. The prevalence and incidence of cysticercosis in swine increase exponentially with proximity to human tapeworm carriers. Free-roaming pigs with access to human feces, usually within 100 meters of their homes, are at high risk of infection (Pray et al. 2016).

### 12.2.3 Epidemiology

*T. solium* taeniasis is highly endemic in developing countries where free-range pigs are raised and poor sanitation is practiced. Local cases have been reported in Latin America, sub-Saharan Africa, Asia, and parts of Oceania. The prevalence of taeniasis varies with the diagnostic methods used being lowest with stool direct microscopy and highest with coproantigen ELISA. Depending on the diagnostic test used, taeniasis prevalence in Latin America ranges between 0.2% and 17%, in Africa between 0.3% and 14%, and in Asia between 0.3% and 8% (Coral-Almeida et al. 2015). However, variations in prevalence are also observed within small geographic regions. Madinga et al. reported a prevalence of taeniasis between 1% and 60% in villages in a small health region of Democratic Republic of Congo using coproantigen (Madinga et al. 2016). A similar study by Garcia et al. showed a prevalence between 0% and 7% in villages of a small district in the highlands of Peru (Garcia et al. 2003). A 5-year report in Los Angeles County in California identified 24 *T. solium* taenia carriers; some of these in households of patients with neurocysticercosis (Croker 2015). These large variations within small regions may be explained by the observed clustering of human and pig cysticercosis around tapeworm carriers, which closes the transmission circle.

Neurocysticercosis, the most serious consequence of *T. solium* taeniasis, is common in developing countries and follows the same distribution of taeniasis. The prevalence of the disease in the general population is difficult to estimate as neuroimaging is necessary to stablish the diagnosis. Fleury et al. evaluated 649 subjects in a neurocysticercosis-endemic area of Mexico using CT scans and found 59 (9.1%) subjects with compatible brain lesions (Fleury et al. 2006). Interestingly, the vast majority of subjects had single calcified lesions and had no symptoms. Moyano et al. reported a 23% prevalence of brain lesions in a general population village without epilepsy in an endemic area of Peru (Moyano et al. 2014). A higher risk of cysticercosis is attributed to taenia carriers and their close contacts. Subjects who raise and butcher pigs and those who sell pork might also be at increased risk of taeniasis and cysticercosis (Garcia et al. 1998).

Cysticercosis is being increasingly diagnosed in developed countries mainly among immigrants from endemic areas. Although a rare event, local transmission of cysticercosis has been reported in developed countries among groups in close contact with tapeworm-carrying immigrants (Del Brutto 2012; Serpa and White 2012).

### 12.2.4 Clinical Significance

*T. solium* taeniasis is asymptomatic in most carriers, but some patients may complain of abdominal pain and diarrhea. The clinical significance of taeniasis due to *T. solium* relates to the potential to develop neurocysticercosis. A significant proportion of tapeworm carriers have positive serology or imaging studies for cysticercosis (Garcia et al. 1998; Lescano et al. 2009). It is estimated that as high as 30% of seizures in endemic areas are caused by neurocysticercosis (Ndimubanzi et al. 2010).

### 12.2.5 Diagnosis

Stool microscopy allows the identification of *Taenia* sp. eggs in tapeworm carriers, but the sensitivity and specificity are low. Egg concentration techniques like the rapid sedimentation and Kato-Katz are more sensitive than other techniques, but do not allow the differentiation between *T. solium* and *T. saginata* eggs as they are morphologically identical.

Species identification may be possible if fragments of the tapeworm are recovered by spontaneous elimination or after treatment. *T. solium* gravid proglottids measure 10 mm × 10 mm approximately, have a mid-lateral genital pore, and have few (<12) primary branches on each side of the central uterine stem.

*T. solium* coproantigen ELISA is significantly more sensitive than microscopy and should be the test of choice to diagnose taeniasis and monitor treatment response (Bustos et al. 2012). Guezala et al. reported on 100% species-specific coproantigen ELISA tests for *T. solium* (Guezala et al. 2009). ELISA for the detection of *T. solium* antigen is also useful to detect the presence of cysticercosis using body fluids (Paredes et al. 2016). Species-specific PCR techniques can also differentiate *T. saginata* from *T. solium* and some of these tests have shown high sensitivity and specificity using DNA extracted from stools of tapeworm carriers (Mayta et al. 2007; Yamasaki et al. 2004).

Serology directed to detect *T. solium* antibodies is used for the diagnosis of cysticercosis. Antibody detection by enzyme-linked immunoelectrotransfer blot assay is the method of choice. The sensitivity of this method is 98% and the specificity 100% in human cases with more than one viable cerebral cyst (Tsang et al. 1989).

### 12.2.6 Treatment

Praziquantel and niclosamide are effective drugs against the adult form of *T. solium*. In regions where *T. solium* is endemic, latent neurocysticercosis may respond to praziquantel and cause severe headaches or seizures. In contrast, niclosamide is not absorbed from the gastrointestinal tract and does not carry this risk.

### 12.2.7 Prevention

Prevention of *T. solium* taeniasis and neurocysticercosis requires integrated interventions aimed at interrupting the transmission. Improving handling of human excreta, identification and treatment of infected animals, and robust meat inspection programs are an important aspect in control (Del Brutto and Garcia 2013). Irradiation of pork meat, freezing at −15 °C for 6 days, and cooking it at 63 °C for 3 min inactivate cysticerci and prevent tapeworm infections (Gamble 1997; USDA 2015).

Identifying and treating tapeworm carriers, especially in certain groups like household contacts of patients with neurocysticercosis or certain food handlers, is paramount to stop transmission of cysticercosis (Del Brutto and Garcia 2013). Of note, *T. solium* proglottids and stools containing eggs should be handled with extreme precaution and following the appropriate biosafety measures to avoid cysticercosis.

## 12.3 *Hymenolepis nana*

*H. nana* is the smallest human intestinal tapeworm and the most common throughout the world. It can complete its life cycle inside the intestine without the need for an intermediary host.

### *12.3.1 Description of the Life Cycle*

The adult tapeworm measures 2–4 cm long and has a life span of between 4 and 6 weeks. Multiple tapeworms attach to the intestinal mucosa by the scolex that has four suckers and one crown of hooks. Several thousand eggs (Fig. 12.3) are produced by the tapeworm every day and passed in the stool. When eggs are ingested by human or the intermediate host (beetle and flea species), they hatch in the intestine, and the released oncospheres or hexacanth larvae invade the microvilli and form cysticercoid larvae. Autoinfection may occur when eggs hatch before excretion into the environment and form cysticercoid larvae in the same host. The cysticercoid contains an invaginated scolex without surrounding fluid, like in other larvae of the Taeniidae family. The cysticercoid larvae mature within 2 or 3 weeks and break the microvilli to release young tapeworms. *H. nana* has a wild life cycle

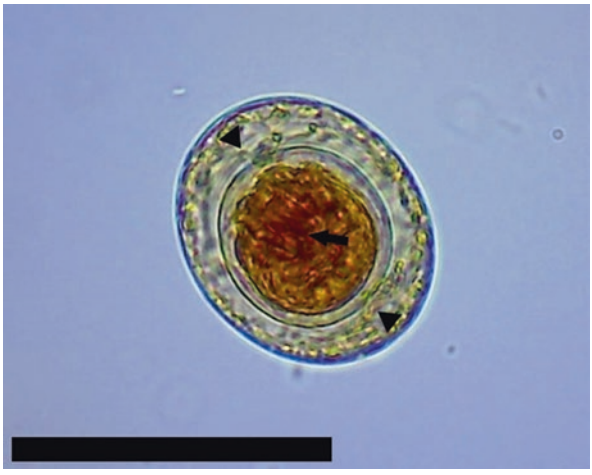

**Fig. 12.3** *Hymenolepis nana* egg. *Large arrow*, hooks. *Short arrows*, polar filaments

that involves mice as the definitive host and beetles as the intermediate host. Some evidence suggests that the strain that infects rodents may be different from the one that infects humans, but this is still a matter of debate.

### 12.3.2 Transmission

*H. nana* eggs are infectious when shed in the stool, and some may hatch in the host's intestine before reaching the environment, which is called autoinfection. A common transmission route is direct ingestion of infective eggs shed by the same individual or by a close contact which is usually a household member. The ingestion of infected insects is a potential transmission route, but does not explain the high prevalence of infection in certain areas. *H. nana* transmission by contaminated food and water is considered part of the infection cycle, but the importance of this route has not been subjected to scrutiny (Rostami et al. 2016).

### 12.3.3 Epidemiology

*H. nana* infection is one of the more common helminth infections worldwide and the most widely distributed cestode. It is significantly more common in developing countries than in developed countries. The prevalence ranges between 5% and 50% among some age groups in areas of Asia, Southern and Eastern Europe, Central and South America, and Africa (Mega et al. 2012; Thaher Al-Marsome 2012; Romero-Cabello et al. 1991). The prevalence in Ethiopia was 13% in elementary school children studied by Tefera et al. and 32% among refugee children in Sudan studied by Hamid et al. (Tefera et al. 2015; Hamid et al. 2015). Other studies have reported 16% and 10% prevalence among children in Egypt and India (Khalil et al. 1991; Mirdha and Samantray 2002). Low socioeconomic status and poor access to sanitation and safe water are associated with the infection (Gelaw et al. 2013; Hamid et al. 2015). Hymenolepiasis is particularly common among children, and its prevalence tends to decrease in adulthood. It is an emerging problem in areas where programs for mass deworming of children use medications inactive for *H. nana*. In developed countries it most frequently affects institutionalized people and those with immunosuppression.

### 12.3.4 Clinical Significance

Infections are often regarded as asymptomatic. However, several studies have linked *H. nana* to diarrhea, abdominal pain, headaches, and irritability. Abdel Hamid et al. studied 500 children 1–5 years old in refugee camps in Sudan and reported that infected children were 9.5 times more likely to report diarrhea than uninfected children (Hamid et al. 2015). Hymenolepiasis in children may also be linked to anemia

and malnutrition (Khalil et al. 1991; Willcocks et al. 2015). The burden of infection estimated by the number of eggs per gram of stool is probably related to symptoms in children with hymenolepiasis, but the cutoff numbers are less well defined than in soil-transmitted helminths.

### *12.3.5 Diagnosis*

The diagnosis of the infection relays on finding the eggs in feces by microscopy. Flotation and sedimentation techniques are used to concentrate the eggs and increase sensitivity. *Hymenolepis* eggs have a thin shell and measure between 30 and 50 μm in diameter. Each egg contains one oncosphere or hexacanth embryo with six hooks surrounded by an inner membrane. The inner membrane has two polar thickenings from which four to eight filaments arise and extend into the space between the shell and the oncosphere (Fig. 12.3). These filaments are characteristic of *H. nana* and are not seen in other *Hymenolepis* species. The proglottids of *H. nana* are rarely found in stool because they disintegrate after breaking off from the strobila. No culture, antigen detection, or nucleic acid detection tests are available for the identification of *H. nana* in stool.

### *12.3.6 Treatment*

Praziquantel and niclosamide are effective drugs against *H. nana*. Praziquantel is administered in two doses separated 10–15 days to decrease relapses. Niclosamide is administered for 7 days because it only has luminal activity and does not affect the cysticercoid larvae within the intestinal villi. Nitazoxanide may be a therapeutic alternative (Chero et al. 2007).

### *12.3.7 Prevention*

Prevention of *H. nana* infection requires improved sanitation, water supply, and handwashing education which may interrupt fecal-oral transmission. Correct food handling practices may decrease contamination of the food chain with infected insects and rodent droppings. Mass deworming programs with praziquantel (directed to control schistosomiasis) decrease the burden of infection in the community (Soares Magalhães et al. 2013). However, the use of albendazole for mass deworming against soil-transmitted helminth infections has little or no effect of *H. nana* prevalence (Horton 2000).

## 12.4 *Diphyllobothrium* Species

*Diphyllobothrium latum*, known as the fish tapeworm, is the most representative species in the genus. It is the longest intestinal parasite to infect the human intestinal tract. The infection is acquired by ingesting raw or partially cooked fish.

### *12.4.1 Description of the Life Cycle*

The tapeworm of *D. latum* can grow up to 15 m long and may live for as long as 20 years. However, other species like *D. pacificum* are much smaller measuring between 0.5 m and 4 m. Their scolex attach to the small intestine mucosa using dorsal and ventral bothria. The gravid proglottids produce unembryonated eggs (Fig. 12.4) that are passed in the feces and require 18–20 days in the environment to mature. High numbers of eggs are passed in stool (up to 1 million per day), and passing proglottid chains is common. Embryonated eggs release coracidia in water, which are ciliated embryos with six hooks. Coracidia are ingested by freshwater copepods and transform into procercoid larvae (the second larval stage). Infective procercoid larvae inside the copepod are ingested by small fish and migrate to the muscle where they transform into the third larval stage called the plerocercoid or sparganum. Finally, when large predatory fish like pike and perch feed on small fish, the plerocercoid or sparganum migrates to the musculature of the bigger fish. The third larval stage is capable of infecting the intestine of fish-eating mammals like human, dogs, foxes, wolves, cats, bears, and pigs. After ingestion of the plerocercoid, a flatworm develops in the small intestine that matures in 4–6 weeks and starts producing eggs.

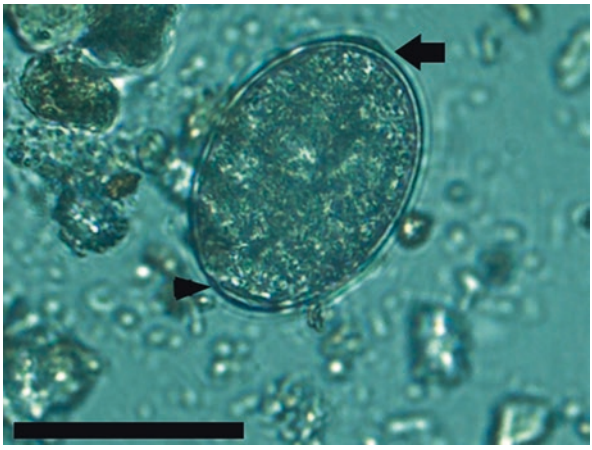

**Fig. 12.4** *Diphyllobothrium* spp. egg. *Short arrow*, operculum. *Large arrow*, abopercular end showing a characteristic small knob

### 12.4.2 Transmission

Transmission occurs by ingestion of raw, smoked, or pickled fish. While *D. latum* is transmitted after ingestion of freshwater fish like perch, pike, or burbot, other *Diphyllobothrium* like *D. nihonkaiense* is transmitted by salmon species and *D. pacificum* by marine fish like bonito and lorna drum. Depending on ecological conditions and population dynamics, a high prevalence of plerocercoid larvae can be found in fish. In Alpine lakes of Switzerland, Italy, and France, the prevalence of infection in fish may reach 33% (Dupouy-Camet and Peduzzi 2004). Kuhn et al. reported a prevalence of up to 45% among trout in Norwegian lakes (Kuhn et al. 2016). Similarly, Rozas et al. described a plerocercoid prevalence between 4% and 66% among feral rainbow trout captured in lakes used for aquaculture in Southern Chile (Rozas et al. 2012). The expansion of aquaculture using suboptimal practices and the increasing popularity of exotic raw fish dishes like ceviche and sashimi favor the transmission of diphyllobothriasis (Cabello 2007). An outbreak of diphyllobothriasis reported in Brazil, where the infection is not endemic, was linked to the consumption of farmed raw Chilean salmon (Sampaio et al. 2005).

### 12.4.3 Epidemiology

*D. latum* is the most common species infecting humans, with a distribution that includes Northern Europe especially in lake areas of Scandinavia, Russia, Northern Japan, the United States principally the upper Midwest and Alaska, Canada, and the southwestern coast of South America (Scholz et al. 2009). Recent reports suggest that *D. latum* is also found in Asia, especially in China (Guo et al. 2012). Although *D. pacificum*, *D. nihonkaiense*, and *D. dendriticum* are less common, these species are emerging human pathogens in the west coast of South America, the northern Pacific basin, and Europe, respectively (Scholz et al. 2009). Other *Diphyllobothrium* species (*D. cordatum*, *D. ursi*, *D. lanceolatum*, *D. dalliae*, and *D. yonagoensis*) have also been reported to infect humans but less frequently.

Recent data on the prevalence of diphyllobothriasis at the community level are scarce. In Finland a small study involving 2000 subjects identified a prevalence of between 0.3% and 3.8% (Kyrönseppä 1993). Navarrete and Torres reported a prevalence of 0.4% in school age children in a coastal community in Chile (Navarrete and Torres 1994). As in Japan, Arizono et al. reported a prevalence of 1 case per 100,000 in the Kyoto and Tokyo regions (Arizono et al. 2009). A recent review of diphyllobothriasis in Europe suggested a decreasing prevalence of *D. latum* in northern countries but an increase in reported cases in Italy and France (Dupouy-Camet and Peduzzi 2004).

### 12.4.4 Clinical Significance

Diphyllobothriasis is usually asymptomatic, and individuals may only become aware of the infection after passing parasite segments in their stool. When the tapeworm reaches a large size, it can cause mechanical bowel obstruction, diarrhea, and abdominal pain. Megaloblastic anemia resulting from vitamin $B_{12}$ deficiency has been associated with diphyllobothriasis, particularly in Northern European countries. *Diphyllobothrium latum* competes with the host for the uptake of vitamin $B_{12}$ (Anonymous 1976). However, the infection alone does not seem to be sufficient to cause the anemia. The "tapeworm anemia" is rare outside Scandinavian countries, and some authors postulate, among other factors, a genetic predisposition (Anonymous 1976).

### 12.4.5 Diagnosis

*Diphyllobothrium* eggs are identified by stool microscopy, and concentration techniques like rapid sedimentation and Kato-Katz increase the sensitivity. *Diphyllobothrium* eggs are oval, operculated, measure 60–75 μm × 45–50 μm, and have a clearly defined wall. The abopercular end usually has a small knoblike protrusion. Egg morphology does not allow the identification at the species level, and recovering the adult tapeworm may aid in speciation. The *Diphyllobothrium* proglottids have a width of ~8 mm and length of 4 mm. Their genital pore is located in the center of the proglottid rather than in the edges as in other tapeworms. The coiled uterus is located in the center of the gravid proglottid and is brown in freshly collected specimens. Although, nucleic acid detection is not used for diagnosis of tapeworm infections, *Diphyllobothrium* DNA analysis allows the identification of the species which has epidemiologic relevance for the evaluation of geographic distribution, potential hosts, and control measures (Scholz et al. 2009).

### 12.4.6 Treatment

Praziquantel and niclosamide are highly effective against *Diphyllobothrium* in single-dose regimens. Niclosamide allows recovering the tapeworm intact for examination and speciation.

### 12.4.7 Prevention

The most common sources of human *Diphyllobothrium* infection are pike, burbot, perch, ruff, salmon, trout, and turbot (Scholz et al. 2009). Infected fresh fish (recently caught or chilled) eaten raw, undercooked, or pickled transmits plerocercoid larvae

to humans and other hosts (Kuchta et al. 2015). Thus, infection with *Diphyllobothrium* is prevented by eating well-cooked fish heated at (60 °C for a few minutes) or fish that has been deep-frozen (at least −10 °C for 24 h). Also, placing fish in a solution of at least 12% sodium chloride (brine) is useful to inactivate the plerocercoids (Raether and Hänel 2003).

## 12.5 *Taenia saginata*

*T. saginata*, the beef tapeworm, is endemic to most regions of the world. Humans are the only definitive host and the infection is generally innocuous. It is important to differentiate *T. saginata* from *T. solium* infections due to their contrasting clinical and epidemiologic implications.

### *12.5.1 Description of the Life Cycle*

The mature *T. saginata* tapeworm measures between 4 and 8 m long and can live in the small intestine of the human definitive host for many years. The gravid proglottids produce eggs that are eliminated in the stool. In addition, eggs may reach the environment inside the proglottids that are motile and often eliminated intact as *Taenia* segments. *T. saginata* eggs are morphologically indistinguishable from eggs of *T. solium*. Eggs are infective upon excretion and contaminate pastures. Cattle are the intermediate hosts and get infected by ingesting the eggs in areas where human stools contaminate the environment. When eggs reach the intestine, they release oncospheres that penetrate the mucosa and gain access to the circulation. Through the blood stream, the oncospheres reach the muscle and other tissue of cattle forming cysticerci. The cysticercus is the larval stage of *T. saginata* and consists of a fluid-filled cyst formed by the invagination of the scolex. When these are ingested by the human host, they evaginate in the small intestine and attach to the mucosa using the suckers in the scolex, and after 5–12 weeks, they become a mature tapeworm.

### *12.5.2 Transmission*

Humans are the only definite host for *T. saginata* taeniasis which is acquired through the consumption of raw or undercooked fresh cattle meat infected with viable cysticerci. Other cattle tissues like the liver and lungs may also contain cysticerci, and, when inappropriately cooked, their ingestion may cause taeniasis. Human usually carry a single tapeworm, but multiple parasites have been described in some subjects. Importantly, the ingestion of *T. saginata* eggs does not cause cysticercosis in humans.

Infection rates in cattle assessed by carcass inspection in abattoirs vary significantly. In France, the reported prevalence was 1.4 cysticerci-positive carcasses/1000 cattle in all the country's metropolitan abattoirs in year 2010 (Dupuy et al. 2014). In Mexico, up to 2.8 cysticerci-positive carcasses/1000 cattle were reported among 17 Mexican states (Cueto Gonzalez et al. 2015). Qekwana et al. reported a prevalence between 6 and 9 cysticerci-positive carcasses/1000 cattle in 26 abattoirs in South Africa between 2010 and 2013 (Qekwana et al. 2016). Lastly, Terefe et al. reported 197 cysticerci-positive carcasses/1000 cattle in one abattoir in Harari Regional State in eastern Ethiopia (Terefe et al. 2014). According to Dupuy et al., one infected cattle carcass can transmit *T. saginata* taeniasis to between 8 and 20 people. Considering that, depending on the quality of carcass inspection, the sensitivity for bovine cysticercosis is between 10% and 50%, the risk of infection may be significant even in developed countries (Dupuy et al. 2014).

### *12.5.3 Epidemiology*

*T. saginata* has a worldwide distribution. It is especially prevalent in Africa, Central and South America, Eastern and Western Asia, and some countries in Europe. The prevalence of *T. saginata* taeniasis around the world between the years 1973 and 2000 was reviewed by Cabaret et al. In Europe and the Americas, the prevalence ranged between 0.01 and 10%, in Africa between 0.01% and 7%, and in Asia between 0.02% and 36% (Cabaret et al. 2002). More recent reports confirm the high prevalence of *T. saginata* in Asian countries, demonstrated by tapeworm morphology and molecular methods (Wandra et al. 2006; Van De et al. 2014).

*T. saginata* taeniasis is more common in areas where cattle are raised. Raw and inadequately cooked beef consumption is the main risk factor (Cabaret et al. 2002). Raw beef dishes like carpaccio, tartare, and yook hwe are delicacies served in developed and developing countries but carry a significant risk for taeniasis. Differences in the prevalence of taeniasis may occur within countries and may be related to local uses and environmental conditions.

### *12.5.4 Clinical Significance*

Patients with *T. saginata* infection often exhibit no symptoms. However, infected subjects often report spontaneously passing proglottids or finding them in their underwear. The mature tapeworm can also cause abdominal pain, discomfort, and diarrhea. Case reports of intestinal obstruction, intestinal perforation, and biliary tree obstruction in *T. saginata* taeniasis are rare (Bekraki and Hanna 2016; Dural et al. 2015).

