Review

Zoonotic helminth infections with particular emphasis on fasciolosis and other trematodiases

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Zoonotic infections are among the most common on earth and are responsible for >60 per cent of all human infectious diseases. Some of the most important and well-known human zoonoses are caused by worm or helminth parasites, including species of nematodes (trichinellosis), cestodes (cysticercosis, echinococcosis) and trematodes (schistosomiasis). However, along with social, epidemiological and environmental changes, together with improvements in our ability to diagnose helminth infections, several neglected parasite species are now fast-becoming recognized as important zoonotic diseases of humans, e.g. anasakiasis, several fish-borne trematodiases and fasciolosis. In the present review, we discuss the current disease status of these primary helminth zoonotic infections with particular emphasis on their diagnosis and control. Advances in molecular biology, proteomics and the release of helminth genome-sequencing project data are revolutionizing parasitology research. The use of these powerful experimental approaches, and their potential benefits to helminth biology are also discussed in relation to the future control of helminth infections of animals and humans.

Keywords: zoonosis; helminth; nematode; trematode; cestode; Fasciola

1. INTRODUCTION

Zoonotic infectious agents are among the most prevalent on earth and are thought to be responsible for >60 per cent of all human infections and 75 per cent of emerging human infectious diseases (Cunningham 2005). The success and widespread epidemiology of these infections can be attributed to a range of human factors including social and dietary changes as well as an increased mobility of the human population (McCarthy & Moore 2000; Vorou et al. 2007). As the human population continues to grow there is an ever increasing need to develop and maintain food products with a high protein content (particularly livestock and fish) under intensive farming situations, which is inevitably leading to a greater spread of animal diseases and their transmission to humans (McCarthy & Moore 2000; Keiser & Utzinger 2005). Improved diagnosis and/or recognition of neglected human infections can account for some diseases apparently emerging or re-emerging in recent times (e.g. human fasciolosis). Climate change has also been suggested as a cause for disease spread and is a concern for the future (McCarthy & Moore 2000).

Zoonotic infections of humans are caused by a wide variety of agents including bacteria (e.g. brucellosis and salmonellosis), viruses (e.g. avian influenza and rabies), parasites (e.g. leishmaniasis, schistosomiasis) and other ‘unconventional’ agents such as prions (e.g. bovine spongiform encephalopathy and variant Creutzfeldt–Jakob disease). Human infections caused by parasitic helminths are of particular importance given the relatively recent acknowledgement of a number of species as important human pathogens (McCarthy & Moore 2000; Mas-Coma et al. 2005; Garcia et al. 2007). In the present review, we will focus on some of the major zoonotic and food-borne helminth pathogens of humans (table 1). With particular emphasis on trematode infections, we aim to highlight their current disease status and show where advances in genomics, proteomics and molecular biology may lead to improved diagnosis and control of these important pathogens.

2. ZOONOTIC NEMATODE INFECTIONS

(a) Trichinellosis

Parasitic nematodes of the genus Trichinella are remarkable as the mature L1 larva occupies two distinct intracellular niches within a single vertebrate host (the intestinal epithelia and the skeletal muscle), whereas the immature L1 larvae are solely extracellular (Despommier 1983). After ingestion of infected meat, the L1 larvae are released from muscle tissue by host-digestive enzymes in the stomach. The free L1 larvae then migrate to the small intestine where they penetrate the intestinal mucosa and undergo four successive molts, becoming mature adult worms within little
more than 24 h. Mating also occurs within this niche and from this site the newborn larvae migrate via the blood and lymphatic systems to skeletal muscle, where they infect the myofibres and develop into the encysted infective L1 stage (reviewed by Despommier 1998).

Human infection occurs following consumption of raw or undercooked meat containing encysted Trichinella larvae. Symptoms are varied (including nausea, vomiting, diarrhoea, fatigue, fever and abdominal discomfort) and the severity of the disease is dependent on the dose of infective larvae ingested. Infected individuals can also suffer from heart and breathing problems and in severe cases death can occur (Bruschi & Murrell 2002; Pozio et al. 2003). The disease is best treated early with a combination of benzimidazoles and anti-inflammatory corticosteroids (e.g. prednisone (Shimoni et al. 2006)).

The range of ambiguous and changing symptoms that occur during human infections often leads to misdiagnosis of the disease. The definitive method for diagnosis of trichinellosis is microscopic analysis of muscle biopsies though this has limited use for detection of light and moderate infections. More recent alternatives include detection of antibodies against Trichinella spiralis excretory–secretory (E–S) proteins in human sera by enzyme-linked immunosorbent assay (ELISA; Moskwa et al. 2000) and polymerase chain reaction (PCR)-based identification approaches (Wu et al. 1999; Zarlanga et al. 2001). The release of the T. spiralis genome sequence draft assembly (Washington University Genome Sequencing Center; http://genome.wustl.edu/home.cgi) together with recent advances in proteomics for the identification of individual Trichinella E–S proteins has expanded the panel of Trichinella antigens that may be used to detect trichinellosis earlier post-infection (Robinson & Connolly 2005; Robinson et al. 2005, 2007). The current status of nucleotide sequence databases for the major zoonotic helminths is summarized in table 2.

In recent years, trichinellosis has been considered to be a truly emerging (or re-emerging) zoonosis owing to increased infection rates as a direct result of changing human dietary trends and the breakdown of veterinary management practices in several developing countries (Pozio 2001; Vorou et al. 2007). Indeed, human cases of the disease have been documented in 55 (27.8%) countries around the world (Pozio 2007). By far, the major causative agent of trichinellosis is T. spiralis (Vorou et al. 2007) although cases of human infection caused by other Trichinella species, including Trichinella pseudospiralis, Trichinella nativa, Trichinella murrelli and Trichinella britovi have been reported (Jongwutiwes et al. 1998; Pozio 2001). The major reservoir host for T. spiralis is the domestic pig which has been responsible for a growing number of outbreaks of trichinellosis in eastern European countries since the break-up of the USSR in the early 1990s (Pozio 2001). The European countries most affected by human Trichinella infection include Poland where there have been a number of outbreaks within the last 8 years (e.g. Golab et al. 2007), the Slovak Republic (Reiterová et al. 2007) and the Baltic states of Lithuania, Latvia and Estonia (Malakauskas et al. 2007). Consumption of raw horsemeat has led to cases of human trichinellosis in Italy and France (Pozio 2001) and even the practice of hunting and eating wild animals (including wild boar and bears) has contributed to a number of human infections worldwide (Ancelle et al. 2005; De Bruyne et al. 2006). For a comprehensive review of the worldwide incidence of Trichinella infections of humans and animals see Pozio (2007).

