9

Food-borne Parasitic Diseases in China

Xue Bai[1], Xiaolei Liu[1], Xiaonong Zhou[2], Jiaxu Chen[2], Xiuping Wu[2], Pascal Boireau[3] and Mingyuan Liu[1]

[1] *Jilin University, Changchun, China*
[2] *Chinese Center for Disease Control and Prevention, Shanghai, China*
[3] *Animal Health Laboratory, Maisons-Alfort, France*

Food-borne parasitic diseases are human and animal diseases that are caused by helminths and protozoans, which are acquired through the consumption of infected or contaminated meat, fish, shellfish, molluscs, plants, water, reptiles, and amphibians. To date, 95 species of food-borne parasites have been identified; these parasites pose significant public health and socioeconomic problems [1]. The World Health Organization (WHO) focuses on food-borne parasitic diseases when conducting surveys to assess food-borne diseases. The organization has found that 7% of the world's food-borne diseases are caused by these parasites, which have become a major threat to human health and a public health problem. The infectious diseases caused by food-borne parasites are often referred to as neglected diseases, and from a food safety perspective, parasites have not received the same level of attention as other food-borne biological and chemical hazards. Nevertheless, they cause a high burden of disease in humans. The infections may have prolonged, severe, and sometimes fatal outcomes, resulting in considerable hardship in terms of food safety, security, quality of life, and negative impacts on livelihoods.

In 2014, Food and Agriculture Organization of the United Nations (FAO) and WHO composed a list of 24 parasites ranked according to their "importance" and their primary food vehicle. Meanwhile, the FAO/ WHO defined global criteria for evaluating the 24 food-borne parasites and rated each parasite according to these criteria: (a) number of global illnesses, (b) global distribution, (c) morbidity – acute, (d) morbidity – chronic, (e) percentage chronic, (f) mortality, (g) potential for increased burden, (h) trade relevance, and (i) socioeconomic impact. Finally, the top ten list was: *Taenia solium* (pork), *Echinococcus granulosus* (fresh produce), *Echinococcus multilocularis* (fresh produce), *Toxoplasma gondii* (meat from small ruminants, pork, beef, and game meat [red meat and organs]), *Cryptosporidium* spp. (fresh produce, fruit juice, and milk), *Entamoeba histolytica* (fresh produce), *Trichinella spiralis* (pork), *Opisthorchiidae* (freshwater fish), *Ascaris* spp. (fresh produce), and *Trypanosoma cruzi* (fruit juices).

Food Safety in China: Science, Technology, Management and Regulation, First Edition.
Edited by Joseph J. Jen and Junshi Chen.

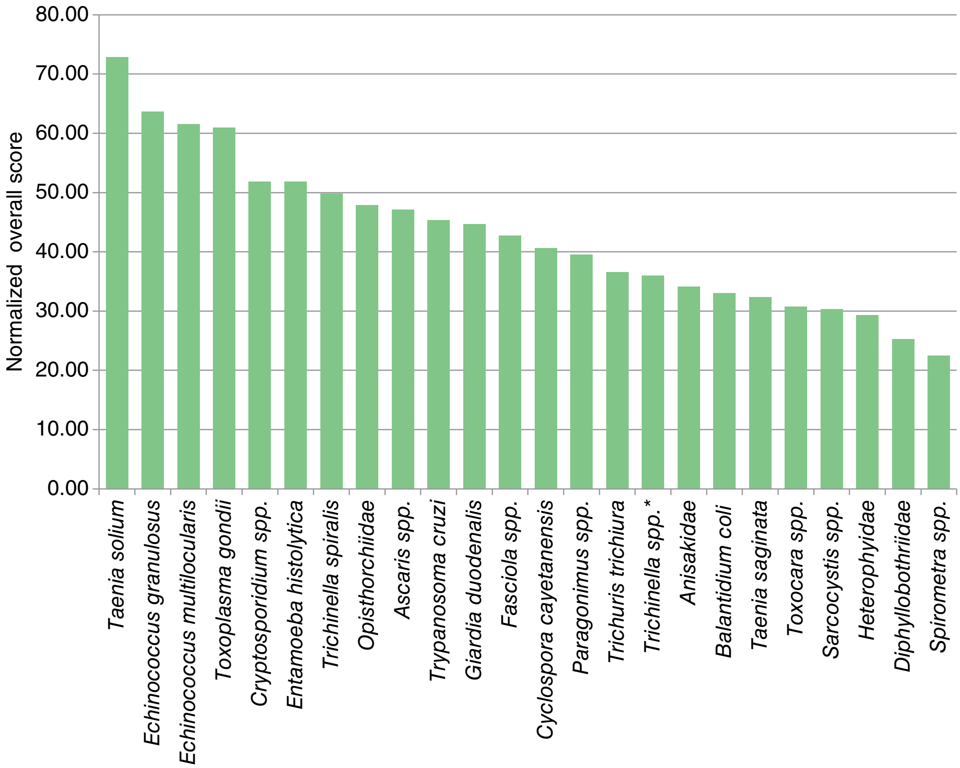

Figure 9.1 Global ranking of food-borne parasites using a multi-criteria ranking tool for scoring parasites and weighting of scoring criteria based on expert preference.