### 12.5.5 Diagnosis

*T. saginata* eggs are 30–35 μm in diameter and have a striated wall characteristic of all human *Taenia* spp. Eggs are detected by stool sedimentation and Kato-Katz tests, but the sensitivity of microscopy is low and does not allow the species identification. Species-specific diagnosis is made by examination of gravid proglottids passed by the patient. *T. saginata* gravid proglottids are 18–20 mm wide and 5–7 mm long, have 15–20 primary lateral branches on each side of the central uterine stem, bilobulated ovaries, and a lateral genital pore. The scolex has four suckers and no rostellum or hooks which differentiates it from *T. solium*.

Coproantigen detection assays using enzyme-linked immunosorbent assay (ELISA) may accurately distinguish between *T. saginata* and *T. solium*. Species-specific PCR techniques have also been described to detect parasite DNA and differentiate *T. saginata* from *T. solium*. PCR tests targeting specific mitochondrial DNA genes like *cox 1* are highly specific and may be able to differentiate *T. saginata*, *T. solium*, and *T. asiatica* (Jeon et al. 2011). PCR tests have mostly been used for epidemiologic purposes, are expensive, and are not commercially available.

Serum antigen detection ELISAs using monoclonal antibodies to *T. saginata* have been developed to detect the infection in cattle. These assays can be very sensitive detecting burdens of <50 cysts per animal, but are not routinely available (Paulan Sde et al. 2013).

### 12.5.6 Treatment

Praziquantel and niclosamide are effective drugs against the *T. saginata* tapeworm. Nitazoxanide is a systemic broad-spectrum antiparasitic with reported activity against *T. saginata* in cases that failed treatment with first-line medications.

### 12.5.7 Prevention

Taeniasis by *T. saginata* is transmitted through the ingestion of raw fresh or chilled cattle products. Avoiding the ingestion of raw or inadequately cooked beef, liver, and beef heart prevents the infection. Identifying *Taenia* carriers and treating them decrease environmental contamination and may decrease transmission. A recent uncontrolled study suggested that mass drug administration with three doses of albendazole decreases the tapeworm carriage prevalence in endemic regions. In addition, preventing environmental contamination by adequate handling of human excreta may also help interrupting the life cycle.

Abattoir inspection programs and enforcement may decrease human exposure to viable cysticerci, preventing new cases of taeniasis. Also, freezing beef at −24 °C for 24 h or cooking it at 63 °C for 3 min inactivates cysticerci and also prevent tapeworm infections.

## 12.6 *Echinococcus granulosus* (Cystic Echinococcosis)

The larval stage of *E. granulosus*, a dog tapeworm, causes cystic echinococcosis in humans. These infections are particularly common in areas of the developing world where sheep farming occurs.

### *12.6.1 Description of the Life Cycle*

The *E. granulosus* tapeworm lives in the small intestine of dogs and other canids. Dogs shed *E. granulosus* eggs in the stool, and these contaminate pastures and the animal fur. Sheep and other herbivores ingest grass contaminated with eggs, which hatch in their small intestine releasing oncospheres. These penetrate the wall of the intestine gaining access to the portal circulation and spreading hematogenously to distant sites, mostly the liver (Fig. 12.5) and lungs. Once the oncosphere has reached its final destination, it forms a cystic lesion or metacestode that grows over several months or years. The wall of the metacestode or endocyst has two layers, the external or hyaline layer and the internal or germinal layer. In addition, the host forms a wall or pericyst that isolates the cyst contents from the immune system. The germinal layer produces protoscoleces and daughter cysts that float in the cyst fluid (hydatid fluid) (Fig. 12.6). Each protoscolex is an invaginated *Taenia* scolex and is capable of producing a new metacestode; thus rupture of the cysts may cause dissemination of the infection. The cycle is completed when dogs are fed sheep-infected viscera and the protoscoleces evaginate in the small intestine of the canid and form adult tapeworms in about 6 weeks.

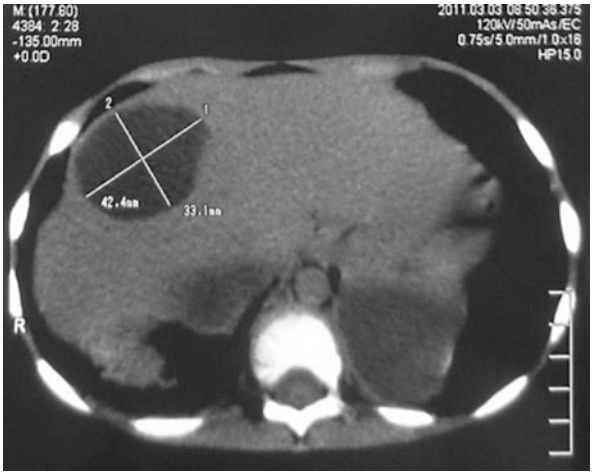


**Fig. 12.5** Patient with liver cystic echinococcosis

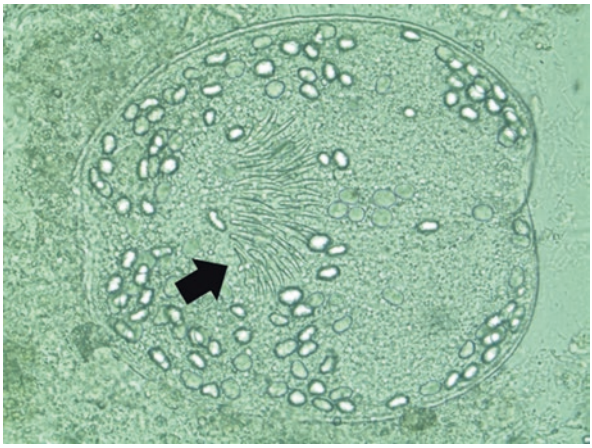

**Fig. 12.6** Protoscolex of *Echinococcus granulosus* cyst. *Arrow*, hooklets

### *12.6.2 Transmission*

Transmission of cystic echinococcosis to humans is strongly linked to contact with dogs and small-scale household farming (Campos-Bueno et al. 2000; Schantz et al. 2003). The infection is likely acquired by ingesting *E. granulosus* eggs in contaminated food (Neghina et al. 2010). In addition, eggs may adhere to the hands of dog owners and be ingested accidentally if poor hand hygiene is practiced. Humans are a dead-end host as canids rarely have access to human viscera.

The prevalence of tapeworm infection in dogs varies significantly with geographic region and animal use. Mastin et al. using coproantigen ELISA reported that about one in four family owned dogs in the Tajikistan region were tapeworm carriers (Mastin et al. 2015). However, in other Asian countries like China, the reported frequencies of taeniasis can reach 66% in stray dogs (Carmena and Cardona 2013). In Lybia, Buishi et al. reported that the proportion of owned dogs diagnosed with echinococcosis by coproantigen ELISA was 20% compared to the proportion of stray dogs with taeniasis that was 26% after necropsy (Buishi et al. 2005). In this study, 100% of sheep dogs were infected with *E. granulosus*. In Peru, Lopera et al. reported a prevalence of taeniasis of 79% in farm dogs by copro-ELISA (Lopera et al. 2003).

### *12.6.3 Epidemiology*

The *E. granulosus* species is a complex composed of at least four clades (*E. granulosus* sensu stricto, *E. equinus*, *E. ortleppi*, and *E. canadensis*). Among these, *E. granulosus* sensu stricto is by far the most common (Alvarez Rojas et al. 2014).

Cystic echinococcosis is still endemic in the Old World, particularly Greece, Cyprus, Bulgaria, Lebanon, Turkey, Iran, and some Western European countries like Spain and Italy. Cystic echinococcosis is an emerging problem in Asia with regions like Tajikistan reporting 28 cases/100,000 population and Tibet reporting 32 cases/100,000 population (Zhang et al. 2015). Cystic echinococcosis is present in 87% of the provinces in China, and an estimated 1% of the population in those areas had ultrasound evidence of infection in 2004 (Zhang et al. 2015). South American countries like Peru report a high prevalence of infection ranging from 9% to 18% using the combined results of liver ultrasound, lung X-rays, and serology (Moro et al. 1997, 1999a, b). Recent studies in the Rio Negro region in Argentine showed a prevalence range between 2.5% and 7% (Salviti et al. 2015; Bingham et al. 2014). A lower prevalence is reported in sub-Saharan Africa; however, the epidemiology of the infection is still to be described in several countries (Magambo et al. 2006). Sporadic autochthonous transmission is currently recognized in Alaska and other states in the United States (Eckert and Deplazes 2004).

### *12.6.4 Clinical Significance*

Human infection affects the liver in two thirds of the cases and the lungs in about one fourth. However, other organs like the heart, brain, bones, or kidneys may be infected. About 80% of infected subjects present with compromise of a single organ. Symptoms are related to the compression of adjacent organs and rupture or infection of the cyst. Some cystic lesions may resolve without therapy in a proportion of patients (Moro et al. 1999a, b). The burden of human cystic echinococcosis is estimated in 1 million DALYs, and the costs associated with the infection are over 4 billion American dollars a year (Budke et al. 2006). In addition, cases have significant fatality rates in developed and developing countries. In Salamanca, Spain, the case fatality rate was 2% and the mortality rate 3.1 for 100,000 inhabitants (Belhassen-Garcia et al. 2014). Chile reported a mortality of 8.5 for 100,000 inhabitants in Aysen and La Araucania regions (Martinez 2014).

### *12.6.5 Diagnosis*

The diagnosis of cystic echinococcosis is made by ultrasound of the liver or chest X-rays, but CT scans are useful for the evaluation of difficult cases or to plan surgical interventions. Serology is used for confirmation, but the sensitivity is low and varies with cyst location, size, and complications (i.e., higher in the liver and in ruptured cysts) (Eckert and Deplazes 2004).

### *12.6.6 Treatment*

Treatment modalities include combinations of antiparasitic agents (albendazole, albendazole plus praziquantel) with percutaneous drainage (i.e., PAIR = puncture, injection, aspiration, and reinjection) or surgical interventions which may include laparoscopic or open procedures. Interventions may cause spillage of cyst contents which rarely leads to anaphylactic reactions but may cause massive seeding of the surrounding organs (Eckert and Deplazes 2004).

### *12.6.7 Prevention*

Close contact with dogs with *E. granulosus* is associated with human infections. Periodic treatment of infected dogs, avoiding feeding dogs with offal, and proper disposal of livestock viscera are measures to control the environmental contamination with *E. granulosus* eggs. The improvement of sanitation, handwashing, and food handling practices may interrupt the transmission of cystic echinococcosis in humans (Lightowlers 2013).

## 12.7 *Spirometra mansonoides* (Sparganosis)

Sparganosis is an uncommon human infection by the larvae of *Spirometra* spp. Sparganosis is a cosmopolitan infection, but human cases are mainly reported in Southeast Asian countries (Liu et al. 2015a, b). The procercoid and plerocercoid larvae of *Spirometra* species can be transmitted to human by ingestion of infected crustaceans in drinking water, raw fish, frog, or snake meat or by direct inoculation via contact of open wounds and conjunctivas with raw infected meat (i.e., poultices). The most common manifestations are subcutaneous, cerebral, or eye nodules that may be migrating and are often associated with hypereosinophilia. ELISA and immunochromatography tests have been developed to diagnose sparganosis, but the diagnosis most often rests on demonstration of the larvae after excisional biopsy (Liu et al. 2015a, b; Wiwanitkit 2005).

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# Chapter 13
# Foodborne Trematodes: *Paragonimus* and *Fasciola*

**Ann M. Adams**

## 13.1 Preface

Digenetic trematodes comprise one of the most common groups of parasitic worms. They have a complex life cycle involving both sexual and asexual reproduction. The parasites require at least two hosts, the first of which is usually a mollusk. Over a hundred species of digenetic trematodes have been recorded from human hosts, but many cases may be spurious or accidental (Crompton 1999). The association between humans and trematodes is long-standing, with reports of *Schistosoma* eggs in Egyptian mummies and of *Clonorchis* eggs in a 2000-year-old corpse from the Chou Dynasty in China.

In Table 13.1, trematodes from six genera are compared, including their infection sites, sizes, and intermediate hosts. These trematodes account for approximately 40 million cases of human infections worldwide. Other mammalian hosts may also be infected, often acting as reservoir hosts for the parasites. Infection of the final host occurs from the consumption of foods contaminated with infectious larvae (metacercariae). The implicated sources of infection are usually freshwater or anadromous fishes, freshwater crustaceans, or aquatic vegetation, depending on the species of trematode involved.

In the present chapter, trematodes of two genera, *Paragonimus* and *Fasciola*, will be discussed in detail including their geographic distribution, life cycles, epidemiology, and clinical aspects of disease. Many of the species of these foodborne trematodes are endemic in developing nations and have significant impact on public health. Developed countries, including the USA, also have foci of trematode infections. Some species are naturally present in the USA, such as *Fasciola hepatica* and *Paragonimus kellicotti*. Others are introduced by the importation of contaminated

A.M. Adams (✉)
U.S. Food and Drug Administration, Kansas City District,
8050 Marshall Drive, Suite 205, Lenexa, KS 66214, USA
e-mail: ann.adams@fda.hhs.gov



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**Table 13.1** Comparison of foodborne trematodes

| Species | Estimate of human cases[a] (millions) | Usual infection site for adult parasite | Size range, adults (mm) | Size range, eggs (μm) | First intermediate hosts | Second intermediate hosts |
|---|---|---|---|---|---|---|
| *Paragonimus* spp. | 20.68 | Lungs | 7–20 × 4–8 | 80–120 × 45–65 | Snails | Freshwater crabs |
| *Clonorchis sinensis* | 7.01 | Bile ducts | 8–25 × 1.5–5 | 26–35 × 12–19 | Snails | Freshwater fishes |
| *Opisthorchis* spp. | 10.33 | Bile ducts | 7–12 × 2–3 | 19–30 × 12–17 | Snails | Freshwater fishes |
| *Fasciola hepatica* | 2.39 | Bile ducts | 20–40 × 8–13 | 130–150 × 63–90 | Snails | Aquatic vegetation[b] |
| *Fasciolopsis buski* | 0.21 | Small intestine | 20–75 × 8–20 | 115–158 × 63–90 | Snails | Aquatic vegetation[b] |
| *Nanophyetus salmincola* | 0.019 | Small intestine | 0.8–2.5 × 0.3–0.5 | 64–97 × 34–55 | Snails | Freshwater & anadromous fishes |

[a]WHO (1995)
[b]Cercariae shed their tails and encyst as metacercariae on aquatic vegetation

foods or infected intermediate hosts, the development of new food habits, and the immigration of peoples from endemic countries.

The control of the trematode populations and prevention of their respective diseases are dependent on understanding the transmission and development of the parasites. Disruption of the life cycles is necessary for the control and eradication of disease, which can be attained through the treatment of infected populations, improved sanitation and agricultural practices, or the alteration of food habits.

## 13.2 *Paragonimus* spp.

### *13.2.1 Introduction*

Trematodes of the genus *Paragonimus* have a long association with their human hosts. Over the course of several paleoparasitological studies in South Korea involving the study of 18 mummified or half-mummified human remains from the Joseon Dynasty (1392–1910 AD), eggs of several species of trematodes were recovered (Shin et al. 2012; Seo et al. 2014). Five of 18 mummies (27.8%) were positive for eggs of *P. westermani*. These mummies were determined to be from the fifteenth to sixteenth and from the seventeenth to eighteenth centuries (Shin et al. 2012). The authors contrasted the infection rates from the mid-Joseon Dynasty with the infection rate for *P. westermani* in 1961 of 0.09%.

WHO (1995) estimated that over 20 million people are infected worldwide by *Paragonimus*; Keiser and Utzinger (2005) estimated that at least 292.8 million people were at risk of acquiring paragonimiasis. Over 40 species of *Paragonimus* have been described although not all may be valid. At least 15 species have been reported from humans, of which eight are considered important (Sinniah 1997; Cross 2001). *P. westermani* is the most studied since it has the broadest geographical distribution and accounts for most cases of pulmonary paragonimiasis. This species has been reported from China, Japan, Korea, Thailand, Taiwan, the Philippines, Indonesia, India, Nepal, and Manchuria. In addition to *P. westermani*, other species of medical importance in China are *P. skrjabini*, *P. hueitungensis*, and *P. heterotremus*. The latter is also distributed in Southeast Asia, including Thailand. *P. miyazakii* is endemic in Japan in addition to *P. westermani*. Two species are endemic in Africa, *P. africanus* and *P. uterobilateralis*. In Latin America, including Peru and Ecuador, *P. mexicanus* is of greatest concern for human infections, although other species have been reported (e.g., *P. amazonicus* and *P. inca*. Note that *P. peruvianus*, *P. ecuadoriensis*, and *P. caliensis* are considered synonyms of *P. mexicanus* (WHO 1995)). Velez et al. (2003) provide a morphological comparison of eggs and rediae of various species of *Paragonimus* in Latin America (including the three species considered synonyms of *P. mexicanus* given immediately above).

The species, *P. kellicotti*, is primarily known from wild and domestic carnivores, especially from cats, dogs, pigs, minks, fishers, foxes, and muskrats in North America, east of the Rocky Mountains (Yokogawa 1965; Ramsden and Presidente 1975; Schell 1985). Infections by *Paragonimus* in North America were reported as early as 1894 in a cat from Michigan (Ward 1894) and a dog from Ohio (Kellicott 1894), respectively, as well as in over 50 pigs in Ohio in 1900 (U.S. Marine-Hospital Service 1900). In the latter cases, lung lesions were reported by meat inspectors for the Bureau of Animal Industry (currently, US Dept. of Agriculture – Food Safety and Inspection Service). These early reports attributed the infections by trematodes of *P. westermani*, but these parasites are now considered to be *P. kellicotti*. Human infections of *P. kellicotti* derived domestically have been reported (Pachucki et al. 1984; Mariano et al. 1986; Procop et al. 2000; DeFrain and Hooker 2002; Castilla et al. 2003), and Weina and England (1990) suggest that such infections may be more prevalent.

### *13.2.2 Life Cycle*

The adult trematode of *P. westermani* is thick and oval shaped, 7–20 mm long, 4–8 mm wide, and 2.5–5 mm thick (Sinniah 1997; Cross 2001). The two suckers are equal in size and the tegument is spinose. The ovary is lobate and anterior of the two deeply lobed testes. The uterus lies opposite of the ovary. The cecae are unbranched and extend to the posterior end of the body. The eggs measure 80–120 μm long by 45–65 μm wide, with an obvious, somewhat flattened operculum situated within a rim. Differentiation of adults of the genus *Paragonimus* includes comparison of

overall size and shape, patterns of lobation of the ovary and testes, and the appearance of cuticular spines (Procop 2009). *P. kellicotti* can be differentiated by the groupings of the tegumental spines.

In addition to humans, other definitive hosts of *P. westermani* include dogs, pigs, cats, tigers, leopards, panthers, and other wild cats (Yokogawa 1965). Depending on the species of *Paragonimus*, weasels, badgers, monkeys, rats, and other carnivorous mammals in addition to those previously mentioned may also act as definitive hosts. Generally, the adult trematodes live in the respiratory tract of their hosts, although extrapulmonary infections can occur and are discussed below.

In infections with *Paragonimus*, two worms are usually present in a cyst in the lungs, although the trematodes are hermaphroditic. Cysts measure up to 2 cm in diameter and are lined with simple cuboidal, nonciliated epithelial cells (Procop 2009). The cysts are produced by the host in response to parasite products, including eggs. Cysts caused by *P. kellicotti* in the lungs measure 1–1.5 cm in diameter in animal models (Lumsden and Sogandares-Bernal 1970; Sogandares-Bernal and Seed 1973). Eggs are passed unembryonated from the adults between 28 and 111 days, depending on the species of *Paragonimus*. Rupture of the cyst into the bronchus or bronchiole releases the eggs into the tracheobronchial tree and results in hemoptysis (Procop 2009). The eggs are then either coughed up in thick sputum or are swallowed and passed out in the feces. The eggs are thick shelled, operculate, and generally oval in shape (Fig. 13.1). If the eggs reach water, they will embryonate and hatch in 2–3 weeks. However, the eggs of *P. kellicotti* depend upon appropriate temperatures to fully develop and hatch, sometimes taking up to 6 months for ciliated miracidiae to leave the eggs. The resulting miracidiae swim in search of appropriate snail intermediate hosts, of the families Hydrobiidae, Pleuroceridae, and Thiaridae (Cross 2001). Numerous species of snails act as hosts for *Paragonimus* spp. in China and Southeast Asia, demonstrating little host specificity. In North America however, only the snail *Pomatiopsis lapidaria* is known as the first intermediate host of *P. kellicotti* (Yokogawa 1965; WHO 1995).

Within the molluscan host, the parasite develops asexually as a sporocyst, then through two generations of rediae, prior to forming cercariae. These cercariae have

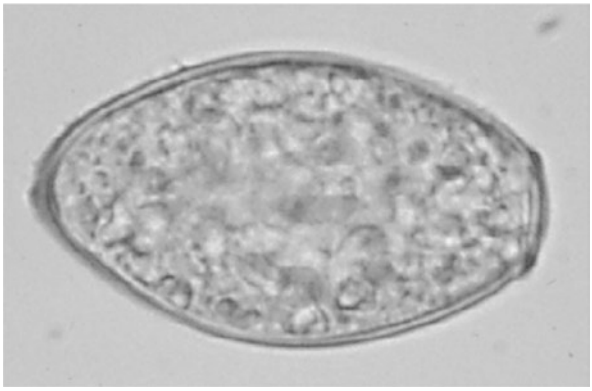

**Fig. 13.1** Egg of *Paragonimus westermani*

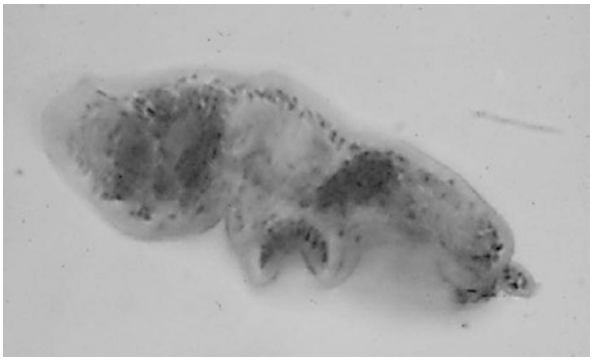

**Fig. 13.2** Cercaria of *Paragonimus westermani* showing small, knob-like tail

small, knobby tails with spines (Fig. 13.2) and are poor swimmers. Therefore, after leaving the snails, the cercariae crawl along the sediment and rocks in search of crustacean second intermediate hosts. Inside these hosts, the trematodes encyst in the viscera, gills, and muscles and form metacercariae. Cercariae of *P. kellicotti* migrate to the heart muscle of the crayfish and develop into encysted metacercariae (Stromberg and Dubey 1978; Fischer and Weil 2015). Over 50 species of freshwater crabs and crayfish have been reported as second intermediate hosts of *Paragonimus* spp., again demonstrating little host specificity (WHO 1995; Keiser and Utzinger 2005). Several species of crayfish of the genera *Orconectes* and *Cambarus* are hosts for metacercariae of *P. kellicotti* in North America (Yokogawa 1965; Diaz 2013). In Latin America, species of freshwater crabs from three genera (*Hypolobocerca*, *Potamocarcinum*, and *Pseudothelphusa*) are recognized as second intermediate hosts of *P. mexicanus* (WHO 1995). In addition to penetration and encystment, experimental evidence indicates that crustaceans may also become infected from consuming infected snails (Noble 1963). A second source of metacercariae can be water contaminated from the discharge of metacercariae from dead or injured crabs (Yoshida 1916). Consumption of the contaminated water or of foods washed in the water can result in infection in the final host. As noted by Yoshida (1916), the liberated metacercariae could survive at least 30 days under optimal conditions.