(b) Anisakiasis

Anisakiasis results from infection with the larvae of the nematodes Anisakis simplex and Pseudoterranova decipiens. In humans, infection occurs following...
The occurrence of human anisakiasis has risen dramatically since it was first described almost 50 years ago with a number of human and environmental factors likely contributing to the rise (van Thiel et al. 1960). The advent of the endoscope has undoubtedly led to the detection of more cases of human anisakiasis and this, together with advances in the immunological diagnosis of the disease, should see that this trend will continue (Rodriguez et al. 2008). Changing dietary trends may also have contributed to the spread of human anisakiasis. Eating raw fish such as sushi (a practice that has been ongoing in Japan for many centuries) has become highly fashionable in many western societies and has led to an increased risk of infection of populations that would not usually be exposed. It has also been suggested that greater numbers of the natural definitive hosts of anisakid nematodes (large marine mammals such as whales, dolphins, sea-lions and seals) brought about by increased regulatory controls over hunting has resulted in greater levels of contamination of fish used for human consumption (McCarthy & Moore 2000).

These trends have clear implications for human health given the severe hypersensitivity reactions that can occur against anisakid antigens (Audicana et al. 2002; Moneo et al. 2007). At least seven different protein allergens have been identified from *A. simplex*, some of which are known to be heat-stable (e.g. Kobayashi et al. 2007). In fact, cooking or freezing of fish containing *A. simplex* antigens may not be sufficient to prevent allergic reactions to the parasite in humans (Audicana et al. 2002). Moreover, it has been reported that ingestion of chickens that were fed on fishmeal contaminated with *A. simplex* antigens caused hypersensitivity reactions in anisakid-sensitized individuals (Armentia et al. 2006). At present, there is no genome-sequencing project for *Anisakis*. However, a small-scale expressed sequence tag (EST) project identified 493 sequences that provide a useful first look at the transcriptome of the infectious *A. simplex* L3 stage larva (Yu et al. 2007a). The continued use of molecular biology approaches to identify and characterize anisakid allergens is central to our understanding of anisakiasis and will aid the diagnosis and treatment of the disease in the future.

### 3. ZOONOTIC CESTODE INFECTIONS

#### (a) Cysticercosis

The major cause of human cysticercosis is the cestode parasite *Taenia solium* (pork tapeworm) although infections of *Taenia saginata* (beef tapeworm) also occur. Human infection occurs upon ingestion of raw or under-cooked meat of the intermediate host containing larval cysts known as cysticerci. In the human intestine, the cysticerci develop into adult tapeworms that release proglottids (containing tapeworm eggs)
that are passed in the faeces and eaten by the intermediate host (Garcia et al. 2007). The disease is found in generally poor regions with poor sanitation and estimates suggest that while around 2.5 million people carry the adult tapeworm, between 20 and 50 million people are infected with *T. solium* cysticerci worldwide (Pawlowski et al. 2005; Kraft 2007). Infection with adult *Taeniae* can often be asymptomatic however it is infection of the central nervous system by cysticerci (neurocysticercosis) that poses the greatest risk to human health. Neurocysticercosis is the most common parasitic disease of the brain and is associated with the occurrence of epilepsy. A recent field study has suggested that neurocysticercosis is responsible for around one-third of all cases of epilepsy in India (one million people; Rajshekhar et al. 2006), and conservative figures suggest that at least 50 000 people die each year as a result of the disease (Maføjane et al. 2003).

Cysticercosis is endemic in many parts of urban and rural Latin America where humans live in close proximity to their swine. Human seroprevalence (indicated by exposure to *Taeonia* eggs but not necessarily an infection) is, on average, 10 per cent in Colombia, Brazil, Mexico, Peru, Honduras, Ecuador, Guatemala, Bolivia and Venezuela but ranges from 1.3 to 36.5 per cent (Pawlowski et al. 2005). National campaigns against human *Taeonia* infections are rare in Latin America (Pawlowski et al. 2005), however, since 2002 the Bill and Melinda Gates Foundation has pledged over US $15 million in sponsorship for a programme to eliminate the disease in an endemic area of Peru (Garcia et al. 2007).

Cysticercosis is fast-becoming recognized as an emerging zoonosis in some developed countries, notably the USA. Sorvillo et al. (2007) report that for the period 1990–2002 there were 221 deaths in the USA as a result of cysticercosis. Although most cases occurred in immigrants from Mexico, 33 (15%) US-born individuals also died from the disease. Human *Taeonia* infections are also a growing problem in parts of Africa and Asia, where it is linked to an increase in pigs farming by small-holders (Boa et al. 2006; Prakash et al. 2007).

Despite the fact that infection rates are on the rise, taeniasis/cysticercosis is considered to be an eradicable disease. The drug of choice for human *Taeonia* infections is praziquantel although niclosamide is also recommended. While these drugs offer a highly effective treatment for the disease, reports of praziquantel- and niclosamide-resistant *Taeonia* populations are emerging (Lateef et al. 2008); hence the use of both drugs should be tightly regulated in the future. Early diagnosis of *Taeonia* infection is crucial for the prevention of cysticercosis in humans and a number of coproantigen (Allan & Craig 2006) and serological tests (Garcia et al. 2007) based on purified parasite antigens are now available. Such tests offer extremely high sensitivity and specificity and are often used in conjunction with magnetic resonance imaging (MRI) or computed tomography (CT) scanning to confirm the presence of *Taeonia* cysts in neurocysticercosis. Recently, a specific nested PCR technique for the diagnosis of *T. solium* infection has been developed and shows promise under field conditions (Mayta et al. 2008). The launch of the *T. solium* genome-sequencing project (Aguilar-Diaz et al. 2006) will aid gene discovery in this parasite and will in no doubt lead to future molecular diagnostic tests for human cysticercosis.