The results of the ranking exercise are presented in Figure 9.1, where the top-ranking parasites are arranged on the x-axis from top to bottom in decreasing rank order and the average weightings (in percentages) are arranged on the y-axis. This figure was obtained from the average of all elicited weightings for the criteria. Among the top-ranked parasites are those that have already been singled out by WHO as neglected tropical diseases (NTD) and identified by the WHO Food-borne Disease Epidemiology Reference Group (FERG) as priorities for further burden of illness studies [2].

Food-borne parasitic diseases are exhibiting new epidemiological characteristics in a society that is filled with economic development, ecological environmental changes, more frequent population flow, as well as diversities in dietary source and style. They have become a major risk factor for food safety and health care, and a global public health problem. In China, there are a variety of food-borne parasitic diseases, with a wide distribution, a high prevalence, and sudden outbreaks, which has resulted in a high burden of disease. This review will summarize information on the epidemic features, diagnosis, and technologies of food-borne parasitic diseases in China. Meanwhile, perspectives are given on the strategies for prevention of food-borne parasitic diseases, combined with foreign management and regulation.

9.1 Epidemic Features of Major Food-borne Parasitic Diseases in China

9.1.1 Various Food-borne Parasitic Diseases

The major food-borne parasites are divided into seven groups, including meat-borne, plant-borne, shellfish-borne, fish-borne, mollusc-borne, water-borne, and reptile- and amphibian-borne parasites. In China, approximately 20 species of food-borne parasitic diseases have been identified, including taeniasis/cysticercosis, trichinellosis, echinococcosis, sarcocystosis, and toxoplasmosis, which are caused by eating raw or undercooked meat (pork, lamb, beef, rabbit, and chicken), gnathostomiasis and diphyllobothriasis, which are caused by eating raw freshwater fish, such as finless eel and loach [3]. China has 56 ethnic groups with different ways of life and customs, and some people have a habit of consuming wild animals and raw meat [4,5]. Therefore, healthy eating habits have been recommended, and the consumption of wild animals is prohibited by legislation. In China, minority groups, such as people of Bai nationality, Dai nationality, and Hani nationality, continue the habit of eating raw or undercooked pork, especially on festival days. In addition, eating some characteristic snacks in the southwest of China, Yunnan Province and Fujian Province can easily lead to taeniasis suis.

Linguatuliasis was considered a rare parasitic disease, but some human diets have changed to include drinking fresh snake blood or eating snake gall and undercooked snakes; therefore, linguatuliasis is becoming increasingly common. When picking water chestnuts, people can easily become infected with fascioliasis, and when eating raw celery, with hepatic fascioliasis. *Cryptosporidium*, *Giardia* and *Cyclospora* are the main parasites causing watery diarrhoea [6]. *Cryptosporidium* is a global pollutant of surface water. Because of its resistance to the standard water chlorination method and its low infective dose (10 oocysts), *Cryptosporidium* can infect large numbers of people at the same time and is a potential biopathogen.

9.1.2 Food Safety Incidents Occur Frequently

Food-borne parasites can be transmitted by the ingestion of fresh or processed foods that have been contaminated with the transmission stages (spores, cysts, oocysts, ova, larval, and encysted stages) via the environment, animals (often from their faeces), or people (often due to inadequate hygiene). With globalization, food-borne parasitic infections are becoming more prevalent nationwide. Improved sanitation, health education, and the establishment of appropriate food safety mechanisms can assist in the control of many of these infections. However, food-borne parasitic infections are still common diseases in developed and developing regions, especially in rural China. Food-borne parasitic diseases cause death and serious diseases in humans and animals nationwide and are of public health significance and socioeconomic importance [7].

Trichinella spiralis has a unique lifecycle in which there is no environmental transmission stage. Thus, all cases are due to the ingestion of meat containing the encysted larvae; meat types typically associated with *T. spiralis* include pork, horse meat, and game. Globally, 65 818 human infections have been reported between 1986 and 2009; most of these were reported for hospitalized patients in Romania, where 42 patient deaths were reported. However, increased exposure may result from human behavioural trends, for example, the consumption of raw horse meat, dog meat, wild boar, and other sylvatic animal meats, as well as practices of free-range animal husbandry (infected animals are asymptomatic). Trichinosis is one of the three parasitic zoonoses in China (trichinosis, cysticercosis, and echinococciosis), but is also a consideration in importing and exporting meat quarantines. In recent years, trichinellosis cases have occurred in some regions of China. On 18 February 2009, an unknown disease broke out in Lanping County of Yunnan Province; nine people were seriously infected, and one person died, which caused public panic and national attention, before finally being diagnosed as trichinosis. In early 2013, trichinosis also broke out in Lancang County of Yunnan Province; 41 families had slaughtered swine within the previous two days, and among the villagers, 108 people had eaten the "raw chops". They presented with different degrees of fever, headache, diarrhoea, calf pain, body aches, facial edema, and other symptoms, finally being diagnosed as infected with *Trichinella*.