When the infected crustacean is eaten raw or undercooked, or the contaminated water or food consumed, the trematodes excyst in the intestine and pass through the intestinal wall. The worms penetrate the diaphragm and pleura and encyst in the lungs. The trematodes of *P. westermani* reach maturity and begin to lay eggs in 5–6 weeks after infection, 5–8 weeks for *P. kellicotti* (Stromberg and Dubey 1978). Although infections may persist up to 20 years, most adult worms die in about 6 years (Sinniah 1997). If the host is not suitable for the trematodes to develop into adults, the worms will encyst in the tissues of that host and remain until consumed by an appropriate host (Sinniah 1997). For example, people in Japan became infected after eating raw meat from a wild boar. Immature worms of *Paragonimus* were found in the muscle of the boar (Miyazaki and Hirose 1976).

### *13.2.3 Epidemiology*

Paragonimiasis is more common in children, with peak prevalence in 10–14-year-olds (Kum and Nchinda 1982; WHO 1995). The prevalence also tends to be higher among males in comparison to females, but the difference is not always statistically significant (Kum and Nchinda 1982; Moyou-Somo et al. 2003). The exception to this observation concerns the use of raw crabs by females in Africa to aid fertility, resulting in higher infection rates. However, Kum and Nchinda (1982) conducted a survey which indicated this practice has decreased, with only 4% of the respondents considering this to be effective. In North America, the male to female ratio of infections by *P. kellicotti* from 1984 to 2010 was 15 to 1 (Diaz 2011, 2013).

In general, humans become infected from the ingestion of infected crustaceans that are either raw or undercooked. Some traditional preparations of crustaceans by marinating, pickling, or salting may give the appearance of cooked flesh, but the metacercariae maintain their infectivity. In Korea, crab marinated in soy sauce is a major source of paragonimiasis, as are crabs soaked in wine (drunken crabs) in China (Cross 2001). Throughout Asia, cultures have various dishes consisting of raw crab, shrimp, and crayfish which provide transmission of the trematode. Cultures with a strong tradition of eating raw fish dishes have a corresponding high rate of paragonimiasis, as evidenced by populations of the Joseon Dynasty in South Korea (Seo et al. 2014). Such a correlation is further supported by historical documents containing many recipes for these dishes (Seo et al. 2008).

Generally, residents of North America diagnosed with infections of *P. westermani* are either immigrants from where *Paragonimus* is endemic or traveled to such areas. Nonnative paragonimiasis has occurred in the USA after the consumption of imported raw crabs purchased from specialty food markets (Procop 2009) or consumed in sushi bars. A resident of California ate live crabs imported from Asia at a sushi bar and was diagnosed with chylothorax caused by paragonimiasis 2 years later. The patient denied traveling outside of the USA or of dining on any crustaceans which may have been infected with the native *P. kellicotti* (Wright et al. 2011). Three patients at the Mayo Clinic in Rochester, MN, were diagnosed with pleural and/or pulmonary infections by *P. westermani* after consuming live crabs in sushi bars (Boland et al. 2011). Some of these patients reported eating live crabs which were placed in their martinis. The authors considered this a novel mode of transmission for *P. westermani* in the USA. Regardless, the mode of transmission is correlated with the consumption of alcohol.

Improper cooking may account for many infections in cultures that infrequently consume raw crustaceans. Kum and Nchinda (1982) reported that only 12% surveyed in Cameroon admitted eating raw crabs; however, the local delicacy consisted of a preparation of crabs and plantain baked in hot ashes. The period of baking was not always sufficient to inactivate metacercariae. The authors also noted that children would roast crabs directly in the fire, but not long enough, since the metacercariae present were still viable. In a later study by Moyou-Somo et al. (2003), also in Cameroon, all children examined reported preparing crustaceans by roasting, boiling, or frying (in oil). However, duration of cooking was not determined by internal temperature or con-

sistency of flesh, but rather by a color change of the crab shell. Sachs and Cumberlidge (1990) related that roasting of crabs by children was also common in Liberia, but that the claws and legs of crabs were often removed prior to roasting and children chewed these raw appendages while food preparation was underway. They proposed that this activity was the predominant route of infection for children and adolescents.

The consumption of raw crayfish is not generally practiced in North America, explaining the paucity of human infections by *P. kellicotti.* Crayfish are commonly eaten in the USA, particularly in the creole culinary tradition in Louisiana, but the crayfish are cooked prior to consumption. Additionally, most of the crayfish consumed are raised in ponds and are not intermediate hosts of *P. kellicotti* (Fischer and Weil 2015). Behaviors resulting in infections by *P. kellicotti* usually occurred on canoeing or camping trips, particularly along the Mississippi River and were primarily associated with the consumption of alcohol (Centers for Disease Control and Prevention MMWR 2010; Diaz 2011).

Cross contamination of cooked foods with raw materials or utensils is another route of transmission for paragonimiasis. In Japan, crab soup is prepared by removing the shells and legs and chopping the bodies with a knife on a chopping block. The crab is strained through a bamboo basket and cooked 10–20 min with vegetables or noodles. A study of the preparation of the soup demonstrated metacercariae on the knife, on the cook's hands, on the chopping block and table, and on the bamboo basket (Yokogawa 1965). Metacercariae can survive for weeks outside of the animal host, so contaminated utensils can be a serious source of infection (Cross 2001).

In addition, juices from crustaceans may be used in food preparation or in traditional medicines. In Korea, raw juice from crabs was used to treat fever and diarrhea. Similarly, in Japan, juices from *Eriocheir japonicus* and *Potamon dehani* were used to treat fever or to make an ointment for urticaria (Yokogawa 1965). Medicinal use of juice from crabs occurs also in South America, with the supernatant from ground crabs of the genus *Hypolobocera* used to treat children (WHO 1995).

In addition to the consumption of intermediate hosts, infections may also occur by the ingestion of tissues from paratenic hosts. Paratenic hosts are animals in which the parasite can survive, but cannot develop further into sexually competent adults. Paratenic hosts for *Paragonimus* are generally mammals, but there is some evidence that birds such as ducks and geese may also serve as paratenic hosts. Procop (2009) related a possible infection by *P. kellicotti* through the consumption of raw, locally caught fish in Illinois, although the source of infection was not confirmed. In Japan, wild boars are known to be paratenic hosts of *P. westermani* and have been reported as the source of infection for hunters in Kyushu (Miyazaki and Habe 1976; Miyazaki et al. 1978).

### *13.2.4 Clinical Signs and Diagnosis*

The severity of symptoms is often determined by the location of the trematodes, the degree of infection, and the progression of the worms through the body of the host. Early in the infection, the human host may be asymptomatic; however, when the

worms migrate from the intestine into the abdomen, diarrhea and abdominal pain may be experienced. Migration to the lungs can elicit an allergic response, with fever, chills, chest pain, urticaria, and eosinophilia (Cross 2001). After maturity in the lungs, eggs may appear in the sputum without the presence of symptoms. Incidental lesions may form in the lungs with a granulomatous reaction that eventually results in fibrotic encapsulation of the adult trematodes. The most common clinical signs of infection are persistent cough, especially in the morning, and the production of gelatinous, odorous, rust-colored sputum. Other symptoms include fatigue, myalgia, fever, and dyspnea (Yokogawa 1965, 1969; WHO 1995). Symptoms of paragonimiasis may be mistaken for bacterial or fungal infections of the lung, autoimmune diseases, and some malignancies (Diaz 2013). Chronic infections can result in hemoptysis, pleural effusion, persistent rales, clubbed fingers, and pneumothorax (Cross 2001). Patients with only chronic cough may be misdiagnosed with bronchitis, bronchial asthma, or bronchiectasis. Similarly, the dark sputum, heavy cough, rales, and hemoptysis of pulmonary paragonimiasis can cause confusion with tuberculosis. Differentiating paragonimiasis from pulmonary tuberculosis can be accomplished with tuberculin skin testing or gamma interferon release assay as well as by specific microscopic and serological tests (Diaz 2013). Chest radiography and CT scans of these infections may add to the possibility of misdiagnosis by mimicking the pleural and parenchymal lesions and solitary nodular lesions of tuberculosis or other diseases (Mukae et al. 2001). Kum and Nchinda (1982) recommended that chest X-rays be used only to evaluate the extent of damage to the lungs from the infections rather than for diagnosis. The literature is replete with reports of patients with paragonimiasis first being treated for tuberculosis (Yokogawa 1965; Pezzella et al. 1981; Weina and England 1990). In Ecuador, 13% of the patients being treated for tuberculosis were actually infected with *Paragonimus* (WHO 1995). Since treatment for the parasite was estimated to be 100 times less costly, the misdiagnoses had an economic impact as well as medical. In the USA, Lane et al. (2012) reported that the median time from the onset of symptoms for nine patients infected with *P. kellicotti*, until the correct diagnosis, was 12 weeks (range 3–83 weeks). Prior to the diagnosis of paragonimiasis, these patients received multiple medications and treatments which were unnecessary and often associated with serious illness.

The lungs are the primary site for infections by *Paragonimus*, but the worms can wander and encyst throughout the body of the mammalian host. Reported extrapulmonary locations for encystment include the brain, spinal cord, liver, eyes, reproductive organs, subcutaneous tissues, diaphragm, pancreas, pericardium, and lymph nodes (Yokogawa 1965, 1969; Cross 2001). The spinal cord and brain are common sites for encystment outside of the lungs. Spinal involvement can result in paraplegia, monoplegia, limb weakness, and sensory deficiencies. Symptoms of cerebral paragonimiasis, usually manifested about 10 months after the appearance of pulmonary signs, are headache, fever, vomiting, seizures, and visual disturbances. Approximately one quarter of patients hospitalized for paragonimiasis have involvement of the central nervous system and may experience *Paragonimus*-induced meningitis. Fürst et al. (2012) estimate that one out of ten cases of cerebral paragonimiasis results in death. In contrast,

in 15 cases of autochthonous human infections diagnosed in the USA between 1984 and 2010, only one patient developed cerebral paragonimiasis (Madriaga et al. 2007; Lane et al. 2009; Diaz 2011, 2013). Cerebral hemorrhage may occur, especially in children under 15 years of age (Sinniah 1997). Oh and Jordan (1967) evaluated the intellectual capabilities of patients with cerebral paragonimiasis in Korea and reported that 90% of afflicted children under 15 years became mentally retarded. Children also frequently experience involvement of the liver. A study in China found 51% of infected children presenting with hepatic involvement (WHO 1995).

Although *P. westermani* may occasionally demonstrate extrapulmonary infections in the abdomen or in subcutaneous tissues, these manifestations are usually caused by *P. skrjabini*, *P. heterotremus*, and *P. mexicanus* because humans are not the most suitable hosts for these parasites (Rim et al. 1994). As a result, migratory subcutaneous lesions may form in the chest, abdominal wall, and extremities.

Microscopic examination of the sputum or stool for the characteristic eggs of *Paragonimus* is the usual approach for the clinical diagnosis of the infection. However, eggs may be intermittently discharged by the patient, decreasing the sensitivity of the procedure (Kong et al. 1998). In light infections, sputum might not be produced, or the patient may habitually swallow it, thereby eliminating the analysis of the sputum as a means of diagnosis. Pezzella et al. (1981) reported the treatment of 11 patients (15–39 years of age) in Korea diagnosed with pulmonary paragonimiasis, of which only two had eggs in their sputum. In an earlier study, eggs were detected in sputum 72% of the time and in stools 63% (Yokogawa 1965). In those cases (13%) of which eggs were found in the stool specimens but not in the sputa, the patients were primarily children or the elderly. In a study in Missouri, USA, involving nine patients infected with *P. kellicotti*, only one patient was positive for eggs in their sputa (Lane et al. 2012).

For those infections in which eggs are not present in the sputa but detected in fecal exams, eggs of *Paragonimus* spp. need to be differentiated from other trematode species given in Table 13.1. Several species of *Paragonimus*, in particular *P. westermani*, occur in similar geographic areas as *Clonorchis* and *Opisthorchis*. The eggs of these three genera all have opercula, but the eggs of the latter two genera are smaller, and their opercula have prominent shoulders (Fig. 13.3). Similarly, the eggs of both *Paragonimus* and *Fasciola* have opercula, but the eggs of *Fasciola* are substantially larger than the former taxon (Jiménez et al. 2012).

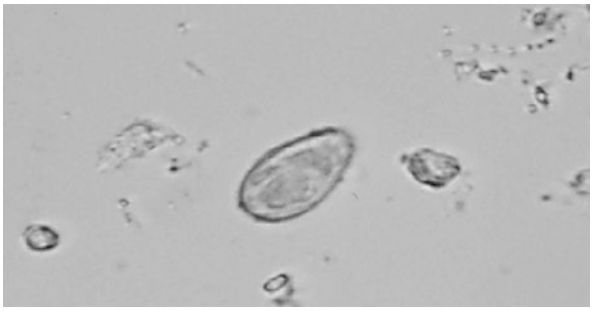

**Fig. 13.3** Egg of *Clonorchis sinensis*, with distinctive shoulders on egg for the operculum

Serological testing, in addition to the microscopical procedures, is of value for confirmation of other tests and for diagnosis in chronic infections of which eggs are difficult to isolate. The interdermal test using the veronal buffered saline (VBS) extract of adults of *P. westermani* is recommended as a screening method (Yokogawa 1965; Cross 2001). The test may be used in surveys or to differentiate paragonimiasis from tuberculosis, tumors, or other nonparasitic conditions. This method will provide positive results for up to 20 years after complete recovery; therefore, follow-up testing with complement fixation tests, ELISA, or immunoblot is recommended (Cross 2001). Earlier work with these serodiagnostic techniques indicated cross-reactivity with antigens from other trematodes, including *Clonorchis*, *Schistosoma*, and *Fasciola* (Yokogawa 1965; Hillyer and Serrano 1983). Advances in these procedures have increased the sensitivity and specificity, such that the cross-reactivity inherent in the earlier procedures have been decreased or eliminated. For example, ELISA methods have been developed using cysteine protease antigens which have high specificity and can differentiate between paragonimiasis and fascioliasis (Ikeda et al. 1996; Ikeda 1998). Similarly, immunoblot for paragonimiasis has been developed and refined such that sensitivity is estimated at 96% and specificity at 99% (Slemenda et al. 1988). In a study of 40 separate cases of paragonimiasis caused by three species of *Paragonimus*, sera from patients with infections with other trematodes or cestodes, with lung cancer, or from healthy subjects failed to react in the immunoblot (Kong et al. 1998).

Molecular testing to detect and differentiate among species of *Paragonimus* has developed substantially since the beginning of this century. Sugiyama et al. (2002) used polymerase chain reaction (PCR) to differentiate between the metacercariae of *P. westermani* and *P. miyazakii*. Using animal models, a PCR method was developed to detect infections by *P. heterotremus* from stool samples (Intapan et al. 2005). The method could detect as few as five eggs in 0.6 g of feces, although the authors also reported cross-reactivity with other species of *Paragonimus*. Using different primers, Yahiro et al. (2008) used a PCR method to further characterize and confirm the strain of *P. heterotremus* in human infections in the Lao People's Democratic Republic as an internal transcribed spacer 2 (ITS2). The authors analyzed ova from the sputa of six confirmed cases of paragonimiasis, with PCR amplifying the ITS2 and its flanking regions. The PCR product was then compared for homology with other sequences in GenBank at the National Center for Biotechnology Information (www.ncbi.nih.gov/blast/Blast.cgi) to identify the species. The sequences were in accord with eight sequences in GenBank attributed to *P. heterotremus.* Other diagnostic technologies to rapidly detect paragonimiasis are the loop-mediated isothermal amplification (LAMP) assays and multiplex protein microarray assays (Diaz 2013). The former was developed to detect and identify the DNA of *P. westermani* from specimens from humans and infected crustaceans. The method detected the adults, metacercariae, and eggs in a rapid and specific manner. The multiplex protein microarray assay was found to be more sensitive and faster than ELISA for five separate species of *Paragonimus*, including *P. westermani*.

### *13.2.5 Treatment*

Several regimens are available for treatment of paragonimiasis. The drug of choice is praziquantel administered at 25 mg/kg, three times a day for 1–2 days (Medical Letter Inc. 1984). Dosage is the same for children as for adults. Praziquantel is also effective treatment for dogs and cats infected with *P. kellicotti* at 23 mg/kg every 8 h for 3 days (Bowman et al. 1991). Bithionol is also efficacious for all forms of paragonimiasis (Yokogawa 1969). The drug is given at a dose of 30–50 mg/kg every other day for 10–15 days (Burton et al. 1982; Sinniah 1997). Niclofolan is also effective in a single oral dose of 2 mg/kg of body weight (Kum and Nchinda 1982). Triclabendazole given orally at 10 mg/kg twice a day for 1–2 days has successfully treated human paragonimiasis in adults and children (Diaz 2013). Triclabendazole is not commercially available since it has not been approved by the US Food and Drug Administration; however, the drug is available from the Centers for Disease Control and Prevention under an investigational protocol (Centers for Disease Control and Prevention 2013). Both albendazole and fenbendazole have been used to treat experimentally infected cats and dogs with *P. kellicotti*, respectively (Dubey et al. 1978, 1979), but aren't usually included in possible drug regimens. When treating cerebral paragonimiasis, corticosteroids should be given to ameliorate the localized immune response to the dead parasites (Cross 2001).

Surgical treatment may be necessary for severe pleural paragonimiasis, not to specifically remove the worms, but rather to alleviate some of the damage incurred during the infection (Pezzella et al. 1981). In these cases, patients were treated pharmacologically 10–14 days before surgery. Surgical treatment of cerebral paragonimiasis could eliminate seizures with the removal of encapsulated abscesses, often including a trematode (Yokogawa 1965). Only 30% of those patients operated upon were considered improved or cured after 2 years, and 21% still experienced seizures after surgery.

## 13.3 *Fasciola* spp.

### *13.3.1 Introduction*

*Fasciola hepatica* and *F. gigantica* are both known to infect humans, although they are primarily parasites of ruminants. The former species has a worldwide distribution, but paleoparasitological studies have shown that *F. hepatica* was common in prehistoric humans in Europe as well as during the Bronze Age, the Gallo-Roman period, and the Middle Ages (Mas-Coma et al. 2005). In contrast, no eggs of *Fasciola* have been found in human coprolites in the New World, indicating that the establishment of fascioliasis in the Americas was fairly recent. *F. gigantica* has a more restricted distribution, being found in Asia, Africa, and Hawaii. The latter trematode was probably introduced to Hawaii with the importation of water

buffaloes from Asia (Alicata 1964). Of the two species, *F. hepatica* more commonly infects humans. Stoll (1947) estimated that there were less than 100,000 cases of fascioliasis worldwide. Fifty years later, estimates are given of 2.4 million human infections in over 56 countries, primarily distributed in China, Egypt, Europe (especially France and Portugal), Iran, and South America (especially Bolivia, Ecuador, and Peru) (Rim et al. 1994; WHO 1995; Crompton 1999). Another estimated 91 million people are at risk of infection (Keiser and Utzinger 2005). Although no consistent quantitative correlation has been shown, areas with high prevalences in domestic animals also tend to have high rates of human infections. For example, in the Altiplano region of Bolivia, infection rates of sheep and cattle range from 25% to 95%. In some villages, 65% of the people were found to pass eggs in their stool, and 92% were serologically positive (WHO 1995).

Fascioliasis is a serious disease in cattle and sheep throughout the world with an enormous economic impact. In 1969, Boray (1969) reported that one quarter of the sheep and cattle (40 million and 5 million, respectively) were grazing on pastures in which the infective metacercariae were potentially endemic. In Great Britain, 53% of the farms had livestock with fascioliasis (Froyd 1975), with adult stock affected more than twice that of young animals. In the USA, 17% of cattle slaughtered in Montana were found to be infected (Knapp et al. 1992). In a survey conducted by McKown and Ridley (1995), 33% of 278 veterinarians in Kansas reported diagnosing at least one case of liver fluke disease in their practice. Foreyt and Todd (1976) disclosed that 2.1% of beef livers examined in Kansas were condemned because of damage by the trematodes.

Fascioliasis causes decreased milk production and weight gain, increased numbers of condemned livers at slaughter, and decreased reproductive performance. The parasite also adversely impacts wool production in sheep. In the USA alone, financial losses due to these trematodes were estimated at $30 million in 1973 (McKown and Ridley 1995); globally, production losses were estimated to cost over US$3 billion (Spithill et al. 1999; Piedrafita et al. 2010). Similarly, total losses due to fascioliasis in animals amounted to US$11 million in Peru (WHO 1995). In Australia, livestock production losses were estimated to be Australian $50 to $80 million per annum in 1999, with anthelminthic treatment costing Australian $10 million (Brockwell et al. 2014). The authors state that over 6 million cattle are considered at risk, primarily in south-eastern Australia. Losses in milk production are estimated to range from 4% to 30% in Australia, depending on the intensity of infection (worm burden) and the nutritional status of the dairy cattle (Charlier et al. 2014). Total losses due to fascioliasis in dairy cattle in Switzerland are estimated at €48.8 per animal (Elliott et al. 2015).

### *13.3.2 Life Cycle*

The adult trematodes are large, somewhat leaf-shaped, with a “cone-shaped” projection on the anterior. *F. hepatica* is generally 30 mm long and 13 mm wide (Fig. 13.4); *F. gigantica* may measure 73 mm long. The ventral sucker is larger than

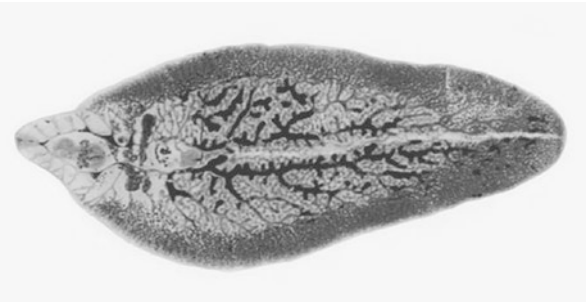

**Fig. 13.4** Adult of *Fasciola hepatica*, showing the distinct shape of the body and the "cone-shaped" projection on the anterior (Photograph by Charles Sterling, University of Arizona)

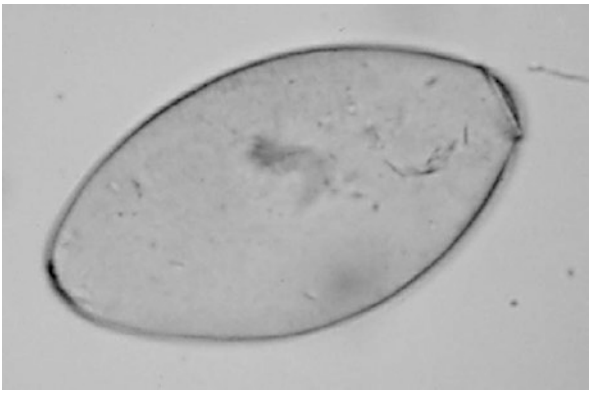

**Fig. 13.5** Egg of *Fasciola hepatica*

the oral sucker and located anteriad, near the base of the "cone." The intestinal ceca are highly branched, as are the two testes. The ovary is smaller, located near the ventral sucker. The operculate eggs of *F. hepatica* measure 130–150 μm by 63–90 μm (Fig. 13.5); eggs of *F. gigantica* are typically larger, 160–190 μm by 70–90 μm (Fig. 13.6). Hybridization between these two species when they coexist in a region can cause confusion when only morphology is used for identification. Hybridization has been confirmed through the use of molecular methods; such methods can also be used to identify the two separate species. Using methods such as PCR-restriction fragment length polymorphism (RFLP) and randomly amplified polymorphic DNA (RAPD)-derived sequences, identification of the two species or their hybrids can be confirmed. Ribosomal DNA from *F. hepatica* and mitochondrial DNA from *F. gigantica* as determined by the molecular methods provide the confirmation of hybridization (Periago et al. 2008; Rokni et al. 2010; Rokni 2014).