Considerable progress has been made in the development of vaccines against *Taenia* (Lightowlers 2006). The most successful vaccines (up to 100% protection) have used antigens purified from *Taenia* oncospheres (Pawlowski et al. 2005) and advances in recombinant protein expression are now allowing the production of protective antigens on a large scale (Cai et al. 2008). In a recent vaccine trial, purified recombinant *Taeniae multiceps* oncosphere antigens (designated Tm16 and Tm18) were administered to sheep. Vaccination with Tm16 alone or together with Tm18 offered significant levels of protection against a challenge infection of *T. multiceps* eggs (Gauci et al. 2008) and it is envisaged that these studies may lead to a combined vaccine against *Taeonia* and *Echinococcus* infections.

(b) *Echinococcus*

Cystic and alveolar echinococcosis (hydatid disease), caused by the cestode parasites *Echinococcus granulosus* and *Echinococcus multilocularis*, respectively, are a major cause of morbidity in many parts of the world including much of Europe, North America and South America (Craig et al. 2007; Garcia et al. 2007). The adult tapeworms reside in the small intestine of the definitive hosts (typically domestic dogs for *E. granulosus* and foxes for *E. multilocularis*) and release eggs that are passed in the faeces and ingested by herbivorous intermediate hosts. The eggs release oncospheres that eventually form cysts in various organs, including the liver and lungs. The definitive hosts become infected after eating these tissues.

*Echinococcus granulosus* infections are more common in humans and typically occur in poor pastoral communities where livestock are slaughtered in open areas with little or no veterinary management practices (Craig et al. 2007). Here, domestic dogs are often fed infected offal (liver and lungs) and act as definitive hosts that pass on the infection to humans (Jenkins et al. 2005). Major endemic regions of *E. granulosus* include parts of South America notably the central and southern Peruvian Andes, southern Brazil, Argentina and southern parts of Chile (Moro & Schantz 2006). *Echinococcus granulosus* also occurs throughout the Middle East and Mediterranean (Romig et al. 2006; Sadjjadi 2006), parts of east Africa (Magambo et al. 2006) and Australia (Jenkins 2006). In contrast, *E. multilocularis* is typically found in central Europe, Russia and Asia (Garcia et al. 2007) where prevalence in humans of up to 15 per cent has been reported in some villages in China (Craig 2006).

In cystic echinococcosis, the fluid-filled hydatid cysts take considerable time to develop and can be well-tolerated by their hosts, which often results in asymptomatic infections (Craig et al. 2007; Garcia et al. 2007). They almost always form in the liver and lungs (90% of cases) and have well-defined
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limits of development. In contrast, cysts of *E. multilocularis* almost invariably form in the liver and often spread to surrounding tissues or organs such as the brain (Craig et al. 2007; Garcia et al. 2007). As for cysticercosis, diagnosis of human hydatid disease is highly reliant on imaging techniques such as MRI, ultrasound and radiology. Although serological tests based on native or recombinant *Echinococcus* antigens are available, a recent study suggests that they may be of limited use in the field (Gavidia et al. 2008). A PCR-based assay has shown promise for the early detection of *E. granulosus* in dogs, so it is likely that future diagnostic tests for human echinococcosis will also make use of molecular biology techniques (Lahmar et al. 2007).

Echinococcosis has long been considered to be an eradicable disease. Unlike cysticercosis, eradication of *Echinococcus* (*E. granulosus*) infections has been achieved in several countries/regions including Iceland, Tasmania, New Zealand, Cyprus and the Falkland Islands as a result of concerted efforts in the field and long-term political support (Jenkins et al. 2005; Craig et al. 2007). In areas where human infections remain, surgical removal of larval cysts is the preferred treatment, however, bezimidazole anthelmintics (such as albendazole) can be effective against both alveolar and cystic hydatid disease with or without surgery (El-On 2003). Future control programmes for echinococcosis are likely to depend on the reduction of transmission of the parasite to humans and there have been a number of developments in vaccine design in recent years. For instance, oral vaccines using recombinant *E. granulosus* proteins (tropomyosin and a paramyosin-like protein) have shown promise, reducing parasite burdens by up to 80 per cent in dogs (Petavy et al. 2008). Currently, sequencing and assembly of a reference genome for *E. multilocularis* is underway at the Wellcome Trust Sanger Institute (http://www.sanger.ac.uk/Projects/Echinococcus/) while a whole genome shotgun is planned for *E. granulosus*. A further 60 000 ESTs from *E. multilocularis* and *E. granulosus* metacestodes will be sequenced for comparative purposes. These unique resources will dramatically expand the biological data for these parasites and will open the door to novel diagnostic methods and treatments for human echinococcosis.

4. ZOONOTIC TREMATODE INFECTIONS

(a) Fasciolosis

(i) Parasites, life cycle and distribution

*Fasciola hepatica* and *Fasciola gigantica* are the major causative agents of liver fluke disease (fasciolosis) in domestic animals in regions with temperate and tropical climates, respectively. Fluke eggs, released by adult parasites residing in the bile ducts of the mammalian host, are carried into the intestine and are passed in the faeces. Embryonation occurs only after the eggs are liberated from the faecal matter and is dependent on a number of physio-chemical factors, although temperature and the availability of moisture are considered most important (Andrews 1999). The eggs hatch and release free-swimming miracidia that find and penetrate the tissues of their molluscan intermediate hosts: *F. hepatica* typically infects the freshwater snail *Galba truncatula* (formerly known as *Lymnaea truncatula*) in Europe and parts of Asia, whereas *F. gigantica* can infect a greater range of snail species including *Lymnaea natalensis* in Africa and *Lymnaea rubiginosa* in Asia (Malone 1997). Within the digestive gland of the infected snail, the parasite undergoes a series of dramatic developmental changes (sporocyst → rediae → cercariae) and multiplications that ends in the release of thousands of free-swimming cercariae. These adhere to and encyst, as metacercariae, on vegetation and are infective to the definitive host. Following ingestion of the vegetation contaminated with metacercariae by the definitive host, the parasites excyst in the small intestine and juvenile liver fluke penetrate through the gut wall and enter the peritoneal cavity. The fluke spend a period of time wandering over the viscera before locating, and penetrating the liver parenchyma. After about 10–12 weeks they enter the bile ducts where they mature and may remain for up to 1–2 years in cattle or as long as 20 years in sheep (Andrews 1999).