In 2006, several tourists ate *Pomacea canaliculata* ("fresh apple snails") in a Beijing restaurant. These tourists presented with fever, severe headache, neck rigidity, and body pain, finally being diagnosed with angiostrongyliasis. Residual *Angiostrongylus cantonensis* larvae were present due to processing problems with the snails.

In alveolar echinococcosis, the occurrence of alveolar echinococcosis in China accounts for more than 90% of the total global burden. The highest prevalence is in the Qinghai-Tibet plateau. Cases of echinococcosis from Xinjiang, Sichuan, Qinghai, Gansu, Ningxia, and Inner Mongolia account for 98.2% of the total number of cases reported in China. In Western China, 5783 cases were reported in 2008 in six provinces with a total population of 96 million people, resulting in an incidence of 6 cases per 100,000.

These frequent food safety incidents remind us to be careful of the food-borne parasite!

9.1.3 Number of Latent Infections is Increasing

From 2001 to 2004, a national survey of the prevalence of parasitic diseases was carried out in China (not including Taiwan, Hong Kong, and Macau), sponsored by the Ministry of Health, China, and involved stratified, random, and mass sampling. The data from that survey revealed two major trends in the epidemiology of parasitic diseases in China. First, the prevalence of intestinal parasites such as *Entamoeba histolytica*, *Fasciolopis buski* and soil-transmitted helminths has declined markedly in comparison to the rates recorded in the first national survey conducted in 1990 [8]. In 2003, the prevalence of hookworms, *Ascaris* and *Trichuris* had declined by 60.7%, 71.3% ,and 73.6%, respectively, and the number of people infected by soil-transmitted nematodes declined from 536 million in 1990 to 129 million in 2003; of these, 85.9, 39.3, and 29.1 million represent infections with *Ascaris lumbricoides*, *hookworms*, and *Trichuris trichiura*, respectively [9]. However, the infection rate with soil-transmitted helminths in China is still unacceptably high in comparison to economically developed countries such as Japan and South Korea.

Second, with regard to the prevalence of food-transmitted parasitic diseases, the fastest-growing food-borne parasitic diseases in China include clonorchiasis, angiostrongyliasis, echinococcosis, trichinellosis, and cysticercosis. The most striking example is clonorchiasis, for which the average national prevalence has increased by 75%

compared to the results of the first national survey, with an estimated 12.49 million people (0.58%) being infected in 2003 compared with 4.7 million (0.36%) in 1990. The prevalence of *Taenia* has increased by 52.49% nationwide, with Sichuan Province and the Tibet autonomous region having the highest increases of 98% and 97%, respectively [10].

9.1.4 Epidemic Areas are Expanding

Globalization is the spread and exchange of people, animals, goods, resources, ideas, and other physical or cultural materials. Globalization also facilitates the spread of infectious diseases, and this can have enormous negative consequences on food security, food safety, and food sovereignty, among which the spread of parasites, including food-borne parasites, ranks highly [11]. Some of these are related to lifestyle changes, including the consumption of raw or undercooked fish and meat, and curiosity about exotic foods and delicacies. An increasingly large transient population has also contributed.

Trichinellosis has become the most important food-borne parasitic zoonosis in China, having a high prevalence in domestic animals and humans. The first outbreak of human trichinellosis was documented in Tibet in 1964. Since then, more than 500 major outbreaks have been recorded in 12 of the 34 Chinese provinces, affecting 25 161 people and leading to 240 deaths. Most of the clinical (88.6%) and fatal (99.6%) cases occurred in southwestern areas (Yunnan, Guangxi and Tibet), where locals have the habit of eating raw pork meat [12].

Human angiostrongyliasis is caused by the larvae of the rat lungworm *Angiostrongylus cantonensis* and can cause eosinophilic meningitis. Humans become infected by ingesting freshwater and terrestrial snails and slugs. The first case of human angiostrongyliasis in mainland China was reported in 1984. Since then, approximately 400 human cases have been reported, including outbreaks of 65 cases in Wenzhou City of Zhejiang Province in 1997; 30 cases in Fuzhou, Fujian Province, in 2002; 28 cases in Yunnan Province between 2003 and 2005; and 131 cases in Beijing in 2006. Other sporadic cases have occurred in the Heilongjiang, Liaoning, Jiangsu, Zhejiang, Fujian, Guangdong, Yunnan, Beijing, and Tianjin provinces [13].

Echinococcosis, including cystic echinococcosis caused by the *Echinococcus granulosus* (*Cestoda; Taeniidae)* and alveolar echinococcosis caused by *Echinococcus multilocularis*, is regarded as one of the most serious parasitic zoonoses in China. A recent nationwide survey by the ELISA method estimated that approximately 380 000 people are infected with echinococcosis, and approximately 50 million are at risk of infection in China [10]. The endemic provinces are predominantly pastoral and semi-pastoral areas, including Inner Mongolia, Ningxia, Qinghai, Jilin, Henan, Sichuan, Guizhou, Yunnan, Shaanxi, Gansu, Tibet, and Xinjiang, but recently, Sichuan has become an endemic area of infection with echinococcosis. Genotyping hydatid cysts from humans and gravid tapeworms from dogs in Xinjiang in northwestern China revealed that the *E. granulosus* G1 genotype was the major source of this human cystic echinococcosis, although the G6 genotype was also present [14].