The definitive hosts of *Fasciola hepatica* (specifically sheep, cattle, and pigs) have been shown to impact the size of the adult trematodes and eggs (Valero et al. 2001). Eggs of *F. hepatica* may be differentiated from those of *Fasciolopsis buski* by the roughened or irregular area at the abopercular end of the shell of *F. hepatica* (Ash and Orihel 1997). Although humans may be infected, definitive hosts are generally ruminants, including cattle, sheep, goats, and swine. Horses may also be hosts, but infections generally lack clinical symptoms and produce low egg counts

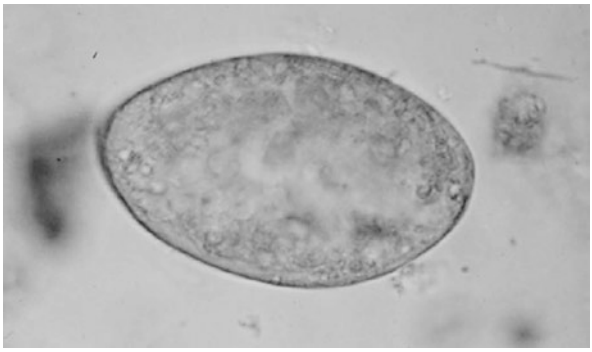

**Fig. 13.6** Egg of *Fasciolopsis buski*

(Alicata 1964, Boray 1969). Rabbits, mice, rats, and guinea pigs are all susceptible to infection. The rabbit has been recognized as a natural reservoir host. *F. hepatica* has demonstrated an ability to adapt to various final hosts during its expansion to most of the continents (Mas-Coma et al. 2005). Examples include the pig in Andean countries, the nutria and the black rat (on Corsica Island) in France, and seven wild animal species (elk, red deer, roe deer, wild boar, beaver, otter, and hare) in Belorussia.

*Fasciola* adults reside in the bile ducts of the liver. Operculated eggs are passed out in the feces of the host and must reach freshwater for continued development. After hatching, a miracidium escapes the egg and searches for a snail host. Suitable snails belong to several genera including *Lymnaea*, *Fossaria*, *Pseudosuccinea*, and *Austropeplea* (WHO 1995). The miracidium penetrates the snail and develops into a sporocyst. Two generations of rediae ensue. Cercariae develop in the daughter rediae and later emerge from the snail and become free swimming. Unlike other trematodes, these cercariae do not infect a second intermediate host. When they encounter vegetation or submerged bark, they shed their tails and encyst on the plants and become metacercariae. If they do not find an object to encyst on, the cercariae will drop their tails and encyst free in the water. When the contaminated vegetation or water is consumed, the trematodes excyst in the small intestine and penetrate the intestinal wall. They travel around the viscera to the liver, where they burrow in and continue to move and feed for a couple of months before entering the bile ducts. Approximately 4 months (range 3–18 months) after ingestion, the worm matures and begins passing eggs (WHO 1995).

### *13.3.3 Epidemiology*

Within populations in endemic areas, adults are more commonly infected than children. Similarly, women have a higher prevalence of fascioliasis than men (Binkley and Sinniah 1997). Women more often pick and gather vegetation and may consume more salads and raw vegetation than men.

In general, people become infected from eating raw vegetation contaminated with *Fasciola* metacercariae. Watercress (e.g., *Nasturtium officinale*) is the most common source of infections for people, although mint, lettuce, parsley, and wild watercress may also be contaminated with metacercariae (Rim et al. 1994; WHO 1995). During a study by Marcos et al. (2006) in the northern Peruvian Altiplano, the principal factor for infection by *F. hepatica* among children was drinking alfalfa juice. In Africa, people may become infected from chewing the leaves of the stimulant *khat* (Chand et al. 2009). Food habits often play a role in the transmission of parasites. For example, southern Europeans frequently consume watercress. When they migrated to Algeria, an outbreak of fascioliasis occurred among the Europeans, even though no infections of native Algerians were reported. Unlike the Europeans, the Algerians did not eat watercress (WHO 1995).

Agricultural practices contribute to the contamination of vegetation by the metacercariae. The use of animal excreta as fertilizer helps distribute the eggs in the environment and increases the chances that the parasites may encounter freshwater and snails. The use of effluent from slaughterhouses or livestock pens as fertilizer for watercress plots increases the concentration and distribution of metacercariae on the plants. Emphasis on "organic" or natural healthy foods has increased the collection and consumption of wild watercress and other vegetation, introducing the infection to higher economic classes in some countries.

Agricultural practices may help maintain or increase fascioliasis among livestock. For example, many farmers allow their livestock to wander freely to graze and to drink from streams and ponds. This provides the parasite eggs ready access to freshwater in order to develop and hatch and gives the livestock direct access to vegetation that may be contaminated with metacercariae. Many farmers also cut forage from wet or swampy areas to feed cattle, in essence feeding the infecting metacercariae to their livestock (Alicata 1964).

Lastly, climate and altitude affect the transmission of fascioliasis. The annual patterns and incidence of rainfall and temperature impact the distribution and prevalence of the snail intermediate host as well as the rate of development of the intermediate life cycle stages of the trematode. In temperate areas, *F. hepatica* follows a defined seasonal pattern of transmission and development. Development of the eggs begins at 10 °C, but the process is slow, taking up to 6 months to fully develop (Rapsch et al. 2008). The rate of development increases as temperatures increase, 2–3 months at 16 °C, 2–3 weeks at around 25 °C, and only 8–10 days at 30 °C. Temperatures above 30 °C are lethal. As the eggs more rapidly mature, the life cycle of the trematode is driven forward, and the risk of infection markedly increases. Temperature also affects the viability of snails which survive and reproduce between 10 and 25 °C. Optimal temperatures for the snail intermediate host are between 22 and 25 °C. Maximum survival time for metacercariae of *F. hepatica* is 6 months at 13 °C, with decreasing survivability as temperatures decrease toward zero or as temperatures increase to or past 25 °C (Rapsch et al. 2008). Because of the impact of temperature on the eggs, metacercariae, and the intermediate hosts, infection rates increase through the summer months and drop off precipitously during the winter in temperate regions. The development of the adult trematodes follows infection by at

least 3 months, so eggs are passed by the final hosts optimally beginning in the spring. Similar seasonality is demonstrated in the USA. Hoover et al. (1984) reported transmission of *F. hepatica* to cattle occurred from June through November, with the peak rates of transmission in October and November in southern Idaho. A somewhat similar seasonal transmission occurred at a cashmere goat ranch in southwest Montana, but transmission of the trematode to the goats occurred only in late autumn – September to November (Rognlie et al. 1996). Transmission of the trematode to sheep in a much warmer climate – Florida – occurred much earlier than in the northern states of Montana and Idaho (Boyce and Courtney 1990). Transmission was almost exclusive to the early part of the year, December to June, with infections of the sheep peaking during February to April. Because of the heat, no transmission occurred during the summer months of May and June.

Transmission and development characteristics of *F. hepatica* are strikingly different at high altitudes (Valero et al. 2012). Snail hosts shed cercariae during all seasons and produce a greater number of cercariae. The snails also survive longer than their lower-altitude cousins. In contrast, adult trematodes at the higher altitudes produce eggs earlier but produce fewer eggs. This may be the result of lower oxygen levels at the higher altitudes.

### *13.3.4 Clinical Signs and Diagnosis*

The onset of symptoms usually occurs 4–6 weeks after ingestion of the metacercariae but can vary depending on the number of parasites ingested and the host immune response (WHO 1995; Dauchy et al. 2006). Light infections may be asymptomatic. Patients often complain of fever, sweating, abdominal pain, dizziness, cough, bronchial asthma, fatigue, general malaise, loss of weight, and loss of appetite (WHO 1995; Binkley and Sinniah 1997). Patients may have gastrointestinal complaints including nausea and vomiting. Allergic reactions such as urticaria and pruritus may also occur. The liver may be tender and enlarged, with jaundice. During the acute infection when the trematodes are migrating toward the bile ducts, children may have severe clinical manifestations such as right upper quadrant or general abdominal pain, fever, and anemia (WHO 1995). These infections in children can be fatal.

The chronic phase of the infection begins when the trematodes reach the bile ducts. Often, this stage of the infection is asymptomatic. After arrival in the bile ducts, the trematodes mature into adults and may cause irritation and inflammation of the ducts. The resulting symptoms may include dyspepsia, diarrhea, jaundice, biliary colic, cholecystitis, and cholelithiasis. If juvenile worms do not migrate properly into the liver, they may cause ectopic fascioliasis in other organs, including the intestines, lungs, heart, brain, and skin (the most common extrahepatic site). Nodules may form in the skin, such as around the abdomen (Binkley and Sinniah 1997) or on the neck (Marcos et al. 2009), sometimes reaching 5–6 cm across.

In an endemic region, fascioliasis is suggested if a patient presents with fever, hepatomegaly, and eosinophilia (Binkley and Sinniah 1997). Patients have been

misdiagnosed as having visceral larval migrans or toxoplasmosis when the fever is intermittent (WHO 1995). Diagnosis is made either by observation of eggs in a fecal sample or from sero-immunological tests. However, diagnosis dependent on the former may be problematic for several reasons. Passage of eggs may be inconsistent, resulting in negative exams. In a study involving patients with a history of consuming watercress, 79% tested positive by serology, but only 4% had positive stool specimens during their hospital stay (Chen and Mott 1990). Symptoms of infection during the acute phase arise within 4–6 weeks (see above), but worms do not mature and pass eggs until approximately 4 months after ingestion of metacercariae. Therefore, the clinical findings cannot be supported by parasitological exams until months later, and the infection has entered into the chronic phase. Patients with fascioliasis may be subjected to exploratory surgery to establish the cause of their disease because of the difficulties in diagnosis (WHO 1995). In contrast, people who consume infected livers of cattle, sheep, or water buffalo may be incorrectly diagnosed as having fascioliasis when eggs are passed in their stools (Ash and Orihel 1997). To prevent the diagnosis of spurious fascioliasis, the patient should be placed on a liver-free diet for at least 3 days and then retested. If eggs are still observed in the feces, the diagnosis of fascioliasis can be supported (Binkley and Sinniah 1997).

Given the lag between the onset of symptoms and the production of parasite eggs, the use of serologic/immunodiagnostic methods is invaluable, particularly during the acute phase. Immunodetection of coproantigens of *Fasciola* rather than the observation of eggs in stool specimens is a viable alternative for diagnosis. Youssef et al. (1991) used partially purified *F. gigantica* worm antigens in counter-immunoelectrophoresis (as known as counterelectrophoresis) to test stool extracts. They found that the test was capable of detecting both early and chronic infections and that false positives from spurious fascioliasis or cross-reactions with other parasitic infections were avoided. In a study involving eight cases of fascioliasis, Dauchy et al. (2006) reported that counterelectrophoresis appeared to be the most useful diagnostic test in comparison to complement fixation or ELISA, because this method detected infections usually a month earlier than the latter two methods. Espino et al. (1998) developed a sandwich enzyme-linked immunosorbent assay (ELISA) to detect coproantigens of *F. hepatica*, which was especially useful if patients with prepatent infections (i.e., not excreting eggs) did not have circulating antigens in their sera. If circulating antigens were present, various forms of ELISA are available for diagnosis, including the sandwich ELISA (Espino et al. 1990), the Falcon assay screening test (FAST-) ELISA (Hillyer et al. 1992), and Micro-ELISA (Carnevale et al. 2001). Those that use excretory-secretory antigens appear to be more sensitive and specific. In particular, those tests that use worm cysteine proteinases such as cathepsin L1 (O'Neill et al. 1998) or the Fas1 and Fas2 antigens isolated by Cordova et al. (1997) have excellent sensitivity and specificity. In addition, these antigens are excreted by the worms at all stages of development. If the trematodes themselves (or portions thereof) have been recovered, differentiation between *F. hepatica* and *F. gigantica* is possible with isoelectric focusing of soluble proteins from the adult worms (Lee and Zimmerman 1993).

Diagnosis in animals usually involves fecal samples, tested either microscopically, immunologically, or more recently, by PCR. Issues with fecal egg counts as a means of diagnosis in animals have similar shortcomings as with human patients, requiring approximately 8 weeks postinfection before eggs are produced and passed in the feces (Martinez-Perez et al. 2012). The authors used a sandwich ELISA to detect fascioliasis in 57% of experimentally infected sheep at 4 weeks postinfection and 100% by 8 weeks. PCR could detect infections earlier – by 3 weeks postinfection – and nested PCR could detect infections by the second week. A study by Arifin et al. (2016) involving naturally infected sheep and cattle contrasted somewhat from that of Martinez-Perez et al. (2012). Both fecal and sera samples were taken. In addition to fecal egg counts and ELISA, Arifin et al. (2016) performed PCR and loop-mediated isothermal amplification (LAMP) on fecal samples. Both molecular methods showed high specificity for DNA of *F. hepatica*, although their PCR method had tenfold greater detection limit than the LAMP method. However, the egg counts and ELISA test both had greater sensitivity than the two molecular methods. The authors attribute the low sensitivity to several factors, including the species of host, the amount of fecal sample, the DNA extraction process (or commercial kit), and the presence of PCR inhibitors in the samples.

Recently, analysis of milk from tanks has been used to monitor dairy herds for fascioliasis and to estimate the negative impact of the trematode infections on productivity among the cows. Duscher et al. (2011) compared results of analyses of individual fecal and sera samples from dairy cows to that of tank milk. Two commercially available ELISA tests were used for the sera and milk samples; the fecal samples were examined microscopically and by a coproantigen ELISA. Both the milk and sera ELISAs were considerably more sensitive than the fecal methods, detecting 60% and 90% of the infected herds, respectively. The authors calculated that a minimum of 20% of the individual cows within a herd must be infected with *F. hepatica* to obtain a positive result from testing tank milk. A correlation between the positive results of both tank milk and individual cows, and milk production was demonstrated by Mezo et al. (2011). The greater the degree of the ELISA result, the less milk per cow was produced per day. For example, an ELISA result of >0.405 (a highly positive herd) in their study corresponded to a decrease of 1.5 kg of milk per cow per day as compared to the production of negative herds. These two studies indicate that testing tank milk from dairy herds is a viable tool to monitor the presence of *F. hepatica* and the impact by the trematode on milk production. Howell et al. (2015) confirmed the relationship between exposure to *F. hepatica* and milk production using samples from tank milk. The study did not find significant associations between fascioliasis and bovine fertility and the butterfat and protein content of the milk or with the presence of other diseases.

**Table 13.2** Pharmacological treatments for *Fasciola hepatica* and *F. gigantica*

| | |
|---|---|
| Bithional | 30–50 mg/kg on alternate days × 10–15 doses[a] |
| Praziquantel | 25 mg/kg × 3 daily for 1 day[a]<br>Or<br>25 mg/kg × 3 daily for 3–7 days[b] |
| Triclabendazole[c] | 10 mg/kg, single dose after overnight fast[d]<br>Or<br>5 mg/kg × 2 postprandially on same day with 6–8 h interval[e]<br>(total dose 10 mg/kg) |

Dosages are the same for adults and children
[a]Medical Letter Inc. (1984)
[b]Binkley and Sinniah (1997)
[c]Not approved for use in the USA (by the US Food and Drug Administration) or in Canada. Available from CDC under an investigational protocol
[d]Apt et al. (1995)
[e]WHO (1995)

### *13.3.5 Treatment*

Praziquantel is the drug of choice for most trematode infections. Some consider the drug to also be effective against fascioliasis; however, the WHO (1995) and others (Wessely et al. 1988; Lui and Weller 1996; Binkley and Sinniah 1997) recommend the use of other pharmaceuticals (Table 13.2). Bithionol is effective in humans and available commercially within the USA, but this drug may be replaced by triclabendazole if the latter is submitted to the US Food and Drug Administration for review and approval. Triclabendazole is a benzimidazole anthelminthic and is effective against the adults in the bile ducts and the immature worms which migrate through the hepatic parenchyma (Wessely et al. 1988; WHO 1995). Usually, a single oral dose is effective, but a second dose may be administered for those infections which persist. Apt et al. (1995) treated 24 patients, with 19 testing negative after 2 months. Three of the remaining five were treated again with positive resolution. The drug was well tolerated and effective. The drug was developed in the 1980s and was found to also be effective in the treatment of cattle, reducing the worm burden and improving weight gain (Fuhui et al. 1989). The drug was considered to be safer and more efficacious than other drugs against fascioliasis, but has not been approved by the US Food and Drug Administration for use in the USA. Unfortunately, through the continued use of triclabendazole over the years in the same population of sheep or cattle and the practice of routine dosing as a control measure, many reports of triclabendazole-resistant infections of *Fasciola* have been reported (Mooney et al. 2009; Kelley et al. 2016). Cases of human infections resistant to triclabendazole have also been reported, first in the Netherlands (Winkelhagen et al. 2012) and later in Chile, Turkey, and Peru (Kelley et al. 2016).

Table 13.3 lists multiple drugs for the treatment of fascioliasis in cattle and sheep. *Fasciola* demonstrates the greatest incidence of resistance among infected animals to treatment by triclabendazole than the other drugs listed. The loss of efficacy of triclabendazole is of concern since the other drugs available are mostly

**Table 13.3** Pharmacological treatments for *Fasciola hepatica* and *F. gigantica* in cattle and sheep

| Drug | Maturity of trematode affected | Reported resistance |
|---|---|---|
| Triclabendazole[a,b] | From early immature to adult | Yes |
| Albendazole[a] | Adult | Yes |
| Clorsulon[a,c,d] | Adult | Yes |
| Albendazole + clorsulon[e] | Adult | No |
| Closantel[a,f] | From late immature to adult | Yes |
| Nitroxynil[a] | Adult | Yes |
| Nitroxynil + clorsulon[a] | From early immature to adult | No |
| Oxyclozanide[a,c,d] | Adult | No |
| Artesunate[g] | Adult | No[h] |
| Artemether[g] | Adult | No[h] |

[a]Kelley et al. (2016)
[b]Not approved for use in the USA (by the US Food and Drug Administration) or in Canada. Available from CDC under an investigational protocol
[c]Elliott et al. (2015)
[d]Elliott et al. (2015)
[e]Martinez-Valladares et al. (2014)
[f]Novobilsky and Höglund (2015)
[g]Keiser et al. (2010) and O'Neill et al. (2015a, b)
[h]Development and testing of drug still underway; for experimental use only at this time

effective against the late immature and/or adult trematodes (Mooney et al. 2009). Both triclabendazole and albendazole are benzimidazoles used as anthelminthics. Albendazole is the drug of choice in Spain for fascioliasis in sheep. Unlike triclabendazole, albendazole is only effective against the adults of *Fasciola* (Martinez-Valladares et al. 2014; Kelley et al. 2016). Interestingly though, these two benzimidazoles are often effective in the treatment of fascioliasis when the other has encountered resistance (Sanabria et al. 2013; Novobilsky et al. 2016). Elliott et al. (2015) recommends the use of clorsulon or oxyclozanide to treat fascioliasis resistant to triclabendazole. They also reported a survey in which 58% of dairy farmers in Australia often combined clorsulon with ivermectin because of clorsulon's nil milk withholding period, allowing milk production to continue during anthelminthic treatment.

Nitroxynil and closantel have few reports of resistance, primarily in Australia (Fairweather and Boray 1999; Hanna et al. 2015). However, similar to the other alternatives to triclabendazole, these drugs are not effective against the more immature trematodes and, therefore, less effective during acute infections. To utilize the targeting of acute and chronic infections, Hanna et al. (2015) proposed the use of triclabendazole in autumn, to treat any portion of the trematode population still susceptible to the drug and to save some animals with acute infections. The authors then recommend the use of nitroxynil or closantel in the winter and spring months to treat the more mature trematodes and chronic infections in the flocks. The latter treatment would also reduce potential contamination of pastures by the trematode.

Artemisinins were originally developed for use against malarial parasites, but the derivatives have demonstrated a broad therapeutic spectrum against other trem-

atodes (e.g., schistosomes, *Clonorchis sinensis*, *Opisthorchis viverrini*), protozoan parasites, and some forms of cancer (O'Neill et al. 2015a). Currently, experimental work is investigating the efficacy and safety of two derivatives, artemether and artesunate, against infections of *Fasciola hepatica.* Artemether is usually prepared with an oil formulation and presents a low bioavailability following intramuscular administration. The formulation probably results in a slow absorption over a longer period of time (Keiser et al. 2010). In contrast, artesunate is characterized by a higher water solubility than artemether and undergoes better absorption when administered intramuscularly. Both artemether and artesunate can be administered orally after dissolution in a mixture of 7% detergent (Tween 80) and 3% ethanol (O'Neill et al. 2015b). Depending on the animal model and strain of trematode used, as well as method of administration and dosage, the efficacy of the two drugs can vary. Artesunate currently appears to be the more effective of the two but displays a concerning degree of toxicity in both sheep and rats (Keiser et al. 2010; O'Neill et al. 2015a). Further study of artesunate to address appropriate dosage and toxicity must occur, but initial studies point to an association between the rapid conversion of artesunate to its main metabolite, dihydroartemisinin, and the damage caused by the trematode to the liver (O'Neill et al. 2015a). The metabolite accumulates rapidly, and the elimination of the metabolite is delayed by the host's compromised liver. Drug activity against the trematodes results in damage to the female reproductive system and egg production (O'Neill et al. 2015b). Artesunate does not appear to seriously impact the tegument of *Fasciola hepatica*, but inspection of the gut of the trematode, including the gastrodermal cells, displayed severe damage (O'Neill et al. 2015a). The authors hypothesize that the artesunate and other artemisinins are taken up orally by the trematode rather than absorbed through the tegument.

### *13.3.6 Vaccine Development*

Similar to other parasitic diseases such as malaria and schistosomiasis, efforts have been underway to develop an effective vaccine against fascioliasis. This is of particular concern due to the increasing resistance of the trematode to drug treatment as addressed above. Also, many consumers are concerned as to drug residues in their food as well as the impact on the environment. Since vaccines stimulate the immune response of the host, no chemical residues impact the food derived from the animal or on the environment.

Early attempts at the development of a vaccine involved the administration of high doses of radiation-attenuated metacercariae to cattle (Molina-Hernandez et al. 2015). The metacercariae were capable of excystation and penetration into the liver, but did not develop into adults. This attempt at conferring immunity to the cattle was unsuccessful; the migration of the larvae from the radiation-attenuated metacercariae did not induce a protective immunological response and did not confer any resistance to challenge infections. Many of the early efforts also used antigens

isolated from adult trematode excretory-secretory products such as cathepsin L proteases or glutathione S transferase (GST). These antigen preparations did result in reducing the number of worms (worm burden) in a host, egg output by the adults, and liver pathology (Molina-Hernandez et al. 2015). Unfortunately, studies of these antigen preparations demonstrated inconsistency of results with some demonstrating 50–89% reduction in worm burden and egg production depending on whether sheep or cattle were inoculated and depending on whether and what kind of adjuvant was also used with the antigens (Morrison et al. 1996; Molina-Hernandez et al. 2015). An approach using a recombinant form of thioredoxin glutathione reductase (TGR), another antigen present in excretory-secretory products, failed to induce any significant immunological protection in cattle (Maggioli et al. 2016). The result was surprising to the authors since earlier tests using the rabbit model were successful. The authors caution that their results question the usefulness of small mammal models in the development of a vaccine for fascioliasis.