*Fasciola hepatica* is of European origins but its geographical distribution has expanded over the last five centuries as a result of global colonizations by Europeans, and the associated continual export of livestock. *Fasciola hepatica* infections in livestock have now been reported on every continent (except Antarctica) with highest rates of infection occurring in temperate countries such as Bolivia, Peru, Iran, Portugal, Egypt and France (Garcia et al. 2007). This expansion has been greatly facilitated by a remarkable ability of the parasite to adapt to new hosts—flukes can develop, mature and produce viable offspring even in very recently encountered species such as llama and alpaca in South America, camels in Africa and kangaroos in Australia (Mas-Coma et al. 2005). *Fasciola hepatica* also infects a wide variety of wild animals including deer, rabbits, hare, boars, beavers and otters, which, collectively, are major reservoir host populations that contribute significantly to the worldwide dissemination of the disease and to its local transmission patterns.

*Fasciola gigantica* is estimated to have diverged from *F. hepatica* approximately 17 million years ago (Irving et al. 2003) and penetrated more tropical regions in Asia and the Far East where it is the predominant parasitic disease of cattle and water buffalo (McManus & Dalton 2006). *Fasciola hepatica* infects more than 300 million cattle and 250 million sheep worldwide and, together with *F. gigantica*, causes significant economic losses to global agriculture; estimated at more than US$3 billion annually through lost productivity, such as a reduction of milk and meat yields (Mas-Coma et al. 2005).

(ii) Human fasciolosis

Although traditionally regarded as a disease of livestock, fasciolosis is now recognized as an important emerging zoonotic disease of humans. Prior to 1992, the total number of reported human cases of fasciolosis was estimated to be less than 3000. More recent
figures suggest that between 2.4 and 17 million people are currently infected with a further 91.1 million living at risk of infection (Keiser & Utzinger 2005). Human infections normally occur in areas where animal fasciolosis is endemic. Transmission occurs where rural farming communities regularly share the same water source as their animals or consume water-based vegetation growing in endemic areas.

The highest prevalence of human fasciolosis is found in the Altiplano region of Northern Bolivia. Infection is highest in children and females, and prevalence can reach over 40 per cent in certain communities (Mas-Coma et al. 2005). The Bolivian Altiplano is the greatest expanse of arable land in the Andes (approx. 3700 m.a.s.l.) and is inhabited mainly by the indigenous Aymaran population (Parkinson et al. 2007). The prevalence of animal and human fasciolosis corresponds to snail distribution, which is restricted to the northwest of the Altiplano. Snail infection is promoted by animal reservoirs such as sheep, cattle, pigs, llamas and alpacas. In Europe, larval development within the snail is halted in winter as low temperatures influence the development of the free living and intra-molluscan stages of the life cycle and outbreaks occur in early spring as daytime temperature increases to above 9°C. In contrast, temperature is not an important limiting constraint in disease transmission in the Bolivian Altiplano as all-year average night-time and day-time temperatures range from 0 to 6°C and 18 to 22°C, respectively. Furthermore, unlike the European mud-snail, snails in the Altiplano reside almost wholly sub-aqua and are observed on aquatic plants during the dry season at a time when animals and humans collect around the shrinking water sources.

During the height of the rainy season, Lake Titicaca and its tributaries overflow causing extensive flooding, optimizing conditions for transmission. A recent meta-analysis of 10 epidemiological surveys from 38 communities in the region showed that human fasciolosis has been endemic there since at least 1984 and is a significant zoonosis in rural communities (Parkinson et al. 2007). Infection levels as high as 67 per cent of the human population were reported in areas adjacent to tributaries of Lake Titicaca where corresponding high levels of animal infection was also observed. As *F. hepatica* can survive for up to 13 years in humans, producing large numbers of eggs (up to 5000 per gram of faeces in some Bolivian children) that are infective to other hosts, humans are likely to play an important role in transmission of the disease (Mas-Coma et al. 1999a). Surveys carried out in Peru, north of Lake Titicaca, show that human fasciolosis is prevalent here too and suggests that human infection exists along the Altiplano corridor as far as Equador (Mas-Coma et al. 2005).

Transmission in humans is linked to their dietary habits since individuals, particularly children, supplement their diet with aquatic plants during daily animal husbandry. The main types of aquatic plants are 'berro berro' (watercress), 'algas' (algae), kjosco and totora (Mas-Coma et al. 1999a, 2005). Drinking untreated water may be a source of infection due to the presence of free-floating metacercarial cysts. Vegetables washed in contaminated water may also become a source of infection. The incidence of infection is almost inevitably aggregated within familial groups that share contaminated food and drink from a common water source (Mas-Coma et al. 2005).

Hyperendemic human fasciolosis has also been reported in the Nile Delta region between Cairo and Alexandria (Esteban et al. 2003). This study suggests that initial World Health Organization estimates of human fluke infection in the Nile Delta region (830,000 people) may fall considerably short given the high prevalence of the disease (up to 19% of the total population) in some villages. Fawzi et al. (2004) reported that in a suburban village in Alexandria, public knowledge of the risks of *Fasciola* infection via consumption of contaminated vegetables was very poor suggesting that sustained public awareness schemes are required if human fasciolosis is to be controlled in the future. A recent report shows that selective chemotherapy of humans over a 4-year period has been effective in reducing the prevalence of human fasciolosis in the Nile Delta (Curtale et al. 2005).