Clonorchiasis caused by the oriental liver fluke *Clonorchis sinensis* is considered one of the major parasitic zoonoses in some parts of China. Humans become infected with *C. sinensis* when they consume raw or undercooked freshwater fish and shrimp infected by *C. sinensis metacercariae*. A recent national survey showed that human clonorchiasis is endemic in 27 provinces (including municipal cities and autonomous regions). The Guangdong Province has the largest number of infected people (approximately 5.5 million) because of the habit of local people of eating raw and undercooked fish [15].

Cryptosporidiosis is one of the emerging parasitic zoonoses in China and is considered by the WHO Neglected Diseases Initiative as an important infectious disease. In the United States, an estimated 8% of the annual food-borne disease burden may be attributed to this parasite. In 1987, the first case of cryptosporidiosis was identified in the city of Nanjing, China. Subsequently, many cases were reported from more than ten provinces. The prevalence of cryptosporidiosis in diarrhoea patients ranged between 1.4% and 13.3% and was most commonly found in children [16]. A recent survey of cryptosporidiosis revealed a prevalence of 3% in children with diarrhoea, and children of one to four years old had the highest prevalence at 5.5%.

9.1.5 Intermediate Hosts are Widespread, and Infection Rates Remain High

Food-borne parasites have a wide range of hosts, whether definitive hosts or intermediate hosts, including mammals, birds, fish, and other animals.

To date, *Trichinella* has been found in 14 species of animals, including the pig, dog, cat, rat, cow, fox, bear, tiger, marten, raccoon, elk, wolf, and wild boar, and is distributed in all Chinese provinces except the Hainan and Taiwan islands [17]. Swine trichinellosis is a serious problem in China because the prevalence is high in some provinces. Among them, Hubei is the most affected province, with a prevalence of 6.76% by direct diagnostic methods (microscopy or artificial digestion) in the slaughterhouses. In Henan Province, the average prevalence was up to 4.27% by direct detection methods in 43 counties, and in some counties, the level remained extremely high (e.g., reaching 50.4% and 36.1% in Xinye and Deng counties, respectively). Dogs are also prevalently infected in northeastern China. The trichinellosis prevalence in dogs is as follows: 9.82% in Jilin Province, 39.5–44.8% in Heilongjiang Province, 23.52% in Inner Mongolia, and 35.6% in Liaoning Province.

In China, approximately 140 species of freshwater fish and four species of shrimp have been recognized as second intermediate hosts for *C. sinensis*. The prevalence of infected fish is still high in some provinces. Cats and dogs are the most important animal reservoirs for human infection and show high prevalence in some provinces [18].

Anisakiasis has become an important food-borne zoonotic parasitic disease and is ranked as a second-class dangerous parasitic disease in entry-exit inspection and quarantine in China. In the first case of human anisakiasis in China, the patient, a 56-year-old male citizen of Dalian, was admitted to hospital with vomiting, peripheral umbilicus and abdominal distension, and frequent mucous diarrhoea. The patient was examined using an electronic gastroscope, which displayed a parasite residing in the stomach, and subsequently, gastroscope-assisted surgery was implemented. By the end of 2011, 194 of the 239 species of fish inspected were found to be infected with *Anisakis* in China; the infection rate was 81.17%. Of 6969 fish tails checked, 2722 tails were infected, and the infection rate was 39.06%. For the 32 species of fish checked, the infection rate was 100% [19].

9.1.6 Great Economic Losses

Food-borne parasites not only lead to enormous economic losses in animal husbandry, the meat industry, agribusiness, and trade, but also pose a severe threat to public health. Diseases caused by *Taenia solium* (ranked 1st in Figure 9.1) and *Echinococcus granulosus* and *E. multilocularis* (ranked 2nd and 3rd in Figure 9.1, respectively) contribute to economic losses in human and animal populations in many parts of the world. If the parasites are ranked only on trade criterion scores, the order of importance changes: *Trichinella spiralis*, *Taenia solium*, *Taenia saginata*, *Anisakidae* and *Cyclospora cayetanensis* are the top five. Infections from these parasites are considered preventable diseases that can be controlled or eliminated and should be prioritized [20].

Taeniasis and cysticercosis are widespread food-borne disease infections with adult and larval *Taenia*, respectively. Nationwide, 550 000 people have been estimated to be infected. The treatment of taeniasis costs 3918.93 CNY/person; treatment for 550 000 people would cost 31.3 billion yuan. Each year, 200 million kilograms of pork are infected by *cysticercus* in China, and the direct economic loss amounts to $121 million [21].