Future efforts in the development of a vaccine for fascioliasis may utilize genome sequencing technologies (Molina-Hernandez et al. 2015). Such technologies may help target different stages of development by the parasite or target different structures of the parasite such as the tegument or the female reproductive organs. Gene sequencing from multiple populations of host and parasite will help address the high level of genetic diversity within *F. hepatica*, as well as aid in the selection of immunological targets which are conserved among the populations of the trematode.

## 13.4 Prevention and Control

The prevention and control of infections caused by foodborne trematodes can be achieved primarily by the disruption of the life cycles of the parasites. Since the cycles are complex, involving multiple hosts and developmental stages, disruption of development and transmission can occur at multiple sites within the cycles.

The elimination of adults and any resulting eggs can be pursued through treatment of populations at risk. A control program may be implemented in an endemic area which relies heavily on drug treatment. WHO (1995) recommends that a program for community-based treatment provides annual drug therapy for the parasite for up to 3 years. The broad efficacy of praziquantel, the drug of choice for many parasitic infections, allows the targeting of multiple species of parasites, even when the specific identity of a helminth cannot be determined. The general safety of the drug and the accepted use for pediatric patients as well as for adults underscores the value of this treatment for helminthiases.

Generally, patients do not become refractive to reinfection by foodborne trematodes. Therefore, education of the affected populace and of physicians who may treat infected patients is an important component of control of paragonimiasis or fascioliasis. Education of at-risk populations should include information about the parasite(s) as well as the means to avoid infections or to receive treatment. Often avoidance requires changes in food preparation or dietary preferences. After a cou-

ple of reports of infections by *P. kellicotti* in campers and canoeists in Missouri, the Missouri Department of Health and Senior Services (MDHSS) distributed posters to camping and vacation areas describing and warning people of paragonimiasis from the consumption of raw or undercooked crayfish (Centers for Disease Control and Prevention MMWR 2010). The Washington University School of Medicine located in St. Louis, MO, also issued a press release soon after the efforts by MDHSS to further publicize and educate at-risk persons.

Changes in food preparation and food habits are often required to keep patients from acquiring new infections. In addition to drug therapy, any impacted community should receive education as to the life cycle of the parasite and dietary changes that could prevent transmission. Mothers in particular should receive training on the importance of children consuming only fully cooked or adequately processed foods. Fürst et al. (2012) report that in poor regions in which *Paragonimus* is endemic, children often catch and eat raw crustaceans while playing or helping their parents. However, changes in traditional preparation and consumption of foods are difficult, and reinfection is not uncommon.

Infections by foodborne trematodes can be prevented if the intermediate hosts are not consumed raw, undercooked, or improperly (or incompletely) pickled, salted, dried, or smoked. Fully cooking crustaceans or plants would neutralize any metacercariae present of *Paragonimus* or *Fasciola*, respectively. This process is time-temperature dependent, e.g., cooking time decreases with an increase in temperature. Cooking or boiling crustaceans to reach an internal temperature of 145 °F (63 °C) is sufficient to kill metacercariae of *Paragonimus* (Diaz 2013).

Freezing is also very effective at neutralizing the metacercariae. The texture of the flesh may change after freezing, and therefore, many people are reluctant to eat raw previously frozen fish. Freezing is also time-temperature dependent, with colder temperatures requiring less time. The type of freezer, the thickness of the fish, and the form of the product (e.g., whole, headed and gutted, or fillets) can affect the time needed to attain the required temperature and thereby increase the time in the freezer.

Food preparation requiring pickling, salting, drying, or smoking may be insufficient to kill the metacercariae. During pickling, the acidity of the solution and the thickness of the fish may be factors in the continued viability of the larvae. Generally, the higher the acidity, the more effective the control measure will be. However, pickling solutions are usually about 4% acid, similar to vinegar. Metacercariae of *O. viverrini* (a species of liver trematode) can survive only 1–1.5 h when placed free in the solution (WHO 1995) but would require much longer if still situated within the flesh of the fish. Conditions are similar for salting in that the effectiveness of the salt is decreased (1) with a decrease in the concentration and (2) with an increase in the thickness of the flesh. The smoking process may include a substantial increase in temperature. If the fish is cooked during the smoking process (also known as hot smoking), the parasites and other pathogens will be killed. However, if the temperature of the product is not raised sufficiently during smoking (e.g., cold smoking), the product is still raw, and the parasites remain viable and infectious (Adams and DeVlieger 2001). Safety of these food preparations can be enhanced considerably by freezing the fish prior to pickling, salting, drying, or smoking (Adams et al.

1997). The previously mentioned change in texture caused by freezing is usually not noticeable after further processing.

In addition to the physical aspects of food preparation, sanitary practices during meal preparation can also contribute to the transmission of the trematodes. Metacercariae can contaminate knives and cutting boards used to chop crustaceans, as well as food processors in more technically advanced kitchens (Procop 2009). The use of contaminated water during food preparation, while washing utensils, or cleaning vegetation for consumption (e.g., watercress) is also known as a possible route of infection for fascioliasis (Mas-Coma et al. 2005).

Agricultural practices, particularly those involving sanitation, have a direct impact on the completion of the life cycles of these trematodes. The use of human feces as fertilizer, also known as night soil, assists in the distribution of parasites and their infective stages. Runoff from areas fertilized with human and animal excreta can inadvertently contaminate nearby ponds and waterways with the parasites and other pathogens. Alternatives include composting or sterilizing the excreta prior to use as fertilizer and the use of other compounds for enrichment, such as rice bran. The diversion of runoff and the use of cleaner water sources for aquaculture ponds also reduce or prevent contamination.

Another approach is the reduction of infected intermediate and reservoir hosts. Regular anthelminthic treatment for farm and companion animals can reduce the available pool of trematode eggs in endemic areas. However, as noted above with triclabendazole for fascioliasis, prolonged use of a single drug can lead to resistance to the therapeutic agent. Restricting access for animals to streams and ponds may also prevent the consumption of metacercariae and the direct introduction of eggs from animal feces into freshwater. Livestock will readily graze on aquatic vegetation, exposing them to metacercariae of *F. hepatica*. The control of snails is difficult. The use of molluscicides is often ineffective since snails can climb up on vegetation to avoid the chemicals and the products can become diluted. Molluscicides are also harmful to fish and the water environment (Cross 2001).

The increase in aquaculture can inadvertently lead to larger trematode populations. An increase in ponds also creates additional snail habitat. The expansion in fish-farming increases the number of potential intermediate hosts. In China, production of freshwater crustaceans increased from 9509 tons to 453, 696 tons between 1992 and 2002 (Keiser and Utzinger 2005). This is an increase of almost 50 times, a substantial increase in possible intermediate hosts for *Paragonimus* spp. Expanded aquaculture may also result in infected crustaceans being sold over a larger area or exported to non-endemic areas. Similarly, transport of edible plants from areas impacted by *Fasciola* to city markets may facilitate the spread of the trematode to unsuspecting populations (Mas-Coma et al. 2005). The parasite may then become established in areas with suitable intermediate hosts and insufficient sanitation. Under these circumstances, the diagnosis and treatment of resulting helminth infections may be difficult because medical personnel would have little experience with these parasitic diseases. *F. hepatica* is known to have originated in Europe. The transport of livestock infected with *Fasciola* has resulted in the introduction and spread of the parasite throughout the world (excepting Antarctica) (Mas-Coma et al.

2005). The authors also note that the European snail host, *Galba truncatula*, was likely spread to the other continents with the commercial exportation of infected livestock, with the snails within mud attached to the feet of the mammals. Exportation of forage for livestock from the affected areas of Europe could also have assisted in the spread of the snail host.

Similar to the transport of infected animals, the immigration of infected peoples to non-endemic regions could also result in the spread of the parasite if susceptible intermediate hosts were available and the immigrants did not practice the best forms of personal sanitation. In a study of latrine deposits spanning the time from 1880 to the 1930s, eggs of several parasites including a liver trematode endemic to Asia were identified (Reinhard et al. 2008). Only latrines located in an area where Chinese immigrants were known to work and live contained eggs of the liver trematode. The authors stated that some immigrants from Asia would also have been infected with other Asian parasites, including *P. westermani*. In addition to immigration, travel to endemic areas can also result in infections. Chand et al. (2009) investigated 11 cases of fascioliasis in the United Kingdom (UK) during 11 months from January 2008 to the end of January 2009. Only six cases had been diagnosed in the preceding decade. Two patients had recently traveled to sub-Saharan Africa from the UK and acquired the infection during their travels. The other nine were immigrants from Somalia, Ethiopia, or Yemen. Only two had not traveled to Africa for over 20 years, and their infections were believed to have been acquired in Europe. Interestingly, six of the nine patients had a history of using *khat*, a leaf with stimulant properties imported fresh into the UK. Metacercariae of *Fasciola* can easily survive among the leaves of *khat.*

Lastly, mention of the possible impact of climate change must be made. Parasites from tropical regions, such as *Paragonimus*, may increase their geographic distribution as temperatures warm in areas previously inhospitable to them. Additionally, their intermediate hosts may also spread into new regions as global temperatures increase. Snow melt and rising water levels may also contribute to the spread of intermediate hosts. These improving environmental conditions due to climate change have also been noted for *F. hepatica* (Molina-Hernandez et al. 2015). In Australia, the presence of snail, *Pseudosuccinea columella*, may enable the geographic range of *F. hepatica* to expand into the northern areas of Queensland (Molloy and Anderson 2006). *P. columella* is more adaptable to tropical environments than the other snails in the region. Transmission of *F. hepatica* from snail hosts from more temperate regions may actually decrease if temperatures rise to levels detrimental to the snails or to the resulting metacercariae (see temperature parameters in Sect. 13.3.3 above).

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# Chapter 14
# The Global Impact of Foodborne Parasites

**Lucy J. Robertson**

## 14.1 Introduction

Among the foodborne pathogens, parasites have, in general, long been considered of lesser importance than viral or bacterial pathogens. This is partly due to many foodborne viral or bacterial infections presenting with acute symptoms, usually gastroenteritis, while foodborne parasitoses present with a variety of symptoms, and for many foodborne parasitoses, disease progression is often chronic. Although acute gastroenteritis may be associated with some foodborne parasites, such as *Cryptosporidium* spp. or *Cyclospora cayetanensis*, even with these, the relatively prolonged incubation period, with several days elapsing between infection and symptoms, means that the foodborne route of infection is difficult to investigate, and frequently, it is difficult, or impossible, to identify the vehicle of infection.

Whereas for some parasitic diseases for which the foodborne route of infection is essential, such as trichinosis, anisakiasis, taeniasis, it may be relatively straightforward to consider the impact provided by foodborne transmission (by whichever metric) of that parasite, for other parasites it is not so straightforward. For many parasites, other routes of infection may also be important, such as hand-to-mouth transmission or waterborne transmission (such as for *Cryptosporidium*, *Giardia*, or *Ascaris*) or even vectorborne transmission as for *Trypanosoma cruzi*. For these parasites, an additional problem is determining the extent to which foodborne transmission is of importance, or whether other transmission routes may be of greater relevance. An answer to this question cannot be obtained by a prevalence survey, and expert elicitation is often used to obtain estimates. However, different experts have very different opinions, which may be influenced by various factors, including

L.J. Robertson (✉)
Department of Food Safety and Infection Biology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, PO Box 8146 Dep., 0033 Oslo, Norway
e-mail: lucy.robertson@nmbu.no



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geography. In addition, for making such estimates, it is necessary that the experts are not only knowledgeable about the parasitosis in question but also on how to make reasonable estimates and be aware of estimating uncertainty (e.g., the credible interval) within these estimates.

Nevertheless, despite these difficulties, recent initiatives suggest that foodborne parasites may be beginning to become a focus of greater attention (Robertson et al. 2013). This chapter gives an overview on different metrics for considering the global impact of a foodborne parasite and then uses different parasites to describe various aspects regarding the impact of different foodborne parasitic infections. This includes the public health impact of some parasites, impacts on global trade associated with some foodborne parasites, and socioeconomic impacts. This is a huge topic, covering not only many aspects of what is meant by impact and approaches to measuring them, but also a vast number of parasites. In the FAO/WHO risk ranking of foodborne parasites (FAO/WHO 2014), initially, over 90 different parasite-food commodity pairs were considered, although this was later reduced to 24.

## 14.2 How Should Global Impact Be Measured and Compared?

The global impact of a foodborne disease can be measured in various terms. These include, but are not limited to, how commonly the parasitic infection occurs (prevalence or incidence), the public health effect (mortality and morbidity), the geographic distribution (localized or widely distributed), various aspects of its economic impact (financial burden associated with diagnosis and treatment, analysis of food products for its presence and mitigation measures, the effects on community or individual economics if an outbreak occurs including health system overload or loss of a family's breadwinner), its transmission and establishment potential, the effects on achieving potential of the infectd hosts regarding both physical (e.g., height) and social (e.g., educational) factors, and the possible impacts on food-trade at both the national and international level. Other metrics that reflect other aspects of global impact could also be considered, but generally, public health aspects that may impact further on all other aspects are considered to be of greatest importance (e.g., public health metrics of prevalence, mortality, and morbidity were given greatest (85%) of the weight in the FAO/WHO risk-ranking exercise conducted in 2012; FAO/WHO 2014). Interestingly, those foodborne parasites that are considered to be of greatest public health importance are not necessarily those for which most resources are directed regarding identification in foodstuffs or which have had significant impacts on global food-trade.

One metric that is commonly used as a measure for public health impact is the DALY (disability-adjusted life year), which basically refers to the number of lost years of "healthy" life, and thereby includes both mortality and morbidity. As many foodborne parasites fall into the category of being "disablers" rather than killers, DALY estimates are one of the few available metrics that take into account the

chronic effects of these infections. It is thus considered to be a number that can be calculated by adding together the figure for years of life lost (YLL) due to premature mortality associated with the particular pathogen (in this case a foodborne parasite) with the figure for years lived with disability (YLD) for people living with that foodborne parasite or the consequences of that infection. Although DALYs are clearly a useful metric, the lack of clear-cut data for morbidity for many parasitoses adds a further difficulty; for example, for *Toxoplasma gondii*, different strain types have apparently different virulence characteristics, as well as different geographical distributions. In addition, while infection with *Giardia* has very few or minor health consequences in the majority of individuals, in some individuals the infection may result in more severe health consequences, including long-term sequelae. The reasons for this wide range of health consequences associated with a specific parasitic infection are not clear, but probably both parasite and host factors have some impact. How to account for these differences in calculating YLD is challenging. Other parameters may also be used, but are perhaps not as widely knowns as DALYs, such as HALE (health-adjusted life expectancy), which also include YLD and YLL:

## 14.3 *Taenia solium* and *Taenia saginata*: Two Similar Tapeworms with Very Different Global Public Health Impacts

Public health impact is generally considered to be the most important impact that a pathogen can exert. Despite their phylogenetic propinquity and clear similarities in morphology and life cycle, the FAO/WHO risk-ranking exercise of foodborne parasites ranked these two parasites very differently, with *T. saginata* (the beef tapeworm) ranked in position 19 out of 24, whereas *T. solium* (the pork tapeworm) ranked in the first position (FAO/WHO 2014) when all aspects were considered (with public health aspects having the greatest weight). However, when only public health aspects were included in the ranking, and without any weighting, *T. solium* and T. *saginata* were ranked at positions three (behind *Echinococcus multilocularis* and *Toxoplasma gondii*) and 22 out of 24. In a more recent consideration of foodborne parasites, using a different approach for gathering and interpreting data and using only public health indices (DALYs) as the ranking tool, the Foodborne Diseases Burden Epidemiology Reference Group (FERG) also ranked *T. solium* as being of greatest impact (based solely on public health aspects) but did not consider *T. saginata* in their assessment at all (Torgerson et al. 2015). This difference between the species is because these two parasites can have very different effects on human health. Humans are the definitive hosts for both parasites, harboring the mature tapeworm in the intestine following ingestion of the infective cysticerci of *T. saginata* in beef or *T. solium* in pork, and taeniasis results in relatively few symptoms and little pathology. However, for *T. solium*, humans can act not only as definitive hosts, but can also be infected by the eggs of *T. solium*, acting as aberrant

intermediate hosts. Cysticercosis, particularly neurocysticercosis (NCC), in which the cysticerci are in the central nervous system, can result in a serious symptom spectrum with high mortality and morbidity. The Global Burden of Disease 2010 Study (2010) estimated that cysticercosis was responsible for a global loss of 503,000 DALYs (with 95% uncertainty intervals (UI) of 379,000–663,000) annually (Murray et al. 2012), while the Foodborne Diseases Burden Epidemiology Reference Group (FERG) estimated that cysticercosis was responsible for 2,788,426 DALYs annually (95% UI of 2,137,613–3,606,582) (Torgerson et al. 2015). This much higher (over five times) burden estimated in the latter exercise is difficult to explain, but the authors (Torgerson et al. 2015) suggest that a major reason is due to the latter estimates assigning a substantial proportion of the epilepsy burden to cysticercosis. Furthermore, the authors suggest that even this higher estimate from FERG may continue to be lower than the actual burden of cysticercosis, due to other important clinical symptomatology associated with NCC that was not included in the calculation. Thus, on a global basis, it appears clear that *Taenia solium*, specifically cysticercosis, has a significant impact on public health. The FERG group did not include *T. saginata* in their analyses at all due to the very mild clinical condition in infected humans, usually asymptomatic, that would mean a disability weight vanishingly close to zero, indicating a very low burden of human disease.

The global distribution of the fundamentally more important *T. solium* is also important, and it would probably be of interest to assess the relative risk of this parasite compared with other foodborne parasites in regions where pork is not part of the diet. Ingestion of pork is essential for the life cycle (for the adult tapeworm to establish in the human intestine, producing the eggs that may either infect pigs, thus continuing the life cycle, or with the potential to cause cysticercosis if ingested by humans). Thus, a total lack of pork consumption should interrupt transmission. However, even in countries where consumption of undercooked pork is unlikely to occur and where pig-rearing facilities are such that ingestion of potentially contaminated human feces are unlikely to occur, the travel of humans with taeniasis due to *T. solium* (which is a relatively benign infection and may not be recognized by the infected person and thus not diagnosed or treated) leaves the door open for environmental contamination and risks of cysticercosis, in which the pig intermediate host is not required. A review article investigating possible impacts of globalization on foodborne parasites describes cases of cysticercosis in Norway and the USA, where endemic *T. solium* cycling is considered absent or very rare (Robertson et al. 2014). Indeed, in the cases in the USA, the unrelated patients with cysticercosis were Orthodox Jews who had never knowingly eaten pork and did not have risk factors for being infected; investigations suggested that the sources of infection were probably, in some of the cases, migrants working in the households with unrecognized *T. solium* infection. The cryptic nature of taeniasis and our globalized society means that although the greatest health burden from this infection occurs in rural or marginalized communities of sub-Saharan Africa, Asia, and Latin America, *T. solium* really does have a global impact. Furthermore, although generally considered to be a non-endemic in much of Europe, solid data are scanty, and officially reported data on porcine cysticercosis, which would be indicative that the life cycle is in place, are

insufficient. A recent article (Devleesschauwer et al. 2015a) suggests that in Eastern Europe, conditions for *T. solium* transmission remain favorable in some locations; with increasing integration across Europe and with a trend toward "organically raised" pigs, this opens the door for transmission to establish further rather than be held in check. Another method by which *T. solium* might be introduced into areas where it is generally considered not to be a problem (e.g., in Western Europe) is by the import of infected pork that is then consumed without adequate cooking. However, an article that discusses these possible routes concludes that entry of a tapeworm carrier into the EU seems a lot more plausible than the import of infected pork and suggests that although establishment of local transmission in the EU is presently very unlikely, trends in human migration warrant the establishment of an early warning system (Gabriël et al. 2015). It is also worth noting that even in countries where consumption of meat is relatively low, if there are problems with the infrastructure, then even a few infections may result in high environmental contamination and onward transmission, due to the huge production of infective eggs from a single worm; *T. solium*, for example, produces around 50,000 eggs per proglottid, and each adult worm may have 1000 proglottids at any one time. A risk-ranking exercise for foodborne parasites conducted solely for India and based on the FAO/WHO approach also identified *T. solium* as being of most importance, despite India being reported to have the lowest meat consumption per capita than any other country (Robertson et al. 2015). Indeed, as with many other countries with higher meat consumption, NCC is a major cause of epilepsy in India also (Goel et al. 2011; Pandey et al. 2014; Robertson et al. 2017).

One problem with identifying import of *Taenia* tapeworms in migrants to countries otherwise considered free of this infection is that the benign nature of taeniasis means that the infection may not be picked up unless there is compulsory screening in place. Perhaps screening of all persons who are working with pigs may be one approach to reducing the possibility of introducing the full transmission cycle. Indeed, although due to the lack of cysticercosis *Taenia saginata* is of lesser concern, there have already been cases published in which this infection is introduced into cattle herds in Europe due to infected migrant farm workers (Anon 2012).

## 14.4 Global Trade Impacts from Foodborne Parasites: *Trichinella*, *Taenia* spp., Anisakidae, and *Cyclospora cayetanensis*

In the FAO/WHO risk-ranking exercise, as well as providing an overall ranking and a public health criteria-based ranking, a ranking was derived based upon the importance of each of the foodborne parasites in international trade (FAO/WHO 2014). In this ranking, *Trichinella spiralis* was ranked most highly (number 7 in the overall ranking and number 9 based on public health criteria with no weighting), with *Taenia solium*, *Taenia saginata*, Anisakidae, and *Cyclospora cayetanensis* ranked in positions 2, 3, 4,

and 5, respectively. In the FERG estimates of the public health importance of *Trichinella* (all species), it was ranked as being of least importance of the 14 parasites considered, being estimated as being responsible for only 550 DALYs (95% UI of 285–934 2) annually (Torgerson et al. 2015). The reason for the higher rankings of the meatborne parasites based on international trade are largely due to the requirements for testing that are economically demanding and may result in carcasses being rejected or condemned (and the resultant economic loss). However, the consensus of scientific opinion appears to be swinging against the continual compulsory testing of all pigs for *Trichinella*; not only do the FERG estimates indicate a low public health impact of trichinellosis, but another publication from some of the same team states that the global burden of trichinellosis is much lower than that of other foodborne parasitic diseases and is in sharp contrast to the high budget allocated to prevent the disease in many industrialized countries (Devleesschauwer et al. b). The prescribed method for analysis of animals for *Trichinella* in EU countries (EU legislation 2075/2005), which is based on magnetic stirrer, acid-pepsin digestion and identification of the larvae, is time-consuming and requires both training and expertise. Another article makes the salient point that the concentration of surveillance efforts on pigs produced in commercial farms is probably directing our resources wrongly (Pozio 2014). Although millions of pigs are tested annually for *Trichinella* spp. at slaughterhouses worldwide, the results are almost always negative. While pigs from herds kept under controlled management conditions are the ones tested, the parasites are mainly circulating in backyard and free-ranging pigs, which are often not tested. When outbreaks occur, they are very frequently associated with hunted wild game, predominantly wild boar (Murrell and Pozio 2011), indicating that investment of funds into the education of farmers, hunters, and consumers might be a more useful use of the resources (Pozio 2014) currently used for testing for *Trichinella* in commercially raised pigs. Another study that investigated the likelihood of exposure to an infectious dose of *Trichinella spiralis* from consumption of pork meat imported from New Zealand to Singapore (Richardson et al. 2009) came to a similar conclusion. In this study, it was demonstrated that routine testing at slaughter is unnecessary for pig offal produced commercially and concluded that the current system in place has created adverse effects on the international trade of fresh lean meat and does not take into account the surveillance measures employed by the various pork-producing countries (Richardson et al. 2009).