Significant levels of human *F. hepatica* infections also occur, with regular outbreaks involving up to 10,000 infections, in the Gilan and Mazandaran provinces that border the Caspian Sea of northern Iran (Rokni et al. 2002; Moghaddam et al. 2004). In this region high animal fasciolosis also exists and, like the Altiplano, animals are free to roam among arable land. Outbreaks in relatively large towns, such as Rasht, have been associated with the consumption of aquatic plants sold by farmers of the surrounding countryside at local markets. Since reports of human fasciolosis are increasing from Turkey it is also plausible that problems of human fascioliasis are widespread around the Caspian Sea (Mas-Coma et al. 2005).

In Europe human fluke infections occur more sporadically, however significant and recurrent outbreaks of the disease occur in France, Portugal and Spain causing 50–100 infections per year (Mas-Coma et al. 2005). Human infection is mostly due to consumption of aquatic plants, such as watercress, on which *Fasciola* metacercariae have settled. While farm-management practices, including the culture of edible aquatic plants in greenhouses, reduce the risk of human infection, the disease remains an important human health problem in these countries. Sporadic cases of human fasciolosis have been diagnosed in the USA, but these are usually imported, i.e. migrants or returning tourists (Graham et al. 2001).

To date, the majority of reported human cases of fasciolosis are due to infections of *F. hepatica*. However, some reports indicate a rise in human infections due to *F. gigantica* in Vietnam (Hien et al. 2001; Mas-Coma et al. 2005). It is likely that a detailed epidemiological survey of the Thailand, Vietnam and Cambodia region would reveal greater levels of human *F. gigantica* infection given the climate, farming practices and dietary trends that promote other trematodiases in southeast Asia. Recently, infections of humans, cattle and buffalo caused by *F. hepatica*–*F. gigantica* hybrids have been identified in Vietnam, which raises important issues for diagnosis (Le et al. 2008).
(iii) Clinical manifestations and diagnosis

Fasciola infection has two major distinct clinical phases: acute fasciolosis corresponds to the migratory stages of the life cycle, whereas chronic fasciolosis is due to the presence of the mature adult flukes in the bile ducts (Mas-Coma et al. 1999b; Garcia et al. 2007). Most symptoms of acute fasciolosis (including fever, abdominal pain, hepatomegaly and a range of gastrointestinal disturbances) result from the destruction of liver tissue by the migrating immature flukes and can lead to abnormal liver function tests. eosinophilia and mild or moderate anaemia are also common features of acute liver fluke infection. Chronic fasciolosis is often sub-clinical or shows symptoms that are indistinguishable from other hepatic conditions such as cholangitis, cholecystitis and cholelithiasis. In endemic areas, F. hepatica infection is often recurrent and lesions due to acute fasciolosis are superimposed upon chronic disease (Mas-Coma et al. 1999b). Few human deaths have been attributed to fasciolosis; cases that do prove fatal usually involve complications such as ulceration of the bile duct and acute haematochilia (Acuna-Soto & Braun-Roth 1987) or very high levels (more than 40 adult flukes) of chronic infection (Chen & Mott 1990).

Definitive diagnosis of human fasciolosis relies primarily on detection of fluke eggs in faecal matter. For this, the thick smear Kato–Katz method (also used for diagnosis of Schistosoma infections) has been widely used and is a popular choice for epidemiological surveys (Mas-Coma et al. 1999b). The test is not completely reliable as eggs are passed sporadically with the bile juices and low infection levels may not be detected. Some progress has been made in PCR techniques for identification of F. hepatica and F. gigantica infections (McGarry et al. 2007). Alternatives to coprology involve immunological methods although these do not distinguish between past and present infections. Several ELISAs for Fasciola have been developed and most rely on the detection of antibodies against fluke-secretory proteins (O’Neill et al. 1999; Espinoza et al. 2007; Marcilla et al. 2008). An accurate serological test using recombinant cathepsin L protease produced in yeast has been developed by Dalton and colleagues (O’Neill et al. 1999). This test can be applied to blood samples taken onto filter paper (Strauss et al. 1999) and has been validated in various regions including Bolivia and Iran.

(iv) Control of fasciolosis

Control of human fasciolosis depends on reducing infection in animals and preventing the contamination of edible aquatic plants with infective metacercariae. This could be achieved by introducing good control programmes that involve widespread drug treatment of humans and animals coupled with educating the affected farming communities on aspects of parasite transmission and the importance of separating animals from areas where food is grown. Since infection is highly age-related, with highest prevalence in children between 8 and 11 years old, drug treatment should be particularly focussed towards children in high-risk areas (local schools being a focal point).

Unlike for other trematodiasis, praziquantel is ineffective against Fasciola infections. Triclabendazole (TCBZ), a benzimidazole derivative, has been the drug of choice for treatment of liver fluke infections in livestock for more than 20 years and is also used to treat human fasciolosis (Fairweather 2005). In contrast to most other fasciolicides, TCBZ has high efficacy (approximately 99%) against both the mature adult worms in the bile duct and the migratory immature flukes, which is significant since the immature flukes cause most tissue damage during their migration through the liver (Fairweather & Boray 1999).

Due to the huge success (and likely overuse) of TCBZ against liver fluke infections in livestock, populations of F. hepatica that are resistant to TCBZ have emerged in Ireland, the UK, The Netherlands, Spain and Australia (Brennan et al. 2007). Being a benzimidazole derivative, TCBZ is thought to act as a microtubule inhibitor (Robinson et al. 2002) while other potential actions include inhibition of protein synthesis and oxidative phosphorylation (Fairweather & Boray 1999). The precise mode of action of TCBZ still remains to be fully determined, although recent studies indicate that TCBZ resistance in F. hepatica is conferred by a combination of an altered drug influx–efflux mechanism (Alvarez et al. 2005; Mottier et al. 2006) together with the ability to metabolize the drug into potentially inert (or less active) metabolites (Robinson et al. 2004; Alvarez et al. 2005) rather than as a consequence of mutations in the genes encoding the putative TCBZ target molecule, β-tubulin (Robinson et al. 2002). It is reassuring that other flucicidal compounds retain their efficacy against TCBZ-resistant fluke populations. For instance, a TCBZ-resistant isolate from Sligo, Ireland was shown to be susceptible to the salicylamide anthelmintic closantel (Coles et al. 2000) and the sulphonamide clorsulon (Coles & Stafford 2001). These studies are encouraging and suggest that existing anti-fluke drugs can still be used where TCBZ resistance is a problem.