Echinococcus granulosus and *E. multilocularis* represent a substantial burden on the human population. Present estimates suggest that cystic hydatid disease, caused by *E. granulosus*, results in the loss of 1–3 million disability-adjusted life years per annum. The annual cost of treating cases and the economic losses to the livestock industry probably amount to $2 billion USD. Alveolar echinococcosis, which is caused by *E. multilocularis*, results in the loss of approximately 650,000 disability-adjusted life years per year. These diseases are perhaps some of the more important global parasitic diseases, with more than 1 million people affected at any one time, many showing severe clinical syndromes [22]. In China, alveolar echinococcosis (AE) was "The Second Cancer in Tibet". The Tibetans do not harm wild dogs and feed them for religious reasons, resulting in freely roaming wild dogs. People become infected with *E. multilocularis* due to contact with the wild dogs [23]. Treatment of a case of hydatid disease costs as much as 2700–8000 CNY/person, which causes great difficulty to local residents and huge economic losses. Meanwhile, animal infections with alveolar echinococcosis cause the loss of approximately 8 million yuan in animal by-products per year in China.

For *Trichinella* (ranked 7th in Figure 9.1), the cost of inspection of food, and prevention and control of food-borne parasites remains high. The cost of inspection for *Trichinella* is approximately 2.2 billion CNY per year in China, 0.62 billion EUR per year in the European Union, and 1.2 billion USD per year in the United States.

More than 700 million domestic animals are at risk worldwide, and economic losses exceed 2 billion USD per year because of fascioliasis.

9.1.7 Severe Threats to Human Health

Infections from food-borne parasites such as *Taenia solium* and *Trichinella* spp. can lead to severe clinical syndromes and are potentially fatal. Some food-borne parasites can infect humans chronically and can even have carcinogenic potential, such as observed with *Opisthorchiidae* and *Cryptosporidium* spp.

The main clinical symptom of human trichinellosis is the muscular phase, which is accompanied by diarrhoea, edema, fever, facial swelling, heavy muscle pains, conjunctivitis, and splinter haemorrhages [24]. Death is now rare, owing to improved treatment, but may result from congestive heart failure due to myocarditis, encephalitis, pneumonitis, hypokalaemia, or adrenal gland insufficiency [25].

Taenia solium is estimated to infect millions of people worldwide. This parasite is unique in that the larval or cysticercus stage can infect humans as well as pigs, and can cause a wide range of debilitating neurological problems, including the potentially lethal neurocysticercosis (NCC), ocular cysticercosis (OCC), and subcutaneous cysticercosis (SCC). The disease can be spread by poor sanitation, poor hygiene, and improper slaughterhouse services. Human neurocysticercosis is increasingly being reported in developed countries, possibly due to increases in globalization and immigration [26].

Approximately 30% of the world population has been estimated to be infected by *Toxoplasma gondi* (ranked 4th in Figure 9.1), and although the majority of infections are asymptomatic, serious complications can occur during pregnancy and in the immunocompromised. Severe toxoplasmosis, causing damage to the brain, eyes, or other organs, can develop from an acute *Toxoplasma* infection [27]. Most infants who are infected while still in the womb have no symptoms at birth, but they may develop symptoms later in life. A small percentage of infected newborns have serious eye or brain damage at birth [28]. Furthermore, the importance of this parasite may increase should chronic conditions, including chronic mental sequelae, be found in association with the infection.

The importance of *Cryptosporidium* spp. (ranked 5th in Figure 9.1) as a food-borne parasite has emerged in part through outbreak investigations that have linked fresh produce, fruit juice, and dairy products to the disease. For most people, symptomatic cryptosporidiosis is characterized by acute watery diarrhoea, often accompanied by abdominal pain, nausea and/or vomiting, low-grade fever, headache, and general malaise. Most patients recover within two to three weeks, but highly immunocompromised patients may suffer chronic illness, leading to severe disease and sometimes death. For most parasitic infections, some treatment is available, but for *Cryptosporidium* spp. infections in the immunocompromised, none is available. Increasing evidence shows that cryptosporidiosis may have long-term effects, such as chronic gastrointestinal conditions. In addition, cryptosporidium oocysts are very resistant to the chlorine commonly used to treat water.

Giardiasis is the most frequently diagnosed intestinal parasitic disease in China and among travellers with chronic diarrhoea. Signs and symptoms may vary and can last for one to two weeks or longer. In some cases, people infected with *Giardia* have no symptoms. Acute symptoms include diarrhoea, gas, greasy stools that tend to float, stomach or abdominal cramps, upset stomach or nausea/vomiting, and dehydration (loss of fluids) [29].

Human anisakiasis is caused by larvae of some genera of the family Anisakidae. The signs and symptoms of anisakiasis are abdominal pain, nausea, vomiting, abdominal distention, diarrhoea, blood and mucus in the stool, mild fever, allergic reactions with rash and itching, and infrequently, anaphylaxis [30].

9.2 Diagnostic Technologies for Food-borne Parasitic Diseases in China

Food-borne parasitic diseases have become one of the important factors affecting public health and food safety. At present, diagnostic methods for food-borne parasites rely on the use of morphological identification and remain highly dependent on light or electron microscopy, which can provide a useful confirmation of clinical infection, and can also be used in surveys of food-borne parasites in endemic regions. However, microscopy lacks sensitivity, is labour intensive, and requires well-trained microscopists for accurate identification and interpretation, particularly for parasites that are morphologically similar, very small in size, or present in very low numbers [31]. Therefore, a rapid, highly sensitive, and specific diagnosis method is a trend in the development of food-borne parasite diagnostics. Modern molecular and immunological techniques have been developed in China and abroad, but the standardization and application of these techniques in inspections and quarantines need to proceed more rapidly. This part describes some of the methods and standards that were previously used and some that are currently used to detect parasites in food.