Furthermore, with respect to import of meat, a review of the last 60 years of international literature identified 43 reports of importation of *Trichinella* spp. infected animals or meat, most of which (60%, 26/43) related to live horses or their meat, with no reports on the detection of *Trichinella* spp.-infected pigs originating from the international trade (Pozio 2015).

Testing of meat for *Taenia* cysticerci is likewise burdensome and therefore expensive, but the method used is relatively more rapid, and thus cheaper, and is based generally on a series of visual inspections in predilection sites, including examining the external and internal masseters; examining the pterygoid muscles and making incisions into each, parallel to the bone and right through the muscle; and examining the cut surfaces. In addition, different organs such as the heart, pericardium, diaphragm, esophagus, and tongue are inspected and, in the case of the tongue, palpated (OIE 2014).

The Anisakidae were also recorded as being relatively important in international trade. This is largely due to fish for export being returned due to the presence of Anisakid larvae in the meat. In the EU Rapid Alert System for Food and Feed (RASFF), the parasite-food commodity most frequently reported is Anisakid larvae in fish products. For example, of 61 cases of parasitic infestation of traded food registered in RASFF from 2012 until 2013, 56 (over 91%) concerned the import of fish in which Anisakid larvae had been detected (Robertson et al. 2014). Nevertheless, Anisakidae are considered relatively unimportant from a public health perspective, being ranked number 17 in the overall ranking of FAO/WHO and number 18 based on public health criteria with no weighting (FAO/WHO 2014). In the FERG foodborne parasite assessment, Anisakidae were not included as foodborne disease caused by Anisakidae was considered to be uncommon. However, the clinical syndrome associated with infection with, or exposure to, Anisakidae is complicated, with an allergenic response an important component in some individuals, and may be accompanied by severe allergic reactions such as urticaria, angioedema, and anaphylaxis (Nieuwenhuizen and Lopata 2013). Furthermore, another research group has postulated that exposure to Anisakidae may be associated with the development of stomach or colon cancer (Garcia-Perez et al. 2015). Given previous evidence of carcinogenesis associated with helminthic infections, such as *Schistosoma haematobium*, *Clonorchis sinensis*, and *Opisthorchis viverrini*, this is perhaps a realistic supposition and would also indicate a complex process involving several different mechanisms with chronic inflammation as a key feature.

*Cyclospora cayetanensis* was also considered relatively important in international trade in the FAO/WHO ranking exercise (FAO/WHO 2014), although it was ranked only 13th out of 24 in overall importance and 19th out of 24 considering only public health significance when weighting was equal (FAO/WHO 2014). *C. cayetanensis* was not considered in the FERG exercise, because, although initially nominated, it was decided to target resources on other intestinal protozoa (*Cryptosporidium*, *Entamoeba*, *Giardia*), as it was considered that the frequency of citations for these parasites had been markedly increasing between 1990 and 2008, whereas citation frequency for *C. cayetanensis* had remained constant over the same period (Torgerson et al. 2015). Given that *C. cayetanensis* was first properly reported as a human pathogen in 1993 (Ortega et al. 1993), this seems rather unlikely reasoning.

The reason for the impact of this parasite on global trade is directly associated with its association with outbreaks in which imported fruit (notably raspberries) and imported fresh leafy vegetables (notably cilantro) particularly from Latin America to Northern America (although cases and outbreaks have also been reported elsewhere) have been the vehicles of infection. These outbreaks have resulted in serious trade restrictions.

The imported raspberry associated outbreaks occurred particularly from around 1995 until around 2000, in both the USA and Canada (Shields and Ortega 2012). For example, in May–June 1996, over 1460 cases of cyclosporiasis were reported in the USA and Canada, or which around 980 were laboratory confirmed, and almost 50% of these cases were associated with clusters in which imported fresh raspberries

were subsequently epidemiologically linked to the infection (Shields and Ortega 2012). Initially, it was considered that the probable vehicle of infection was not raspberries, but strawberries from California, and it has been reported that this erroneous implication resulted in an economic hit of 20–40 million US dollars in lost strawberry sales (Powell 1998). However, following the recognition that the vehicle of infection was more likely to be raspberries imported from Guatemala, and in the wake of more similar outbreaks, in 1997 the import of Guatemalan raspberries to the USA was suspended. The estimated income loss to Guatemalan producers and workers was around 10 million US dollars, and over 70 raspberry farms were obliged to close (Robertson et al. 2014). Canada did not apply such strict sanctions at first, but following further outbreaks in Toronto and Ontario (Shields and Ortega 2012), a similar ban was imposed. Currently, the Canadian ban remains in place for import of cultivated fresh raspberries from Guatemala between mid-March and mid-August, and import of wild fresh raspberries (and blackberries) are banned in total (Canadian Food Inspection Agency 2013), despite the absence of outbreaks of cyclosporiasis associated with Guatemalan raspberries in more recent years. Furthermore, the combination of trade restrictions and the requirement for implementation of production controls provided other countries with the opportunity to appropriate a considerable proportion of Guatemala's previously major role as a raspberry supplier. The impacts on global trade of this particular foodborne parasite extend further, however, than the immediate impacts of restrictions, resulting in more prolonged and deep-seated effects that affect international trade in the particular commodity concerned – in this case raspberries and other berries. The extent of the illnesses and outbreaks of cyclosporiasis, combined with the media associated with these events, is likely to have resulted in consumers altering their perceptions of raspberries, and possibly other berries, to considering them now as a food with a higher level of safety risk. This modification will, in turn, alter preferences both at the consumer level and at the national level – the USA now puts a requirement for not simply raspberries, but *Cyclospora*-free raspberries, and therefore is more likely to accept as imports raspberries from countries where *Cyclospora* is not considered to be an endemic pathogen (Buzby 2001).

However, the "*Cyclospora* story" does not end with raspberries imported from Guatemala. In the last three years (2013–2015), statewide outbreaks of cyclosporiasis in the USA have been associated with another commodity, cilantro, although not exclusively so; other fresh produce (lettuce mixes) were also implicated in some cases (Abanyie et al. 2015). The salient aspects of these 3 consecutive years of outbreaks of *Cyclospora* infection are summarized in Table 14.1. Here, it is clear that although the first year produced the most cases, it is the repetition of these outbreaks and the implicated vehicle and origins that have led to the import control being applied in 2015. Although such a restriction is likely to have serious consequences for some of the farms, it is intended that they also result in improvements in the hygienic state and facilities at the implicated farms. Inspections of cilantro farms and packinghouses in Mexico by the FDA and Mexican authorities apparently

**Table 14.1** Three years (2013–2015) of cyclosporiasis in the USA associated with cilantro imported from Mexico

| Year of outbreak | 2013 | 2014 | 2015 |
|---|---|---|---|
| Reference | Abanyie et al. (2015), CDC (2013) | CDC (2015a) | CDC (2015b) |
| No. persons infected | 631 | 304 | 546 |
| No. hospitalized (deaths) | 49 (0) | 7 (0) | 21 (0) |
| No. states reporting infection | Most cases in Texas but also cases from 24 other states and New York City | Most cases in Texas but also cases from 19 other states and New York City | Most cases in Texas but also cases from 30 other states and New York City |
| Most cases reported | Mid-June to August | June–August | May–August |
| Implicated vehicles of infection | Cilantro and salad mixes (not all vehicles positively identified) | Cilantro (for cases in Texas) | Cilantro (for some cases) |
| Country of origin of implicated vehicles | Mexico | Mexico | Mexico |
| Effects on trade | Naming of implicated farm by FDA<br>Voluntary suspension of export to USA for two weeks from implicated farm<br>Border surveillance for cilantro by FDA increased | Naming of implicated state by FDA<br>Increased sampling of cilantro at the US/Mexico border by FDA | FDA import alert instituted regarding import of cilantro from Puebla, Mexico, from April 1 through August 31 annually, unless from a firm listed on the Green List of the alert |

identified various weaknesses in good farm practice, including lack of toilets or handwashing facilities, lack of running water, soap or paper towels, dirty surfaces, and confusing, possibly fraudulent, documentation. However, whether these outbreaks and associated measures may have a more prolonged impact on trade in fresh produce from Mexico due to concerns from the US-based consumers, as was seen in the Guatemalan raspberry events, remains to be seen.

## 14.5 Socioeconomic Impacts from Foodborne Parasites: *Taenia solium*, *Echinococcus* spp., *Cyclospora cayetanensis*, and Opisthorchiidae

Another inter-parasite classification that was published with the FAO/WHO ranking is described as socioeconomic or "distributional" impacts (FAO/WHO 2014). In this ranking, the experts considered the extent to which the various listed 24 foodborne parasites affect economically vulnerable communities, with focus on how each of the specific parasites may reduce household or community productivity, or the ability of a household to produce and/or purchase food. The intention was that the emphasis should be directed to vulnerable communities, such as pastoral communities, small fishing communities, rural populations in developing countries and migrant populations in developed countries, and various minority indigenous populations such as Inuit in Canada, aboriginals in Australia, etc. (FAO/WHO 2014). Solid data on which such assessments and comparisons can be made are scant, and, the very nature of the criteria for this ranking means that specific foodborne parasites that are of particular relevance only to a marginal community may be neglected. It could be argued that the reason that FERG did not consider *Trypanosoma cruzi* to be a high priority in their assessment (Torgerson et al. 2015) is because of its relatively localized nature (foodborne transmission having been documented from just five countries in South America), despite being associated with considerable clinical disease, including deaths.

Nevertheless, although making such estimates is difficult, it is very pertinent. The impact of many of the foodborne parasitic diseases falls most heavily on the poor and their often under-resourced health systems (Welburn et al. 2015). For zoonotic foodborne parasites, it is often those populations that live in greatest contact with their animals that are most at risk of infection – and such infections can be of considerable clinical significance. The burden of caring for a family member incapacitated, either short term or long term, by such infections can push households further into a cycle of poverty, and it is clear that the death of a breadwinner may have a devastating impact on a household, particularly in regions where social welfare support is lacking or weak (Welburn et al. 2015).

By not taking these factors into consideration when estimating disease impacts, regardless of etiology or route of infection, then investment and funding may be unintentionally prioritized away from such diseases (Welburn et al. 2015). Foodborne parasites are among these "neglected" diseases for which the importance may become hidden. Although the estimation of a global disease burden provides a logical method of comparison, it does not result in an equitable strategy by which investments should be prioritized. Furthermore, a lack of good or accurate data may result in under-evaluation, and hence, devaluation of some diseases when health priorities are being set (Welburn et al. 2015).

These sentiments were likely the basis for attempting to include these impacts in the FAO/WHO exercise (FAO/WHO 2014), and, despite the difficulties regarding the paucity of data, the foodborne parasites that were ranked of having greatest

socioeconomic impact in the FAO/WHO exercise were *Taenia solium*, *Cyclospora cayetanensis*, *Echinococcus granulosus*, *Echinococcus multilocularis*, and the Opisthorchiidae. The rationale behind this listing is not clearly provided; however, clues are provided in the separate sections on each parasite within the report (Annex 7) (FAO/WHO 2014). For example, neurocysticercosis is not only a serious public health problem, but the attendant epilepsy or seizures that occur in a substantial proportion of cases (>75%, Carabin et al. 2011) are associated with serious social stigma in some communities. For example, a study from Nigeria revealed that people with epilepsy (regardless of etiology) face multiple social and economic challenges and, given that witchcraft/evil spirits were often considered to be causal, these individuals were often isolated or ostracized (Osakwe et al. 2014). These factors can also act as barriers to diagnosis and treatment. A study from South Africa determined that economic impact due to neurocysticercosis was due largely to working time lost due to epilepsy and the requirement for seeking medical treatment (Carabin et al. 2006). Other studies have also indicated that the human economic burden of this infection is probably in excess of that which can be sustained by impoverished rural communities (e.g., an approximate cost per case of cysticercosis of 194 Euro has been estimated for Cameroon; Praet et al. 2009). In addition to the monetary costs associated with the human aspects of cysticercosis, carcass devaluation or condemnation will also contribute to the expense that will be borne by resource-poor communities, frequently smallholders in developing countries. With pig production in smallholder farms being a rising trend across the developing world, neurocysticercosis is increasingly becoming an emerging public health problem and also an agricultural problem. Losses in the average value of pigs of between 25% and 50% due to porcine cysticercosis have been suggested for Western and Central Africa (Zoli et al. 2003). Although probably not all porcine cysticercosis is diagnosed, a study from Democratic Republic of Congo indicated that highly infected pigs are excluded at a certain level in the trade chain, with pig farmers and/or buyers choosing those with low levels of infection over those found positive by tongue inspection at village level (Praet et al. 2010). This may act to reduce the likelihood of onward transmission, but obviously has a negative impact on those who are unable to sell their pigs.

The socioeconomic impact of infection with *Cyclospora cayetanensis*, which was ranked next highly after *Taenia solium*, is largely due to the considerable impact of outbreaks in North America on farming communities in Latin America. Information on these outbreaks has been described in greater detail in the global trade impact section of this chapter. Suffice it to say, the closure of export markets and the dramatic fall in demand for specific export products has ramifications on those communities that depend on these crops for their livelihoods. Although a number of articles have examined in some detail the effects of the outbreaks of cyclosporiasis associated with Guatemalan berries in some detail, including how these had a positive effect on the export trade from other countries such as Chile and Mexico (e.g., Calvin et al. 2002), the emphasis has been on the export productivity of the country as a whole, and there is scant information available on how the collapse of the export market impacted on those communities where the farms were located.

*Echinococcus granulosus*, which causes cystic echinococcosis (CE), was ranked after *Cyclospora*, regarding its socioeconomic impact (FAO/WHO 2014). However, it is stated that due to the agriculture, trade, and market factors, it is not possible to understand the total socioeconomic effect of the disease that encompasses more than animal and human health. For example, although the most obvious expense due to CE is the medical treatment, there is also the additional cost of loss of edible offal from agricultural animals (Torgerson 2003). Nevertheless, it has been proposed that a greater cost is the lack of full recovery for many patients treated for CE, resulting in their quality of life being significantly and permanently diminished, with accompanying loss of income, possibly through a job that is relatively poorly paid, along with the likelihood of additional expenses due to increased ill health (Torgerson 2003). Without including these almost unmeasurable expenses, the monetary costs of CE (including both the human health costs and the agricultural losses) have been estimated for various countries (e.g., Iran, Fasihi Harandi et al. 2012; India, Singh et al. 2014; Tunisia, Majorowski et al. 2005; Peru, Moro et al. 2011) where the annual costs were estimated at around US $232.3 million, US $212.4 million, US $10–19 million, and US $ 6.4 million, respectively. These differences between countries reflect not only actual differences in costs, which are obviously also by country size, but also methods of estimation. However, as stated in the article from India, in calculating human losses, social harm and psychological distress were not taken into account (Singh et al. 2014). It should be noted that various factors that are often associated with impoverished rural populations, such as limited community knowledge of disease transmission, small-scale household animal production, home killing of livestock, and feeding of dogs with uncooked offal, are all factors that could promote the life cycle of *E. granulosus* (Zhang et al. 2015), pushing those families in this situation deeper into poverty due to ill health and loss of life.

*Echinococcus multilocularis*, which causes alveolar echinococcosis, was ranked closely after *E. granulosus* by FAO/WHO regarding socioeconomic impact (FAO/WHO 2014). A serious associated problem in pastoral minority communities in sparsely populated regions of China (including Inner Mongolia, the Tibetan Plateau, etc.) is the lack of diagnosis, which may result in treatment being inadequate or absent, and thus with a very poor prognosis. Again, factors associated with impoverished rural populations, such as poor levels of hygiene, close contact with dogs, and a lack of appropriate facilities for the correct and rapid diagnosis or adequate treatment, are all factors that promote the life cycle (McManus et al. 2011). Although several articles have been published that analyze the costs associated with *E. granulosus*, considerably fewer assess the costs associated with alveolar echinococcosis – a simple "PubMed" literature search using the search terms "cystic" and "echinococcosis" and "cost" provided five times as many relevant articles as a similar search using the terms "alveolar" and "echinococcosis" and "cost." Part of this difference is due to several papers that examine the veterinary costs of cystic echinococcosis; as the intermediate hosts of *E. multilocularis* are rodents rather than livestock (the intermediate hosts for *E. granulosus*), no veterinary burden is associated with the former parasite. However, leaving the veterinary impacts aside, it

seems probable that the global distribution of the two infections is also important. Whereas *E. granulosus* is globally distributed, apparently occurring in every continent except Antarctica, the distribution of *E. multilocularis* is limited to the northern hemisphere. Although the economic impacts of this parasite have been in addressed in marginalized communities in China, the majority of articles concerned with the costs of *E. multilocularis* are concerned with reducing the expense of methods for identifying the parasite in fox feces or the cost of deworming of dogs in northern Europe. It seems likely that more relevant data on the socioeconomic impacts of alveolar echinococcosis is probably published in Chinese; this is unfortunate as although being of limited veterinary relevance only (in dogs), the human impact in those societies for which social infrastructure is fragile, the socioeconomic impact can be enormous.

Parasites in the family Opisthorchiidae were also ranked relatively highly as foodborne parasites with socioeconomic impact. These parasites include the liver flukes *Opisthorchis viverrini*, *O. felineus*, and *Clonorchis sinensis*, along with the more minor flukes in the same family, *Metorchis bilis*, *M. conjunctus*, *M. orientalis*, and *Pseudamphistomum truncatum*, and the focus in the FAO/WHO exercise was largely directed towards the three more widespread flukes. These flukes are all transmitted to humans via consumption of undercooked fish containing viable metacercariae, and thus all infections in humans are foodborne. The socio-impact on vulnerable populations is particularly concerned with an association with the development of cholangiocarcinoma (CCA) in a proportion of those infected. Thus, although the parasitic infection may not be immediately life threatening, cancer can develop within 30–40 years after infection, and death usually occurs 3–6 months after diagnosis – pharmaceutical treatment is lacking, and surgery complicated and often not available in developing countries (Andrews et al. 2008). In Thailand alone, the cost of opisthorchiasis and CCA is estimated to be around US$ 120 million annually in medical care and lost wages (Kaewpitoon et al. 2015). Furthermore, because the disease is more prevalent in adult males, particularly agricultural workers (Kaewpitoon et al. 2015), the potential cost to families and communities is enormous, as men tend to be the principal financial earners. A similar picture emerges for *Clonorchis sinensis*, although its official recognition as a definite carcinogen only happened within the last decade (Qian et al. 2015). Again, the principal patients are adult males, and among the estimated infected 15 million people, 13 million are in China. In societies where untreated human and animal feces are used to feed fish in traditional aquaculture, the life cycle is promoted. In such places, consumption of raw fish and crayfish is also a deeply embedded traditional practice, and often strongly encouraged to protect traditional cultural habits; furthermore, many people believe that raw fish is highly nutritious and that consumption of hot spices or alcohol will kill any infectious agents (Qian et al. 2015). Although these traditions seem to be in line with less developed societies, intriguingly, economic development appears to be an important factor in promoting disease transmission, as greater prosperity enables people to afford to eat raw fish, both at home and in restaurants (Qian et al. 2015).

## 14.6 Concluding Remarks

There are many foodborne parasites that impact globally on human health and well-being. Attempting to define these impacts is a challenge, not only because there are different ways to visualize impact, but also because the relevant data for many parasites is scattered or lacking.

Attempting to compare the impacts of foodborne parasites is equally fraught – the parasites are so diverse, with different life cycles, transmission modalities, clinical symptoms, and varying effects on trade and produce. Some parasites are largely only infectious to humans, whereas others may be zoonotic. In this chapter, just a few of the multitude of foodborne parasites are singled out for their relevance under different ways of considering impact – but all groups of parasites, protozoa, cestodes, trematodes, and nematodes, are nevertheless represented. Another author would likely have made a different selection of foodborne parasites in order to illustrate global impact, and the selection made here is by no means inclusive. Nevertheless, it is hoped that this selection illustrating some, but by no means all, indices of impact at least provides food for thought in demonstrating the importance and significance of foodborne parasites to the world population.

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# Chapter 15
# Burden and Risk Assessment of Foodborne Parasites

**Brecht Devleesschauwer, Pierre Dorny, Christel Faes, Arie H. Havelaar, Paul R. Torgerson, and Niko Speybroeck**

## Abbreviations

| | |
|---|---|
| DALY | Disability-adjusted life year |
| FBP | Foodborne parasite |
| FERG | Foodborne Disease Burden Epidemiology Reference Group |
| GBD | Global Burden of Disease |
| MCDA | Multi-criteria decision analysis |
| MPRM | Modular process risk model |
| QMRA | Quantitative microbiological risk assessment |

B. Devleesschauwer (✉)
Department of Public Health and Surveillance, Scientific Institute of Public Health (WIV-ISP), Brussels, Belgium
e-mail: brecht.devleesschauwer@wiv-isp.be

P. Dorny
Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

Department of Virology, Parasitology and Immunology, Ghent University, Merelbeke, Belgium
e-mail: pdorny@itg.be

C. Faes
Interuniversity Institute for Biostatistics and Statistical Bioinformatics, Hasselt University, Hasselt, Belgium
e-mail: christel.faes@uhasselt.be

A.H. Havelaar
Emerging Pathogens Institute, Institute for Sustainable Food Systems and Department of Animal Sciences, University of Florida, Gainesville, FL, USA
e-mail: ariehavelaar@ufl.edu

P.R. Torgerson
Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
e-mail: paul.torgerson@access.uzh.ch

N. Speybroeck
Institute of Health and Society (IRSS), Université catholique de Louvain, Brussels, Belgium
e-mail: niko.speybroeck@uclouvain.be



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SMPH Summary measure of population health
YLD Years lived with disability
YLL Years of life lost

## 15.1 Preface

Burden and risk assessment play an increasingly important and accepted role in defining control policies for foodborne parasites (FBPs). Burden assessment is a top-down approach, starting from available epidemiological data, while risk assessment is a bottom-up or predictive approach, starting from exposure and dose-response data (Newsome et al. 2009; Stella et al. 2013). Both methods however share a common goal of generating estimates of the health and economic impacts of the concerned hazards. These estimates can be used to generate an evidence-based ranking of the impact of FBPs (i.e. risk ranking) and a baseline against which the effects of interventions can be evaluated (Devleesschauwer et al. 2014a). Risk assessment further provides a scientific framework for evaluating the potential effects of intervention measures and, by combining with economic models, the expected efficiency of such measures.

In this chapter we review methods for quantifying disease burden, for quantifying health risks and for ranking risks. We present applications to FBPs but acknowledge that these methods can be applied to any *hazard* (i.e. any biological, chemical or physical agent able to cause harm or adverse effects). Burden assessment is even more general, as it can also be applied to *outcomes* (such as diarrhoea or epilepsy) and *risk factors* (such as unsafe water or lack of sanitation).