The spread of TCBZ resistance has also fuelled the search for novel fasciolicidal compounds. A TCBZ derivative, designated compound alpha, has proven to be highly active against immature and adult F. hepatica (Fairweather 2005) and has recently been shown to disrupt the tegument of flukes from a TCBZ-resistant isolate (McConville et al. 2007). Other experimental fasciolicides such as artemether and OZ78 also have activity against TCBZ-resistant flukes (Keiser et al. 2007).

An alternative or additional advance in the control of animal fasciolosis would be the development of anti-Fasciola vaccines. Given the current political climate regarding the deposition of agrichemical residues, together with more onerous legislation governing the development of new anthelmintic drugs, it is increasingly likely that the future control of helminth infections may rely heavily on this approach. Although a range of vaccines against F. hepatica infections have been developed by several laboratories (reviewed by McManus & Dalton 2006; table 3) none are commercially available at present. However, since the
incidence of animal fasciolosis is increasing in Europe and anthelmintic resistance continues to spread, it is more likely that a vaccine will offer a level of protection that is comparable with the existing anti-fluke drugs and, hence, become more financially attractive to pharmaceutical companies. This is a critical issue as the manufacture and marketing of any future anti-helminth vaccine product is likely to depend on the ability of researchers to attract a strategic alliance with an industrial partner (Dalton & Mulcahy 2001).

(y) Recent advances in fasciola biology
At present, there is no large-scale genome-sequencing project for *F. hepatica*. However, a total of 11,066 ESTs from four separate cDNA libraries (comprised of 4265, 702, 2767 and 3332 reads) constructed from mRNA isolated from adult *F. hepatica* are currently available from the Wellcome Trust Sanger Institute (ftp://ftp.sanger.ac.uk/pub/pathogens/Fasciola/hepatica/ESTs). The existing EST sequence databases have yet to be formally annotated, and are likely to remain so, but they are already proving to be a valuable resource for the discovery of novel *Fasciola* genes (Russell et al. 2007). The level of redundancy of the various fluke EST databases has not been addressed, although around 15 per cent of the first database to be released (655 out of 4265 sequences) is represented by cDNAs encoding cathepsin L proteases (M. W. Robinson, personal observations). *Fasciola hepatica* cathepsin L proteases have functions in parasite virulence including tissue invasion and suppression of host-immune responses and are the most abundant proteins found in fluke E/S products (Morphew et al. 2007; Robinson et al. 2008). Recently, McGonigle et al. (2008) reported the first successful use of RNA interference technology against *F. hepatica* and demonstrated that cathepsin L and cathepsin B proteases are necessary for penetration of the host intestinal wall by juvenile flukes.

As with other helminth pathogens, the application of proteomics has significantly improved our knowledge of *F. hepatica* secretory proteins and thus has widened the panel of antigens available for diagnostics and vaccine discovery (Jeffries et al. 2001; Morphew et al. 2007; Robinson et al. 2008). Proteomics is also a particularly valuable tool for studying changes in parasite protein expression; for example, proteomic analysis of *F. hepatica* following in vitro culture with TCBZ-sulphoxide has led to the identification of fluke proteins (including a 70 kDa heat-shock protein) that are specifically upregulated in a TCBZ-resistant isolate (Brennan et al. 2007).

(b) Schistosomiasis
Schistosomiasis (bilharzia) is a tropical disease caused by digenean trematodes of the genus *Schistosoma*. The impact of the disease on the human population is vast as it is endemic in 70 countries and more than 207 million people are believed to be infected worldwide with a further 779 million people currently living at risk of infection (Steinmann et al. 2006). The species responsible for most human infections are *Schistosoma mansoni* which is endemic throughout Africa and South America, *Schistosoma haematobium* which also occurs in Africa and the Middle East and *Schistosoma japonicum* which causes schistosomiasis in China, the Philippines and Indonesia (Gryseels et al. 2006). Although the major hosts for *S. mansoni* and *S. haematobium* are humans, a range of animals including sheep, cattle and horses act as natural definitive hosts for *S. japonicum* and, hence, this parasite can be considered truly zoonotic.

Transmission of the schistosome parasite to the definitive host occurs in fresh water. Free-swimming cercariae, released from the freshwater snails that act as intermediate hosts, penetrate the skin of the definitive host and migrate in the blood through a number of tissues before they eventually come to reside in the mesenteric blood vessels of the intestine. Here, the adult worms pair and, following sexual reproduction, the female parasitises release thousands of eggs that make their way through the intestine or bladder wall and are passed in the faeces or urine. On contact with water, the eggs release miracidia that infect the intermediate host, thus completing the life cycle (Gryseels et al. 2006). Many eggs, however, are carried to the liver where they become trapped and induce T cell-dependent immune responses that lead to the development of granuloma that destroy the liver architecture and eventually cause hepato-megaly and splenomegaly (Malenganisho et al. 2008).

The current drug of choice for use against human schistosomiasis is praziquantel. The drug was developed in the 1970s and, due to its extremely low toxicity against mammalian cells and high efficacy against all *Schistosoma* species, it has become the most widely used against the disease (Caffrey 2007). At present, praziquantel-resistant schistosome populations do not seem to be posing a major problem for chemotherapy of human infections. However, reports of human infections that do not respond to standard praziquantel treatments suggest that resistance may be appearing in the field (Alonso et al. 2006).