9.2.1 Chinese Standards

Currently, for the detection of food-borne parasites, four national standards exist: the inspection for parasites in food for import and export (SN/T 1748-2006), standard examination methods for drinking water – microbiological parameters (GB/T 5750.12-2006), protocol for the isolation and identification for parasite eggs or oocysts from kimchi and other plant foods (SN/T 1908-2007), and a protocol for quarantine techniques for parasites in freshwater fish (SN/T 25003-2010).

9.2.2 Morphological Identification

In China, according to the different food sources, different quarantine items and methods exist to test for food-borne parasites in imported and exported foods:

1) Direct microscope examination methods are mainly used for fast detection of larvae and cysticercus of *Trichinella spiralis* in meat and fish. Under a trichinoscope, larvae will appear coiled within an individual muscle cell, and the muscle cell typically appears oval in shape as a result of the formation of the capsule.
2) Artificial digestion is used in the inspection of meat for *Trichinella spiralis*, and the international testing method for *Clonorchis sinensis* in fish still uses pressing microscopy and the pepsin digestion method.
3) The candlelight method is used for testing for metacercariae, *Gnathostoma* cysts, *Angiostrongylus,* and *Diphyllobothrium plerocercoids* in fish.
4) The extrusion and candle method is used to test for metacercariae in the meat of translucent fish.
5) The mechanical separation method is used for testing for metacercariae, *Gnathostoma* cysts, *Angiostrongylus larvae,* and *Diphyllobothrium plerocercoids* in fish.
6) The concentration method is used for testing for the eggs of *Ascaris* and *Trichuris trichiura* on fresh vegetables.

9.2.3 Immunoassays

Immunoassays have the benefits of technical simplicity, rapidity, and cost effectiveness. In recent years, latex particle agglutination tests, co-agglutination tests, colloidal gold immune chromatography (GICA), enzyme-linked immunoassays (ELISA), and direct immunofluorescence antibody assays have been available for use in food-borne parasite inspections. However, immunodiagnostic assays commonly are hampered by antigenic cross-reactivity (among related or distinct taxa) and low specificity and often do not allow for distinction among current infection, past infection, and/or exposure [32, 33]. Recently, some rapid assay kits have been shown to exhibit low sensitivity in detecting the full range of parasites within a genus [34].

A type of chromatography card to test trichinosis (pork TS card) has been developed, which is specific, sensitive and rapid (3–12 min), suitable for detecting the blood of pigs, dried blood, serum, and tissue fluid, and can be used for screening *Trichinella* infection in pork or for monitoring sites [35]. Thiruppathiraja used anti-oocyst antibody and alkaline phosphatase double gold particles to establish a rapid immune-dot blot probe (IDBA) technology; the detection of *Cryptosporidium* in the water and environment shows a minimum detection of 10 oocysts/ml, which is 500 times the sensitivity of conventional methods [36]. A competitive enzyme-linked immunosorbent assay using the rabbit polyclonal antibody has also been developed; it can detect the larvae of *Anisakis* in seafood, and the lowest detection limit is approximately 5/1 kg [37].

9.2.4 Molecular Biology Detection

The advent of molecular tools, particularly those based on the polymerase chain reaction (PCR), resulted mainly in common PCR, multiplex PCR, PCR-ELISA, nested PCR, real-time PCR, and gene chip, which have provided a major advance for the food industry because of the ability to detect low levels of pathogens on food [31, 38].

9.2.4.1 Polymerase Chain Reaction (PCR)

A single PCR test for the simple and unequivocal differentiation of all currently recognized genotypes of *Trichinella* has been developed. The technique was developed further to distinguish genotypes at the level of single muscle larvae using a nested, multiplex PCR. In this PCR, the entire internal transcribed spacer region, as well as the gap region of the expansion segment V of the large subunit ribosomal DNA, is amplified concurrently in a first-round PCR using primer sets specific for each region, followed by the multiplex PCR for final diagnosis [39]. Multiplex PCR utilizes more than one set of primers in a reaction and has been used for the simultaneous detection of multiple pathogens in one sample [40, 41]. However, limitations of PCR include inhibitors in the foods, which can result in false positives. Food-derived PCR inhibitors include Ca^{2+}, fats, glycogen, and phenolic compounds [42]. The presence of proteases in cheese and milk may also inhibit PCR [43, 44], and the detection of *Cryptosporidium* in water and food samples is often hampered by the occurrence of organic and inorganic substances that can potentially be PCR inhibitors [45]. PCR-ELISA allows the fast and non-radioactive detection of PCR products on the microplate. Kellogg used PCR-ELISA to detect *Toxoplasma* contamination in water [46]. This method can provide positive, confirmed results in less than a day. Fewer than 50 oocysts can be detected following recovery of oocyst DNA. The development of a PCR detection method to detect the *T. gondii* oocyst will provide a useful technique to estimate levels present in surface waters.