## 15.2 Quantifying Disease Burden

### *15.2.1 Health Impact*

Quantifying health impact may be based on disease occurrence (prevalence or incidence) or on the number of deaths (mortality). However, these *simple* measures of population health do not provide a complete picture of the impact of FBPs on human health (Batz et al. 2012; Devleesschauwer et al. 2015a). Indeed, while certain parasitic infections may be very common, their clinical impact may be limited. Infections with a highly prevalent parasite such as the pinworm, *Enterobius vermicularis*, for instance, have a very low burden because most of the cases are mild to asymptomatic and self-limiting (Knopp et al. 2012). Likewise, ignoring the age at which people die and thus not considering how many years of healthy life might be lost due to death results in not fully capturing the impact of mortality. Disease severity, defined by the impact on quality of life and the duration of the concerned symptoms, and the

life expectancy at the age of death should thus be accounted for when quantifying burden of disease. Furthermore, simple measures of population health do not combine the impacts of morbidity and mortality. This prohibits a comparative ranking of highly morbid but not necessarily fatal diseases, such as chorioretinitis due to congenital toxoplasmosis, and highly lethal diseases such as alveolar echinococcosis. The absence of a correct ranking complicates decisions on resource allocation priorities.

To overcome the limitations of simple measures such as incidence and mortality, *summary* measures of population health (SMPHs) have been developed as an additional source of information for measuring disease burden. The disability-adjusted life year (DALY) is currently the most widely used SMPH in public health research. Originally developed to quantify and compare the burden of diseases, injuries and risk factors within and across countries, the DALY summarises the occurrence and impact of morbidity and mortality in a single metric (Murray and Lopez 2013; Devleesschauwer et al. 2014b). The DALY is the key measure in the Global Burden of Disease (GBD) studies and is officially adopted by the World Health Organization for reporting on health information (Murray et al. 2012; WHO 2013).

The DALY is a health gap measure, measuring the healthy life years lost due to a disease or injury against some idealised health profile. DALYs are calculated by adding the number of years lived with disability adjusted for the severity of the disease (YLDs) and the number of years of life lost due to premature mortality (YLLs):

YLD = number of incident cases × duration until remission or death × disability weight

YLL = number of deaths × residual life expectancy at the age of death

An alternative formula for calculating YLDs was introduced by the GBD 2010 study (Murray et al. 2012):

YLD = number of prevalent cases × disability weight

This formula reflects a prevalence perspective instead of an incidence perspective. The incidence perspective assigns all health outcomes, including those in future years, to the initial event (e.g. exposure to a certain FBP). This approach therefore reflects the future burden of disease resulting from current events. In the prevalence perspective, on the other hand, the health status of a population is assessed at a specific point in time, and prevalent diseases are attributed to initial events that happened in the past. This approach thus reflects the current burden of disease resulting from previous events. Although both perspectives are valid, the incidence perspective is more appropriate for FBPs, because it is more sensitive to current epidemiological trends, including the effects of intervention measures (Murray 1994; Devleesschauwer et al. 2015a).

Different approaches can be taken for calculating DALYs, depending on whether the interest lies in quantifying the burden of a health outcome, a hazard or a risk factor (Devleesschauwer et al. 2014c). A natural choice for quantifying the health impact of FBPs is the *hazard-based* approach. This approach defines the burden of

**Table 15.1** Health states associated with foodborne parasites, based on Havelaar et al. (2012), Devleesschauwer et al. (2015a) and Torgerson et al. (2015)

| Foodborne parasite | Health state |
|---|---|
| *Cryptosporidium* spp. | Diarrhoeal disease |
| *Entamoeba* spp. | Diarrhoeal disease |
| *Giardia* spp. | Diarrhoeal disease, postinfectious irritable bowel syndrome |
| *Toxoplasma gondii* | Congenital toxoplasmosis: intracranial calcification, hydrocephalus, chorioretinitis, central nervous system abnormalities; acquired toxoplasmosis: acute fever-like illness, post-acute illness, chorioretinitis |
| *Ascaris* spp. | Ascariasis infestation, abdominopelvic problems, wasting |
| *Trichinella* spp. | Acute trichinellosis: diarrhoea, facial oedema, myalgia, fever, headache |
| *Echinococcus granulosus* | Cystic echinococcosis: pulmonary, hepatic, central nervous system problems |
| *Echinococcus multilocularis* | Alveolar echinococcosis: abdominopelvic problems |
| *Taenia solium* | Epilepsy, chronic headache, hydrocephalus |
| Foodborne trematodes | Abdominopelvic, central nervous system, pulmonary problems |

a specific FBP as that resulting from the health states, i.e. acute symptoms, chronic sequelae and death, that are causally related to the concerned parasite transmitted through food and which may become manifest at different time scales or have different severity levels (Mangen et al. 2013). The starting point for quantifying DALYs is therefore typically the construction of a *disease model* or outcome tree, which is a schematic representation of the various health states associated with the concerned hazard, and the possible transitions between these states (Devleesschauwer et al. 2014c).

Table 15.1 shows selected causally related health states for ten important FBPs, highlighting the diverse nature of symptoms and sequelae linked to FBPs (Havelaar et al. 2012; Devleesschauwer et al. 2015a; Torgerson et al. 2015).

Disease burden can be calculated at different levels, ranging from the global to the individual level. Recently, the Foodborne Disease Burden Epidemiology Reference Group (FERG) of the World Health Organization quantified the *global* burden of foodborne disease (Havelaar et al. 2015), including FBPs (Torgerson et al. 2015). Several authors have estimated the burden of FBPs at *country level* to support national decision-making (Polinder et al. 2012). Recently, Trevisan et al. (2017) estimated that in Tanzania, *Taenia solium* neurocysticercosis results in nearly 20,000 new cases of epilepsy per year, leading to over 200 deaths and over 30,000 DALYs. Some studies also include burden estimates at the *individual level*, i.e. the number of DALYs per case. Figure 15.1 contrasts the population and individual level burden of three FBPs in Nepal, highlighting the importance of congenital toxoplasmosis at both levels (Devleesschauwer et al. 2014d).

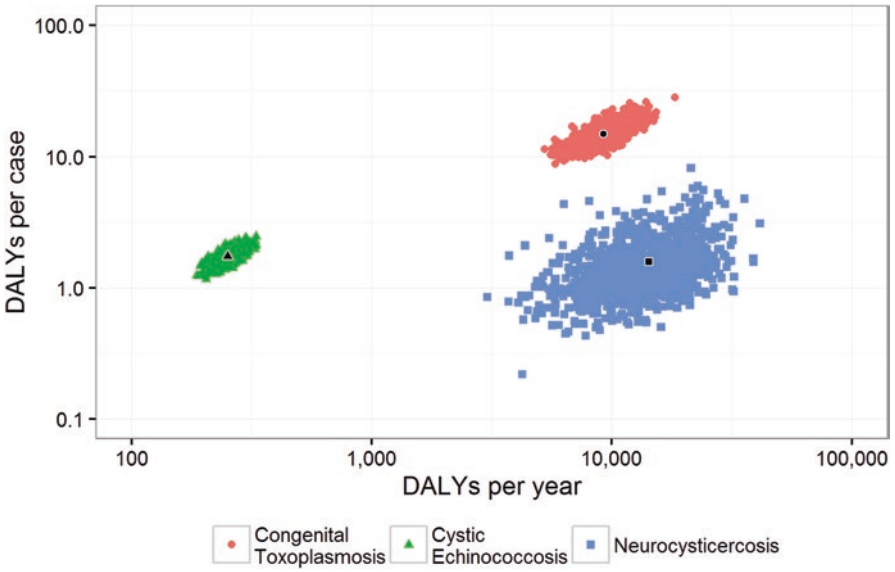


**Fig. 15.1** Population level (x axis) versus individual level (y axis) disease burden of three foodborne parasites in Nepal (Adapted from Devleesschauwer et al. 2014d)

### *15.2.2 Economic Impact*

As for health impact, different approaches exist for estimating the economic impact of FBPs. The most commonly applied approach measures the *cost of illness* from a societal perspective, taking into account that FBPs have an impact on several stakeholders in the society (Mangen et al. 2015). In cost-of-illness studies, a distinction is typically made between direct and indirect costs on the one hand and healthcare and non-healthcare cost on the other hands (Mangen et al. 2010). *Direct healthcare costs* are related to the resources provided by the healthcare sector, such as healthcare provider consultations, diagnosis, medication and hospitalisation. *Direct non-healthcare costs* are related to the resources used for healthcare that are not born by the healthcare system, such as over-the-counter medications and other patient copayments, and travel expenses to visit a healthcare provider. *Indirect non-healthcare costs* include productivity losses due to absenteeism or job loss of patients and their caregivers. *Indirect healthcare costs*, finally, are related to medical consumption in life years gained due to life-saving or death-postponing interventions (van Baal et al. 2011) but are rarely included in cost-of-illness studies.

In Tanzania, the cost of illness of epilepsy due to *Taenia solium* neurocysticercosis was estimated at over 5 million USD, with over 90% due to inactivity-related indirect costs (Trevisan et al. 2017). In the Netherlands, the cost of illness of *Cryptosporidium* spp., *Giardia* spp. and *Toxoplasma gondii* was €8 million, €11

million and €55 million, respectively, accounting for 16% of the economic impact of all considered foodborne pathogens (Mangen et al. 2015). Indirect non-healthcare costs were the most important component of *Cryptosporidium* spp. and *Giardia* spp. cost of illness, while direct healthcare costs were the dominant component of the cost of illness of *T. gondii*.

In addition to the costs linked to their health impact, FBPs may incur an economic impact due to surveillance and other regulatory activities in place to monitor and prevent infection. In the EU, for instance, surveillance of pigs at slaughterhouse level for *Trichinella* spp. induces an estimated annual cost of €25 million (Torgerson 2013), while the health impact of trichinellosis is negligible (Devleesschauwer et al. 2015b). As many FBPs are zoonotic, livestock production losses due to clinical or subclinical infection further add to the economic burden. In Tanzania, the impact of lower prices for infected pigs was estimated at 2.8 million USD, accounting for 35% of the total economic impact of *T. solium* in the country (Trevisan et al. 2017).

Although FBPs are of global concern, there are relatively few assessments of their global economic impact. Budke et al. (2006) estimated global monetary losses resulting from human and livestock cystic echinococcosis. Human-associated direct and indirect costs resulted in a global loss of 764 million USD, while livestock-associated losses due to liver condemnation and reductions in carcass weight, hide value, milk production and fecundity resulted in a global loss of 2 billion USD. Murrell (1991), Roberts et al. (1994) and Torgerson and Macpherson (2011) reviewed the economic impact of FBPs in selected countries.

## 15.3 Quantitative Microbiological Risk Assessment

Risk is defined by the Codex Alimentarius Commission as a function of the probability of an adverse effect consequential to a hazard in food and the severity of that effect (CAC 1999). Following this definition, integrative disease burden metrics such as DALYs and cost of illness are the most appropriate metrics for quantifying risk (Mangen et al. 2007). Nevertheless, risk is often expressed by simple metrics such as incidence of exposure or illness. Risk assessment is the scientific process that aims to examine this risk, in either a qualitative or quantitative way. Qualitative risk assessment results in nonnumerical risk estimates such as "negligible" or "high" risk or in semi-quantitative risk estimates (Ross and Sumner 2002; Newsome et al. 2009). Such models are typically simple and quick to implement but include several subjective steps and do not allow for a full quantification of uncertainty and variability (WHO/FAO 2009). The remainder of this section will therefore focus on quantitative risk assessment. Quantitative microbiological risk assessment (QMRA) aims to quantify the human health effects resulting from exposure to microbiological hazards including parasites, viruses, fungi, bacteria and their toxins. In contrast to chemical risk assessment, QMRA is a relatively young research field, with the emergence of applications to waterborne pathogens in the early 1990s (Rose et al. 1991) and to foodborne pathogens in the mid-1990s (WHO/FAO 2009). Current

QMRA methods are able to consider the uncertainties and variability inherent to any available information and propagate these to the final risk estimate, thus providing an objective scientific basis for decision-making (Lammerding and Paoli 1997). As a result, QMRA methods are increasingly promoted at national and international level to safeguard public health and facilitate free trade (Schroeder et al. 2007), especially in areas of water quality (Macler and Regli 1993) and food safety (Stella et al. 2013). Following the trend towards risk-based standards for foodborne pathogens, the Codex Committee on Food Hygiene explicitly calls for a risk-based approach in their guidelines for the control of *Trichinella* spp. and *Taenia saginata* in meat (FAO/WHO 2014).

QMRA fits into a larger process of risk analysis (Fig. 15.2), which is further characterised by *risk management* (i.e. the identification, selection and implementation of control policies) and *risk communication* (i.e. the mutual interaction between scientists, risk managers and the general public). QMRA itself is classically divided in four components (Fig. 15.2; Buchanan 1998; CAC 1999; Lammerding and Fazil 2000; Buchanan et al. 2000). *Hazard identification* is the process of identifying the biological hazards capable of causing adverse health effects following exposure, as well as the nature of these adverse health effects. *Exposure assessment* is the evaluation of the likely intake of the biological agent via relevant exposure routes. *Dose-response assessment* (the quantitative form of hazard characterisation) is the evaluation of the functional relationship between the ingested dose and the probability of an adverse response, such as infection, illness or death. *Risk characterisation*, finally, is the estimation of the probability of occurrence and severity of the

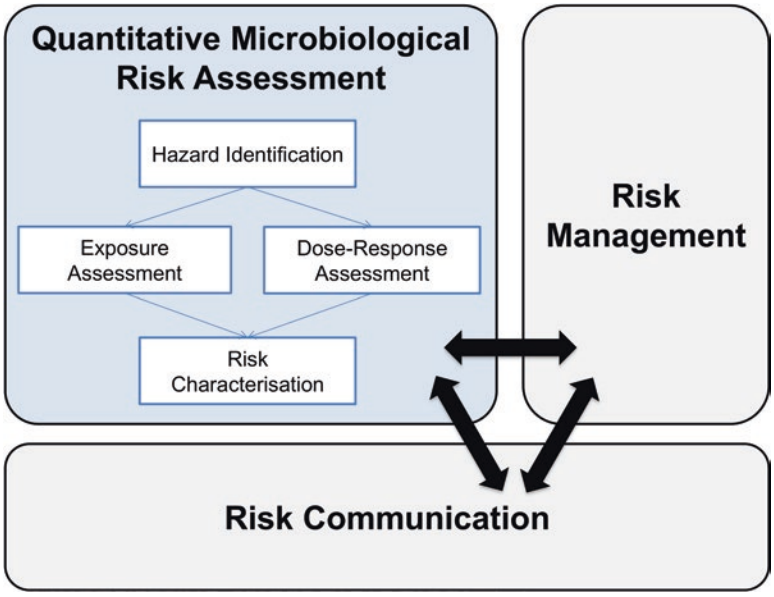


**Fig. 15.2** Risk analysis framework for microbiological hazards

potential adverse health effects in a population, based on the results of the preceding three components.

### 15.3.1 Hazard Identification

Hazard identification is the first step in a formal risk assessment and aims to identify which hazards are present in the food and which adverse health effects they are able to cause. This activity is therefore mainly a qualitative review of available information. The main focus of hazard identification is typically to determine which health states are causally related to the concerned hazards, thus corresponding to the disease models used in disease burden quantifications. This assessment is less involved for FBPs than, for example, chemicals, as the cause-and-effect relationship can typically be observed in individual cases, allowing for categorical attribution. For instance, a case of diarrhoea can be attributed to *Giardia* spp. if a high number of cysts are observed in the stool, or a case of epilepsy can be attributed to *Taenia solium* neurocysticercosis if cysticerci are witnessed in the brain.

However, when the FBP elevates the risk of a disease that occurs in the population from other causes as well, attribution can only be made at a population level and not on an individual basis. This is the case for certain chronic sequelae that may be linked to FBPs, such as irritable bowel syndrome, *Giardia* spp., schizophrenia and *Toxoplasma gondii*. As a result, there is considerably more uncertainty and debate, surrounding these causal relationships. When categorical attribution is not feasible, a valid approach for quantifying the association between exposure and outcome is to use a counterfactual analysis in which the current disease outcomes with current exposure are compared to the disease outcomes under an alternate exposure scenario (e.g. a minimum risk exposure which could be zero or some accepted background level; Prüss-Üstün et al. 2003). This allows for the calculation of a population attributable fraction or population attributable risk, which is a measure of the association between exposure and outcome at the population level. Estimates of the population attributable fraction for *Toxoplasma gondii*-associated schizophrenia, for instance, ranged between 9% (Torgerson et al. 2015) and 21% (Smith 2014).

### 15.3.2 Exposure Assessment

Exposure assessment aims to evaluate the expected dose, $d$, of the ingested hazard (WHO/FAO 2008). Under the assumption of independence, this dose can be obtained as the product of the concentration of the hazard in the ingested medium (e.g. meat, water, aerosol, etc.), $\mu$, and the amount of medium consumed, $m$. Typically, the concentration $\mu$ of the hazard in the medium is not exactly known but must be estimated from random samples from the medium of interest, in which the

concentration, count or the presence/absence of the hazard is measured. By fitting parametric models to these external data sources, the concentration in the medium, $\mu$, can be estimated (Haas et al. 2014). Unless the point of consumption is the only interest, exposure assessment should also describe the relevant exposure pathways along the farm-to-fork chain and the different processes that affect the probability and level of exposure of the consumed end product (WHO/FAO 2008).

#### 15.3.2.1 Exposure Assessment from Concentration Data

Different parametric models may be appropriate to describe the concentration $\mu$ of a FBP. In general, any non-negative continuous probability distribution function may be a possible model. In literature, the most commonly applied distribution has been the log-normal model. Alternative models are the gamma, Weibull, inverse Gaussian (aka Wald) and generalised inverse Gaussian model (Haas et al. 2014).

Depending on the analytical sensitivity of the test, assays may exhibit a certain detection limit or lowest measurable concentration. Samples for which the true concentration is below this detection limit are called below-detection-limit samples. Such samples are an example of left-censored data and require adapted statistical approaches (Haas et al. 2014).

#### 15.3.2.2 Exposure Assessment from Count Data

Count assays provide the most informative data for estimating hazard concentrations, reporting both the number of organisms detected in the sample and the sample quantity (e.g. volume, weight, surface area, etc.), which may differ across samples. Haas and Rose (1996), for instance, presented *Cryptosporidium* oocyst counts from a water supply in the United States. Samples were collected once a week over a one-year study period, with sample volumes ranging from 18 to 227 litres. Surveillance of *Trichinella* spp. in pigs may be done by artificial digestion of a pooled sample of 1 g diaphragm pillar meat from each of 100 pigs, quantifying exposure as the number of larvae in a sample of 100 g (Commission Regulation (EC) No 2075/2005).

The most basic assumption to model $\mu$ from count data is that the distribution of hazards in the sample follows a Poisson distribution with mean $\mu V$, implying that the hazard is distributed randomly in the medium. Under the Poisson model, the probability that a sample of quantity $V$ contains $k$ organisms is given by:

$$P_P\left(x=k\right)=\frac{\left(\mu V\right)^k}{k!}\exp\left(-\mu V\right)$$

Alternative models relax the Poisson assumption of randomness and account for extra-Poisson variability. Such models are discrete mixture distributions $P_M(x)$ of the general form:

$$P_M\left(x=k\right)=\int_k^0 P_P\left(x=k|\mu V\right)h\left(\mu|\beta\right)d\mu$$

in which $P_P(x = k)$ is a Poisson model and $h(\mu)$ is a non-negative continuous probability density function describing the variability in $\mu$, as described above. Assuming a gamma model for the mean concentration $\mu$ leads to a negative binomial model for the counts, while the other models presented above would lead to a Poisson log-normal, Poisson-Weibull, Poisson-inverse Gaussian and Poisson-generalised inverse Gaussian model, respectively (Haas et al. 2014; Jongenburger et al. 2012). Except for the Poisson log-normal and Poisson-Weibull model, analytical solutions exist for these hierarchical mixture models (Haas et al. 2014). Bayesian methods allow modelling these hierarchies explicitly, hereby avoiding the need for an analytical solution.

#### 15.3.2.3 Exposure Assessment from the Presence/Absence Data

Dilution or titration assays report the presence or absence of the hazard in a certain quantity of the concerned medium. Letting $y$ denote the presence/absence of the hazard, the probability of observing the hazard in the sample can be modelled as a Bernoulli process:

$$P\left(y\right)=\pi^y\left(1-\pi\right)^{1-y}$$

where $\pi$ is the probability that the sample contains one or more organisms, $P(k \geq 0) = 1 - P(k = 0)$. Again, if we assume a Poisson model to describe the occurrence of organisms in the sample, this yields $P(k = 0) = \exp(-\mu V)$. The probability of observing the hazard in the sample is therefore given by the following Bernoulli-Poisson mixture model:

$$P\left(y\right)=\left(1-\exp\left(-\mu V\right)\right)^y\left(\exp\left(-\mu V\right)\right)^{1-y}$$

#### 15.3.2.4 Farm-to-Fork Pathway Models

Exposure assessment often requires a description of the pathway from production to consumption. Indeed, microbiological hazards can enter foods at many points in the chain, and their prevalence and concentration may change along the chain. The transmission of pathogens along a farm-to-fork pathway may be modelled using mathematical models in which the output of the previous step is the input of the next step. To formalise this idea, Nauta (2008) introduced the *modular process risk model* (MPRM), which models the food pathway as a sequence of well-defined modules. Each module corresponds to one-*process step*, which in term is defined by

one or more *basic processes*. These basic processes can be *product handling processes*, such as mixing, partitioning, removal and cross-contamination, or *microbiological processes*, such as growth and inactivation. To support the modelling of microbiological processes, an online database of microbial growth and survival curves, *ComBase*, has been developed (Baranyi and Tamplin 2004).

In a QMRA model for *Toxoplasma gondii* in the Netherlands, Opsteegh et al. (2011) used bradyzoite concentration and portion size data to estimate the bradyzoite number in infected unprocessed portions for human consumption. To estimate the number of bradyzoites per processed portion, they applied reduction factors for salting, freezing and heating (i.e. corresponding to the microbiological process of inactivation). Guo et al. (2015) estimated the exposure risk to *T. gondii* from various meat products consumed in the United States based on a qualitative farm-to-retail pathway model. The included modules were farm, abattoir, storage and transportation, meat processing, packaging and retail, with two product handling processes, i.e. removal and cross-contamination, considered in the different modules.

### *15.3.3 Dose-Response Assessment*

The dose-response assessment, also referred to as the quantitative component of hazard characterisation, aims to describe the relationship between the ingested dose of the hazard and the extent of the associated adverse health effects (Buchanan et al. 2000). The dose-response relationship for microbiological hazards shows some essential differences with that of chemical hazards.

First, a clear distinction needs to be made between infection and illness. In QMRA, infection is defined as the invasion (and multiplication) of the microbiological hazard in the host and the reaction of the host to these events. Infection may be ascertained by the detection of the hazard in the host's tissues, secreta or excreta or by detecting antibodies against the hazard. Infection does not necessarily lead to disease and may remain asymptomatic. Whether or not illness develops depends on the balance between the hazard's virulence and infectivity and the host's susceptibility. In severe cases, illness will be followed by death. Unlike for chemical hazards, it is postulated that even one microbiological organism is sufficient to cause illness, even though the likelihood of illness increases with increasing numbers of ingested hazards (Teunis and Havelaar 2000).