Compared with the African schistosomes (*S. mansoni* and *S. haematobium*), *S. japonicum* is particularly pathogenic and being a zoonotic infection a larger proportion of the human population is at risk. Recent studies suggest that the burden of human disease due to *S. japonicum* infection is much greater than thought previously.

<p>| Table 3. Leading vaccine candidates for <em>F. hepatica</em> infections (summarized from McManus &amp; Dalton 2006). |</p>
<table>
<thead>
<tr>
<th>antigen type</th>
<th>host</th>
<th>protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>leucine aminopeptidase</td>
<td>native</td>
<td>rabbit 89.6</td>
</tr>
<tr>
<td>fatty acid-binding protein</td>
<td>recombinant</td>
<td>rabbit 81.0</td>
</tr>
<tr>
<td>glutathione S-transferase</td>
<td>native</td>
<td>sheep 65.0</td>
</tr>
<tr>
<td>cysteine protease (FhCL1/FhCL2)</td>
<td>native</td>
<td>cattle 65.0</td>
</tr>
</tbody>
</table>

*Only the highest level of protection obtained in the various trials for each antigen is shown.*
(King et al. 2005; Finkelstein et al. 2008). This is particularly relevant to China where more than 40 million people are susceptible to infection (McManus & Dalton 2006). An ongoing control programme over the last 50 years including treatment with praziquantel has had a major impact on the spread of the disease in China. However, data from the third national survey of schistosomiasis in China, carried out in 2004, suggests that although the total number of people infected in endemic areas has fallen to approximately 726,112 human infections, the rate of human infection has increased by almost 4 per cent (Zhou et al. 2007). Environmental changes resulting from the construction of the Three Gorges Dam on the Yangtze River in Southern China have been attributed with the creation of conditions favourable for schistosome transmission (McManus & Dalton 2006; Zhou et al. 2007). Consequently, control of human schistosomiasis remains a major political issue in China.

Control of *S. japonicum* requires an understanding of local transmission dynamics, which can even vary between villages within the same region. Transmission is not only determined by local ecology and snail populations, but also by the relevant contribution of reservoir hosts as *S. japonicum* can infect many different non-human mammals. Recent studies on transmission in the Philippines, where approximately 6.7 million people are believed to be threatened by the disease, indicates that even rats may play an important role in contributing to the infection cycle (Riley et al. 2008). Furthermore, the study indicates that water buffaloes may have less impact on the transmission cycle in the Philippines than they are reported to have in China. Understanding the role of each host, their availability and abundance is important particularly for implementing vaccine or drug treatment programmes aimed at both humans and the reservoir hosts (He et al. 2001; Riley et al. 2008). This is further complicated by the possibility that large genetic variability may exist between strains of *S. japonicum* in different regions of countries (He et al. 2001).

The gold standard for diagnosis of human schistosomiasis is microscopic examination of eggs in stool samples. The Kato–Katz or thick smear method is often used in the field and gives an egg count that correlates well with actual worm burdens and morbidity (Gryseels et al. 2006). Antibody-based methods that detect human immunoglobulins against schistosome egg or worm antigens are also used. However, these do not distinguish between previous and current infections, and, because of problems with specificity, they are often poorly suited to field conditions (Yu et al. 2007b). Recently, a highly sensitive PCR-based assay, for detection of *S. japonicum*, has been developed in a pig model of infection (Lier et al. 2008). This approach is particularly useful in cases with very low faecal egg counts, however the cost per sample may restrict its use in developing countries.

With the current absence of reliable and effective alternatives to praziquantel, the future control of human schistosomiasis may rely on the development of a vaccine. A number of anti-schistosome vaccine candidates that induce significant levels of protection in animals are currently under investigation. The majority are parasite membrane proteins or enzymes and include glutathione *S*-transferases, paramyosin, very low density lipoprotein-binding protein, serine protease inhibitors, fatty acid-binding protein, triose-phosphate isomerase and a 23 kDa integral membrane protein (reviewed by McManus & Dalton 2006). Most schistosome vaccine candidates are delivered as purified recombinant proteins, however some DNA vaccines have also shown promise (Dai et al. 2007). The search for novel drug targets and vaccine candidates will no doubt be enhanced by the availability of schistosome genome sequence information. A draft assembly of the *S. mansoni* genome (Haas et al. 2007) has been produced by the Wellcome Trust Sanger Institute (http://www.sanger.ac.uk/Projects/S_mansoni/) and, in an equivalent project, the genome of *S. japonicum* has been sequenced by the Chinese Human Genome Center in Shanghai (http://lifecenter.sgst.cn/sj.do). This information, together with associated transcriptomic and proteomic projects (Oliveira 2007; van Hellemond et al. 2007) provide the most complete biological dataset currently available for a helminth parasite. Armed with this wealth of information, we can be optimistic about the control of human schistosomiasis in the future.

(c) Fish-borne trematodiasis: clonorchiasis, opisthorchiasis and paragonimiasis

Fish-borne trematodiasis caused by liver flukes (*Clonorchis sinensis* and *Opisthorchis viverrini*) and lung flukes (*Paragonimus* spp.), are emerging as a major public health issue in many parts of the world, with particular prevalence throughout Southeast Asia. For instance, the human incidence of clonorchiasis has tripled since the mid-1990s with an estimated 15 million people infected in China alone (Lun et al. 2005). Moreover, figures from 2005 reveal that 601.0, 293.8 and 79.8 million people are living at risk of infection with *Clonorchis sinensis*, *Paragonimus* spp. and *Opisthorchis* spp., respectively (Keiser & Utzinger 2005).