9.2.4.2 Quantitative PCR (qPCR)

The invention of quantitative PCR (qPCR) has overcome several limitations of conventional PCR and led the way to rapid enumeration of food-borne pathogens [33, 34]. In qPCR, the amplified product is detected using fluorescent dyes. These fluorescent dyes are linked to oligonucleotide probes, which bind specifically to the amplified PCR product. This not only allows highly sensitive and specific detection of the target sequences, but also enables very accurate quantitation of the target sequence [34, 47]. One study evaluated whether freshwater bivalves can be used to detect the presence of *Toxoplasma gondii* in water bodies. The presence of *T. gondii* was investigated in mussel tissues by qPCR [48]. By using real-time fluorescence quantitative PCR, the detection limit for *Trichinella spiralis* is approximately 0.01 larvae/1 g of tissue homogenate [49]; for *Anisakis*, 1 mg body tissues/25 g fish samples [50]; and for *Cryptosporidium* oocysts in water samples, < 10 oocysts [51]. This PCR is a reliable, specific, and sensitive detection method.

9.2.4.3 Loop-Mediated Isothermal Amplification (LAMP)

LAMP employs four primers that have a total of six binding sites on the target DNA. It uses a robust polymerase (BST) to amplify the target DNA (or RNA by inclusion of reverse transcriptase) proceeding to an autocycling strand displacement mechanism while at a constant temperature and producing detectable product in approximately 1 h [52]. The procedure is robust, rapid, and able to amplify from a single copy to 10^9 in 1 h at constant temperature, typically in the range of 60–70°C [52, 53]. LAMP can also be applied to nucleic acid extracts of unpurified samples or even to samples without nucleic acid extraction, which demonstrates its general insensitivity to extraneous materials other than the target; that is, *Toxoplasma* oocyst DNA have been detected efficiently in crude faecal nucleic acid extracts [53]. A rapid, sensitive, and specific method for detecting the food-borne trematode *Opisthorchis viverrini* from stool samples using LAMP was developed to obtain results within 40 min, using a heat box or a water bath to maintain the temperature at 65 °C [54]. LAMP assays have also been developed for the detection of *Cryptosporidium* and *Giardia* in water and faeces.

Different PCR methods that have two major advantages have been developed in the past few years [18, 55, 56]. First, PCR shows high performance in the diagnosis of low-intensity infection. Second, the technique allows *C. sinensis* to be distinguished from other trematode species. The LAMP technique has been developed to detect *C. sinensis* infection in intermediate hosts [57, 58]. Studies to diagnose human *C. sinensis* infection with LAMP are warranted in view of the simplicity of this technology compared with PCR.

9.2.4.4 DNA Chips

A DNA chip (also commonly known as a DNA microarray or biochip) is a collection of microscopic DNA spots attached to a solid surface. Scientists use DNA microarrays to measure the expression levels of a large number of genes simultaneously or to genotype multiple regions of a genome. Each DNA spot contains picomoles (10^{-12} moles) of specific DNA sequences, known as a probes (or reporters or oligos). These can be a short section of a gene or another DNA element, used to hybridize a cDNA or cRNA (also called anti-sense RNA) sample (called a target) under high-stringency conditions. Probe-target hybridization is usually detected and quantified by the detection of fluorophore-, silver-, or chemiluminescence-labeled targets to determine the relative abundance of the nucleic acid sequences in the target.

Wang and colleagues designed oligonucleotide chips with specific probes based on genera, species, and sub-species. This chip, combined with multiple PCR, successfully identified *Entamoeba histolytica Schaudinn*, *Giardia lamblia*, and *Cryptosporidium parvum* [59]. Brinkman researched and developed DNA chip technology to detect five types of pathogenic microorganisms in natural water, such as *C. parvum* and *C. tyzzeri*, to facilitate the timely assessment of the risk of exposure to water-borne pathogens [60].

9.3 Management and Regulation of Food-borne Parasitic Diseases in China

Internationally, the incidence rate of parasitic diseases is thought to be a major marker to measure the level of civilization and social development of a country. World trade, climate change, and population movement are important indicators challenging the management of parasitic diseases. At present, in China, the outlook on the prevalence and diagnosis of parasitic diseases is not optimistic. Management and regulation of food-borne parasitic diseases have become difficult because of lagging diagnostic technology, the lack of professional personnel, and an imperfect food safety control system. Since mid-December 2011, a series of persons complaining of fever and hepatalgia were admitted to local hospitals in Yunnan Province. The patients were suspected to be infected by *Fasciola gigantica*. This incident also shows that the prevention and control of rare parasitic diseases is still neglected [61].

In the face of new situations, new problems and the complexity of food-borne parasitic diseases, we should investigate and determine the epidemic status of food-borne parasitic diseases in China, actively work to improve the level of diagnosis and treatment of food-borne parasitic diseases, and strengthen prevention and control efforts. Furthermore, to control emerging and re-emerging parasitic diseases, techniques for detection, surveillance and infection source-tracking must be further improved.