A second important distinct feature of the dose-response assessment step for microbiological hazards is the type of data typically used to establish dose-response curves, i.e. human feeding trials or outbreak data (Mena 2006). Extrapolation of animal studies for determining dose-response is less commonly applied, in contrast to chemical risk assessment. In feeding trials, participants are given a range of doses (through ingestion, inhalation or direct contact), and a human health endpoint of interest (infection and/or illness) is determined. Typically, due to ethical constraints, healthy adults are used, and a relatively high amount of (low virulent) dose is administered to be able to use as few participants as possible but still observe the

adverse event. However, in typical contamination situations, people will be exposed to lower doses of (more virulent) hazards. An important issue in dose-response modelling is therefore the prediction of the probability of the adverse health effect in low-dose ranges (Havelaar and Swart 2014).

A third distinct feature is that microbiological organisms are distinct or discrete particles, which is accounted for when constructing dose-response models. Finally, unlike chemical hazards, repeated exposures may induce acquired immunity, thereby reducing the risk of infection and/or illness (Havelaar and Swart 2014).

#### 15.3.3.1 Dose-Infection Models

Different models have been proposed to model the relationship between exposure and infection. It is common in QMRA literature to distinguish between *mechanistic* models, which have a biological basis, and *empirical* models, for which no biological basis is apparent (Buchanan et al. 2000).

Currently, the exponential and Beta-Poisson models, both introduced by Haas (1983), are recommended for foodborne and waterborne hazards (Schroeder et al. 2007). Both models are mechanistic models, derived from a biological reasoning underlying the relationship between dose and infection. This biological process is assumed to consist of distinct sequential steps. First, a host must ingest one or more viable hazard. Then, some of these ingested hazards must survive the host defences and multiply to cause infection. Further assumptions can be made about the minimum number of surviving organisms $k_{\min}$ needed to initiate infection. Models in which $k_{\min} = 1$ are called *single-hit models*. Empirical evidence shows that, under certain circumstances, single hazards are indeed able to cause infection; a single viable egg of *Echinococcus* can, for instance, cause infection and disease (Gemmell 1990). Hence, the common consensus is to derive mechanistic dose-infection models that are single-hit models. However, for nematodes such as *Trichinella* spp., there must be at least one male and one female survivor to initiate infection, requiring adapted modelling approaches (Teunis et al. 2012).

The exponential dose-response model assumes that the ingested doses among exposed hosts are Poisson distributed and that each ingested hazard is associated with an equal, independent probability of initiating a response:

$$\Pr\left(\text{infection}|\text{dose}\right) = 1 - \exp\left(-rd\right),\ r > 0$$

where $d$ is the ingested dose and $r$ is a rate parameter equal to the probability of a single organism initiating a response.

Messner and Berger (2016) extended the basic exponential dose-response model with a parameter $\pi$ reflecting the susceptible fraction of human hosts, leading to an exponential *with immunity* model:

$$\Pr\left(\text{infection}|\text{dose}\right) = \pi\left(1 - \exp\left(-rd\right)\right),\ r > 0,\ 0 < \pi < 1$$

When $r$ is assumed to equal 1, i.e. each hazard is capable of initiating infection, this model reduces to a fractional Poisson model, which is also the probability of exposure given an average dose $d$:

$$\Pr\left(\text{infection}|\text{dose}\right) = \pi\left(1-\exp\left(-d\right)\right),\ 0<\pi<1$$

In the Beta-Poisson model, ingested doses are assumed to be Poisson distributed as well, but now the probability that an individual organism initiates a response is assumed to follow a Beta distribution. As a result, the Beta-Poisson model allows to characterise variability of the host-pathogen survival probability. The exact Beta-Poisson model is given by:

$$\Pr\left(\text{infection}|\text{dose}\right) = 1-{}_1F_1\left(\alpha,,\alpha+\beta,,-d\right),\ \alpha>0,\ \beta>0$$

where ${}_1F_1$ denotes the Kummer confluent hypergeometric function and $\alpha$ and $\beta$ are the parameters of the Beta distribution. Furumoto and Mickey (1967) proposed an approximation to the confluent hypergeometric function, resulting in the more commonly used approximate Beta-Poisson model:

$$\Pr\left(\text{infection}|\text{dose}\right) = 1-\left(1+d/\beta\right)-\alpha,\ \alpha>0,\beta>0$$

For many datasets where $\beta \gg 1$ and $\alpha \ll \beta$, the differences between the approximation errors provided by applying the approximate Beta-Poisson as compared to those of the hypergeometric function are negligible. However, Teunis and Havelaar (2000) showed that this approximation is in fact not a single-hit model and that it becomes problematic at low doses when the assumptions postulated by Furumoto and Mickey (1967) are not met. Furthermore, even when the most likely values of $\alpha$ and $\beta$ do satisfy the conditions, the assumptions may not be met for every individual pair of parameter samples in the uncertainty set. Therefore, they promote the use the exact Beta-Poisson model.

In addition to the mechanistic models, various so-called empirical models are used to describe the relationship between ingested dose and expected response (Buchanan et al. 2000). These models do not arise from biological reasoning but follow the logic that the probability of a response should be zero at dose zero, reach one when dose becomes very high and increase monotonically in between. These are properties of sigmoidal functions, including cumulative distribution functions. Some of the empirical models described in literature are the log logistic, log probit, extreme value, Weibull and gamma-Weibull model (Haas et al. 2014; Holcomb et al. 1999).

#### 15.3.3.2 Dose-Illness Models

In contrast to dose-infection models, less work has been performed on models describing the relationship between dose and onset of *illness*. Historically, researchers have assumed that the probability of illness following infection is independent

of the dose (Rose et al. 1991; Haas et al. 1993; Haas and Rose 1996). Under this assumption, the probability of illness given dose can be factorised as follows:

$$\Pr(\text{illness}|\text{dose}) = \Pr(\text{illness}|\text{infection})\Pr(\text{infection}|\text{dose})$$

Pouillot et al. (2004), for instance, modelled the probability of illness following *Cryptosporidium* spp. infection in the immunocompetent as a Beta (9,11) distribution, which has a mean of 45%. For the immunocompromised, they assumed a probability of illness of 100%.

In general, however, the dose-illness relationship is given by Namata et al. (2008):

$$\Pr(\text{illness}|\text{dose}) = \Pr(\text{illness}|,\text{infection}|,\text{dose})\Pr(\text{infection}|\text{dose})$$

According to Teunis et al. (1999), the probability of illness given infection and dose can in theory follow any of three distinct shapes, i.e. increasing, decreasing or constant. When the probability of illness is increasing, the resulting dose-illness relationship has a similar shape as the dose-infection models, so that these models can be applied to model dose-illness. A general empirical framework for modelling all possible dose-illness relationships has been proposed by Namata et al. (2008) and Bollaerts et al. (2008), who used fractional polynomials and generalised linear mixed models to model dose-illness relationships for *Campylobacter* and *Salmonella*. So far little work has been done on mechanistic dose-illness models, especially not for FBPs. Buchanan et al. (2000) presented a mechanistic dose-illness model composed of three compartments, i.e. gastric acidity barrier, attachment/infectivity and morbidity/mortality. Havelaar and Swart (2014) discussed dose-illness models that can incorporate both the effects of dose-dependency and acquired immunity in the probability of illness given infection.

#### 15.3.3.3 Dose-Response Models for Foodborne Parasites

Table 15.2 provides an overview of proposed dose-response models for FBPs. To date, most dose-response assessments of FBPs have focused on intestinal protozoa, particularly *Cryptosporidium* spp., and were typically performed in the context of waterborne transmission. Recently, dose-response models have been introduced for *Toxoplasma gondii* and for macroparasites such as *Ascaris lumbricoides* and *Trichinella* spp.

Information on *Cryptosporidium* spp. dose-response first became available from three feeding studies in healthy human volunteers initiated in 1993 at the University of Texas (DuPont et al.1995) ; Chappell et al. 1999); Okhuysen et al. 1999). These studies used three distinct *Cryptosporidium parvum* isolates, referred to as the Iowa, TAMU and UCP strains. Messner et al. (2001) fitted exponential dose-response models to these datasets and performed a random effect meta-analysis of the three

**Table 15.2** Estimated dose-response models for foodborn parasites

| Foodbome parasite | Response | Model | Reference |
|---|---|---|---|
| *Cryptosporidium parvum*, Iowa strain | Oocyst detection | Exponential ( $\hat{r}$ = 0.00419) | Teunis et al. (1999) |
| *Cryptosporidium parvum*, Iowa strain | Clinical definition of infection | Exponential ( $\hat{r}$ = 0.00526) | Messner et al. (2001) and Teunis et al. (2002a) |
| *Cryptosporidium parvum*, Iowa strain | Clinical definition of infection | Hypergeometric ( $\hat{\alpha}$ = 0.801, $\hat{\beta}$ = 56.24) | Teunis et al. (2002a) |
| *Cryptosporidium parvum*, Iowa strain; immunocompromised mice | Oocyst detection | Exponential ( $\hat{r}$ = 0.354) | Pouillot et al. (2004) |
| *Cryptosporidium parvum*, TAMU strain | Clinical definition of infection | Exponential ( $\hat{r}$ = 0.0573) | Messner et al. (2001) and Teunis et al. (2002a) |
| *Cryptosporidium parvum*, TAMU strain | Clinical definition of infection | Hypergeometric ( $\hat{\alpha}$ = 1.831, $\hat{\beta}$ = 18.06) | Teunis et al. (2002a) |
| *Cryptosporidium parvum*, UCP strain | Clinical definition of infection | Hypergeometric ( $\hat{\alpha}$ = 1.17e−5, $\hat{\beta}$ = 8.15e−6) | Teunis et al. (2002a) |
| *Cryptosporidium* spp., pool of seven datasets | Clinical definition of infection | Exponential with immunity ( $\hat{\neq}$ = 0.737, $\hat{r}$ = 0.608) | Messner and Berger (2016) |
| *Cryptosporidium* spp., pool of seven datasets | Clinical definition of infection | Fractional Poisson ( $\hat{\neq}$ = 0.737) | Messner and Berger (2016) |
| *Cryptosporidium* spp., pool of seven datasets | Clinical definition of infection | Beta-Poisson ( $\hat{\alpha}$ = 0.116, $\hat{\beta}$ = 0.121) | Messner and Berger (2016) |
| *Giardia* spp. | Cyst excretion | Exponential ( $\hat{r}$ = 0.0198) | Rose et al. (1991) |
| *Toxoplasma gondii*, type II; mice | Bioassay positive | Exponential ( $\hat{r}$ = 0.001535) | Dubey (1997), Derouin et al. (2005), Opsteegh et al. (2011), and Guo et al. (2015) |
| *Toxoplasma gondii*, type II; mice, with scaling factor | Bioassay positive | Exponential ( $\hat{r}$ = 0.001535 × 0.005) | Guo et al. (2015) |
| *Toxoplasma gondii*, type II; mice | Bioassay positive | Beta-Poisson ( $\hat{\alpha}$ = 1.479, $\hat{\beta}$ = 582.4) | Guo et al. (2015) |

(continued)

**Table 15.2** (continued)

| Foodbome parasite | Response | Model | Reference |
|---|---|---|---|
| *Toxoplasma gondii*, type II; mice, with scaling factor | Bioassay positive | Beta-Poisson ( $\hat{\alpha}$ = 1.479, $\hat{\beta}$ = 582.4/0.003) | Guo et al. (2015) |
| *Ascaris lumbricoides*; exposure through crops | Egg excretion | Beta-Poisson ( $\hat{\alpha}$ = 0.104, $\hat{\beta}$ = 1.096) | Navarro et al. (2009) |
| *Ascaris lumbricoides*; exposure through soil | Egg excretion | Beta-Poisson ( $\hat{\alpha}$ = 0.104, $\hat{\beta}$ = 0.044) | Navarro et al. (2009) |

datasets, highlighting the important heterogeneity between the three involved strains. Teunis et al. (2002a) fitted exponential and hypergeometric dose-response models to the individual and pooled datasets and developed a two-level dose-response model to simultaneously model the heterogeneity between and within isolates. By including the IgG level as a covariate in the single-hit model, they further explored the effect of immunity on the relationship between dose and infection or acute illness (Teunis et al. 2002b). Recently, Messner and Berger (2016) compiled seven human challenge dose-response studies for *Cryptosporidium* spp., including five studies using *C. parvum* and one each using *Cryptosporidium hominis* and *Cryptosporidium muris*. The best fitting models indicated that human susceptibility may be a more important source of heterogeneity than virulence differences. Pouillot et al. (2004), finally, proposed an exponential dose-response model for the immunocompromised, based on a feeding study in immunocompromised mice.

The only *Giardia* spp. dose-response model available in literature is given by Rose et al. (1991). They fitted an Exponential dose-response model to data from human feeding studies published in the 1950s. As noted by Teunis et al. (1999), these results indicated a high single-hit probability of infection but, as none of the infected persons developed gastroenteritis, did not provide any information on the probability of illness.

In a QMRA for meat-borne *Toxoplasma gondii* infection in the Netherlands, Opsteegh et al. (2011) used an exponential dose-response model established for *T. gondii* type II in mouse experiments (Dubey 1997); Derouin et al. 2005). Guo et al. (2015) found that this dataset was reasonably well explained by the exponential dose-response model and less so by the Beta-Poisson dose-response model. They also computed scaling factors so that these mouse-derived models could predict *T. gondii* infection in humans.

Schönning et al. (2007) and Machdar et al. (2013) modelled the dose-response relationship of *Ascaris lumbricoides* as an exponential model with $r = 1$, corresponding to the maximum risk curve (Teunis and Havelaar 2000). Navarro et al. (2009) estimated a Beta-Poisson dose-response model for *A. lumbricoides*, by combining *A. lumbricoides* infection rates from the Mezquital Valley in Mexico with data on *Ascaris* egg concentrations in crops and soil.

Takumi et al. (2009) and Teunis et al. (2012) analysed nine published outbreaks of human trichinellosis to determine the dose-response of *Trichinella* spp. in humans, using seroconversion as response outcome. They extended the Beta-Poisson dose-response model by allowing for extra-Poisson variation in the ingested dose and by taking into account that both male and female worms need to be ingested to initiate infection. Their model resulted in a single-hit infection probability of around 1%.

### *15.3.4 Risk Characterisation*

Risk characterisation is the final stage of QMRA and generates an estimate of the likelihood and extent of the adverse health effects a population will experience due to concerned hazard. This is achieved by integrating exposure and dose-response assessments, where the outcomes of the former analysis serve as an input to the latter analysis (Buchanan et al. 2000).

A crucial aspect of risk characterisation is the proper reflection of the level of confidence in the final risk estimates. In this context, the identification and quantification of uncertainty and variability play an important role, as well as a clear articulation of all assumptions and their support. Uncertainty relates to a lack of knowledge about the system (i.e. model uncertainty) or the parameters characterising the system (i.e. parameter uncertainty). Uncertainty can be mitigated by gaining knowledge, e.g. by collecting information on a larger sample size. Variability on the other hand relates to the stochastic nature of the system and cannot be reduced by increasing the sample size.

In earlier years, uncertainty was explored through scenario or one-way sensitivity analyses, generating, for instance, an average, conservative and worst-case risk estimate (Jaykus 1996). Improvements in computational power have now resulted in Monte Carlo or Latin hypercube simulation methods becoming the standard approach for propagating parameter uncertainty. In brief, these methods simulate random draws from probability distributions reflecting the uncertainty in the input parameters and use these random draws to establish a distribution of plausible risk estimates. Two-dimensional Monte Carlo methods may furthermore be used to additionally, but separately, capture the effects of variability (Miconnet et al. 2005; Pouillot and Delignette-Muller 2010).

Recently, Bayesian methods are increasingly used in QMRA to propagate parameter uncertainty (Delignette-Muller et al. 2006; Smid et al. 2010; Williams et al. 2011). With increasing computational power, Bayesian models may now be developed to capture the often complex processes leading to human exposure (Greiner et al. 2013; Schmidt et al. 2013).

A variety of software tools are being applied in the context of QMRA, some of which specifically developed for the purpose of risk assessment. To date, the most comprehensive tool for QMRA is FDA-iRisk, a web-based quantitative risk

assessment system to estimate and compare the risk of foodborne illness from microbial and chemical hazards (Chen et al. 2013). iRisk integrates seven model elements, i.e. definition of foods, hazards and population groups; process models (cf. MPRM); consumption models; dose-response models; and health outcomes (with default DALY per case values for selected hazards). The different model inputs are integrated through Monte Carlo simulations to quantify variability in the resulting risk estimates.

Other QMRA tools are dedicated to risk characterisation, exposure assessment or dose-response assessment. Risk characterisation is often performed in **Excel** with the add-on **@RISK**, which provides random number generators for a variety of probability distributions and functionalities for sensitivity analysis and plotting. Other add-ons, such as **PopTools**, **Ersatz** and **Crystal Ball**, provide similar features. **Analytica** is a visual software environment for the quantitative analysis of influence diagrams, which may also be applied in the context of QMRA. Other analysts developed models in general scientific software environments such as **R**, **SAS** and **MATLAB** (WHO/FAO 2008). Several **R** packages provide specific QMRA functions, e.g. **mc2d**, which is dedicated to two-dimensional Monte Carlo (Pouillot and Delignette-Muller 2010) and **fitdistrplus**, which allows fitting univariate models to (possibly censored) data.

## 15.4 Risk Ranking

In a time of increasing (recognised) threats and decreasing financial resources, there is a growing need to rationally allocate available means. Consequently, risk ranking is increasingly used within the food safety risk analysis framework (Stella et al. 2013). The aim of these exercises is to prioritise for decision-making certain hazards, hazard-commodity pairs or exposure routes for a given hazard, based on their perceived importance. As different stakeholders have their own prioritisation objectives and beliefs, the outcome of such exercises is necessarily context dependent. Consequently, there is no unique or intrinsically correct ranking of risks.

The main purpose of burden and risk assessments is often to rank different hazards according to the health or economic impact. Figure 15.3 shows the FERG ranking of FBPs according to their global health impact, quantified in terms of DALYs (Torgerson et al. 2015).

Using a single criterion to rank risks may however be insufficient as diseases vary greatly in incidence, clinical manifestations, control measures, transmission potential and socio-economic impact in animals and humans. *Trichinella* spp., for instance, have a near negligible health impact in Europe, while their economic impact remains important due to continuous monitoring and trade implications (Devleesschauwer et al. 2015b). It may therefore be required to base the ranking of risks on multiple criteria (Mangen et al. 2010). To quantify the disease burden of various foodborne hazards in the Netherlands in 2011, Mangen et al. (2015)

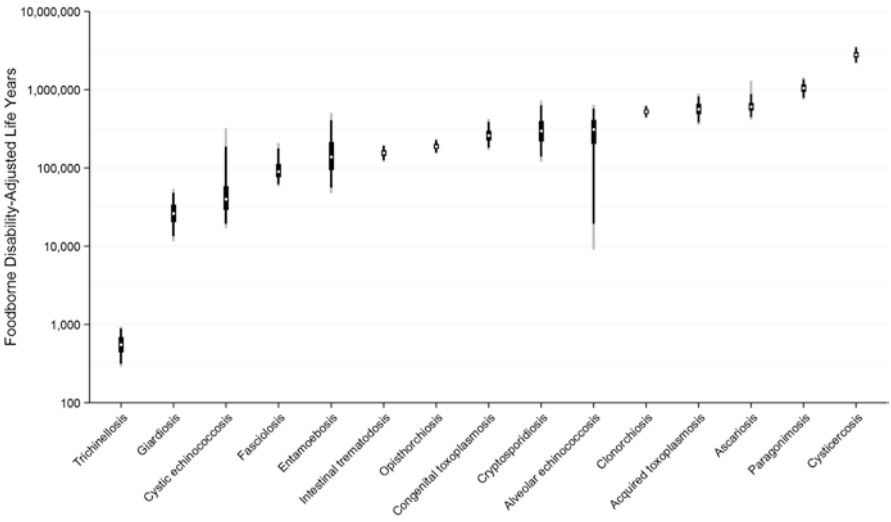


**Fig. 15.3** Disability-adjusted life year-based global ranking of foodborne parasites according to Torgerson et al. (2015)

calculated DALYs and cost-of-illness estimates, both at the population and individual level. The different criteria led to different rankings, with some hazards scoring high on multiple criteria.

Ideally, however, a risk ranking exercise should result in a single ranking taking into account multiple criteria. Some authors combined DALYs and economic impact estimates by assuming one DALY to correspond to an economic loss equal to the per capita gross national product (Torgerson et al.(2008). This approach belongs to the family of *multi-criteria decision analysis* (MCDA) methods. In MCDA, an overall importance measure is constructed based on different criteria, which are assigned weights reflecting their perceived contribution (Cardoen et al. 2009; Havelaar et al. 2010; FAO/WHO 2014; Robertson et al. 2015). As these weights imply a normative choice, the definition of weights should reflect social preferences or expert opinion. The selection of criteria to be scored typically depends on expert opinion. The (quantitative, semi-quantitative or qualitative) scoring of the criteria can be based on existing data or on expert elicitation. Despite its subjective perfume, MCDA provides a transparent and consistent framework for ranking risks (Anderson et al. 2011). It also allows including criteria for which no quantifications are available or possible. In the joint Food and Agriculture Organization of the United Nations and World Health Organization multi-criteria-based ranking of FBPs, trade relevance and impacts on economically vulnerable communities were included, in addition to criteria related to health impact. Figure 15.4 shows the outcome of this exercise, confirming the importance of *Taenia solium* at a global level.

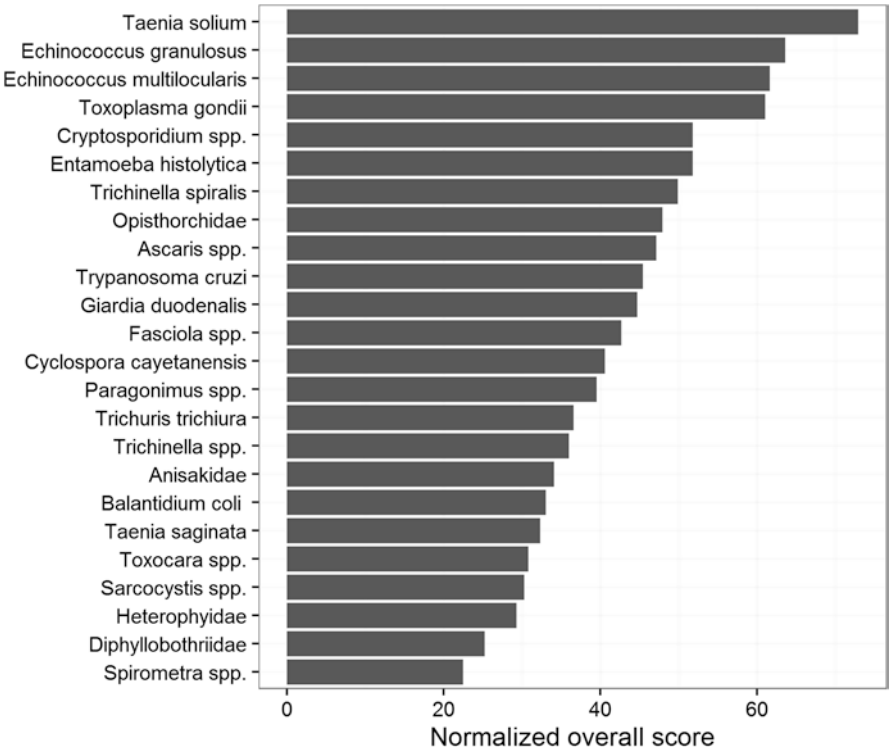


**Fig. 15.4** Multi-criteria-based global ranking of foodborne parasites according to FAO/WHO (2014)

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