The parasites that cause clonorchiasis, opisthorchiasis and paragonimiasis share very similar life-cycles and are all transmitted to humans by the consumption of raw or undercooked freshwater fish and crustaceans. In general, eggs passed in the faeces of infected humans embryonate in freshwater and hatch miracidia that infect the first intermediate host, freshwater snails. Within the snail, the miracidia goes through several developmental stages (sporocysts, rediae, cercariae) before free-swimming cercariae are released that then infect and encyst as metacercariae within the second intermediate hosts (typically freshwater fish). Humans become infected by eating fish carrying the trematode metacercariae and, therefore, communities living near bodies of freshwater suitable for transmission have an estimated 2.15-fold greater risk of infection than those living farther from water (Keiser & Utzinger 2005). Increased human trematodiasis are also likely to occur owing to the expansion of aquaculture. In 2004, 32 per cent (approx. 52.5 million tons) of global fish production came from aquaculture (Brander 2007). As this figure is projected to rise to...
50 per cent by 2030 (Brander 2007), the emergence/re-emergence of fish-borne trematodiasis looks set to continue in the foreseeable future.

Diagnosis of the various fish-borne fluke infections depends largely on the microscopic examination of eggs in human faecal samples, but this approach requires significant training and experience and is not always practical (Chai et al. 2005). Consequently, a number of alternative diagnostic methods are being developed. Molecular assays, using PCR, have been developed for Opisthorchis viverrini (Wongratanacheewin et al. 2002), Paragonimus spp. (Intapan et al. 2005) and C. sinensis (Parvathi et al. 2007) that offer improved sensitivity. Advances in recombinant protein expression are facilitating the development of ELISA-based detection methods. Recombinant antigens showing encouraging results in these applications include a secreted C. sinensis lysophosphatidic acid phosphatase (Hu et al. 2007) and a major egg antigen from P. westermani (Lee et al. 2007). Medical imaging techniques such as MRI and CT have also been used for the diagnosis of trematodiasis (particularly clonorchiasis) and are a valuable alternative to more conventional diagnostic tests (Choi & Hong 2007).

As for schistosomiasis, the drug of choice for fish-borne trematode infections is praziquantel. At present, praziquantel-resistant fluke populations are not thought to be widespread, however reports are beginning to emerge of low cure rates for the drug against clonorchiasis (Tinga et al. 1999) and paragonimiasis (Sumitani et al. 2005). Relatively few vaccine candidates for clonorchiasis, opisthorchiasis or paragonimiasis have been investigated. DNA vaccines encoding a C. sinensis cysteine protease (similar to mammalian and trematode cathepsin L proteases) and a C. sinensis fatty acid-binding protein have induced reasonable levels of protection (31.5% and 40.9%, respectively) in rats following challenge infection with C. sinensis metacercariae (Lee et al. 2006a,b). Zhao et al. (2007) have shown that paramyosin from P. westermani is expressed on the tegumental surface of the parasite and can successfully induce host-immune responses suggesting that it may be a good vaccine candidate.

Long-term infection with fish-borne liver flukes, such as C. sinensis and O. viverrini, is associated with the aetiology of a number of complications including pyogenic cholangitis, biliary calculi, cholecystitis, liver cirrhosis and pancreatitis though the most serious is cancer of the bile duct or cholangiocarcinoma (Chai et al. 2005). Throughout East Asia cholangiocarcinoma is highly prevalent in areas where liver flukes are endemic and there is presently no stronger link between a eukaryotic parasite and human cancer (Sripa et al. 2007). The mechanism of liver fluke-induced carcinogenesis has yet to be determined, although secretory proteins from O. viverrini induced proliferation of cells in culture suggesting that the parasites actively secrete carcinogenic molecules (Thuwajit et al. 2004). Proteomic analysis of parasite secretory proteins may lead to the identification of carcinogenic molecules from fish-borne liver flukes; however, the success of such a project will depend on the availability of fluke nucleotide sequence datasets.

At present, there are no large-scale genome-sequencing projects for any of the fish-borne trematodes. Valuable sequence information has been generated from several independent EST projects: approximately 5000 ESTs have been generated from O. viverrini adult flukes (Laha et al. 2007) and around 500 ESTs each are available from diploid and triploid P. westermani adults (Kim et al. 2006). Two adult C. sinensis EST libraries have been sequenced generating around 2400 sequences (Cho et al. 2006) and 415 sequences (Lee et al. 2003) each. Recently, a further 419 ESTs have been identified from the C. sinensis metacercarial stage (Cho et al. 2008) providing a useful preliminary dataset for comparative studies.

5. CONCLUDING REMARKS

Despite advances in our understanding of the biology of helminth parasites, zoonotic helminth infections remain endemic in many parts of the world. Most cases of zoonotic infection are preventable through good farm management, personal hygiene and food preparation practices. However, large-scale drug treatment of infected humans and animals together with widespread public education programmes is also likely to be required to decrease infection rates, especially in developing countries. This will only be possible with sustained political support.

The need for basic laboratory research on zoonotic helminths is stronger than ever. Recent advances in technology, particularly in the so-called post-genomics arena, have created opportunities for the identification of proteins expressed by helminth parasites. Of particular interest are those proteins that act at the host–parasite interface (E–S proteins), which have roles crucial for successful parasitism. As discussed, they are often leading drug targets and vaccine candidates as well as the principal components in diagnostic tests. These proteins are often difficult to identify due to the fact that many parasites are relatively intractable to study using conventional culture and analysis techniques and to the often low abundance of the proteins themselves. Increasingly, proteomic analysis is being used as a means to circumvent these difficulties and there are now a growing number of studies using a range of helminths that demonstrate the value of this approach. This, together with the ongoing release of helminth genome sequence information, is revolutionizing parasitology research by providing important datasets as well as the principal components in diagnostic tests. These proteins are often difficult to identify due to the fact that many parasites are relatively intractable to study using conventional culture and analysis techniques and to the often low abundance of the proteins themselves. Increasingly, proteomic analysis is being used as a means to circumvent these difficulties and there are now a growing number of studies using a range of helminths that demonstrate the value of this approach. This, together with the ongoing release of helminth genome sequence information, is revolutionizing parasitology research by providing important datasets for functional genomics studies that will allow us to dissect the interactions between parasites and their hosts at the molecular level. Armed with these powerful experimental approaches, we can be optimistic regarding the control of zoonotic helminth infections in the future.

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