9.3.1 Formulation of Laws and Regulations

The development and implementation of a series of laws and regulations for the prevention and control of infectious diseases in humans, domestic animals, and wild animals is in progress. These laws and regulations will define the responsibilities of governments at all levels and will help them report, control, treat, and take other emergency measures against food-borne parasitic diseases in humans, domestic animals, and wild animals [62].

At present, the inspection and quarantine of food-borne parasites in China is improving gradually. For water, the “Water quality standards for urban water supply (CJ/T206-2005)” and “Standards for drinking water quality (GB5749-2006)”, which were published in China in 2006, added two non-routine procedures for the identification of *Giardia* and *Cryptosporidium*, and proposed the immunomagnetic separation of fluorescent antibody method as a corresponding detection method; this method provides the conventional indicators, with the limit value for *Cryptosporidium* (per 10 l is the optimum) < 1 and for *Giardia lamblia* (per 10 l is the optimum) < 1.

In China, food safety law stipulates that food must not contain pathogenic microorganisms and parasites, and the meat hygiene inspection trial procedures jointly issued by different ministries (health, agriculture, and foreign trade) and the ministry of commerce stipulate those parasites in the meat after slaughter that require quarantine, which include *Trichinella spiralis, cysticercus* and *sarcocys*tis [7]. The inspection method for

Trichinella spiralis and *sarcocystis* is diaphragm tableting microscopy. Examination of *cysticercus* is mainly from visual observation of incisions of masseter muscle, waist deep muscle, and the diaphragm, to allow viewing of any rice-like, grey, transparent cysticercus packages. However, the detection rate of these methods is high, and segmenting of the meat is not suitable. Therefore, health departments and food supervision departments need to revise and supplement the current standards and regulations to clarify the types and stages of parasite infection and to facilitate practical operation and application.

Meat inspection for trichinellosis and cysticercosis is required in European Union countries, as described under Regulation 854/2004 for pigs and cattle at slaughter and in six other export countries, including Australia, Canada, Japan, New Zealand, Norway, and the United States [31].

9.3.2 Establishment of the Disease Reporting and Surveillance Systems

The complexities connected to the epidemiology and life cycle of each parasite play a central role in identification, prevention, and control of the risks associated with food-borne parasitic diseases. Surveillance for parasitic diseases is complicated by the often prolonged incubation periods, sub-clinical nature and unrecognized, chronic sequelae. The established disease-reporting systems for humans, domestic animals, and wild animals are used to collect, collate, and analyze the epidemiological information on animal epidemic diseases and are responsible for monitoring the diagnosis of animal diseases. Using the present reporting system, hospitals and clinics can immediately and directly report cases through the internet, allowing public health officials to have information on diseases, which provide the foundation for the design and implementation of control strategies and measures [63].

The United States has many participants in its food-borne disease-monitoring system. In 2011, the US Centers for Disease Control (CDC) list of nationally notifiable infectious conditions contained only four water-borne and food-borne parasites: *Cryptosporidium*, *Cyclospora*, *Giardia*, and *Trichinella* [64]. Most CDC monitoring system data come from public health agencies in various states or regions. In the United States, agencies responsible for safe drinking water, produce, seafood, and meat include the US Environmental Protection Agency (USEPA), the Food and Drug Administration (FDA), the US Department of Commerce's (USDC) National Oceanic and Atmospheric Administration (NOAA), and the US Department of Agriculture (USDA) [31].

After World War II, more than 70% of Japanese people were infected with intestinal parasites, and that situation was similar to the situation in some developing countries today. However, after 30 years, Japan had eliminated the main food-borne parasitic disease because parasite-control programmes were introduced that were devised and conducted by parasitologists. Meanwhile, the Tokyo Public Hygiene Association (TPHA) and the Japan Association of Parasite Control (JAPC) were formed, and school-health-based parasite control was initiated nationwide [65]. These measures effectively controlled parasite epidemics.

9.3.3 Establishment of the International and National Veterinary Reference Laboratories or Collaboration Centres

Establishment of a reference laboratory was needed for important food-borne parasitic diseases, allowing studies in basic research and the application of epidemic prevention.

This laboratory was needed to promote the study of the technology of diagnosis, prevention, control, and eradication of animal diseases, and development of a final strategy [66]. The European Union set up reference laboratories, which are responsible for training and final confirmation of suspected samples; work labs are responsible for the testing of samples. With the improvement of veterinary science and technology, an increasing number of reference laboratories have been established. In May 2015, in China, The World Organisation for Animal Health (OIE) collaborated to establish a centre for food-borne parasites in the Asian-Pacific region in the Institute of Zoonoses of Jilin University to provide more comprehensive monitoring and detection of food-borne parasitic diseases. By the end of 2015, the OIE had set up a total of 12 reference laboratories and three collaborating centres in China.

9.3.4 Implementation of Special Projects and Increasing the Investment in Important Food-borne Parasites

A special project for the prevention and control of important food-borne parasites has been conducted with an increase in the financial support from central and local governments. The aims of this project were to reduce incidence of and mortality from food-borne parasitic diseases and to improve governmental emergency response and capabilities of disease prevention and control. The project has also focused on the detection of food-borne diseases and laboratory monitoring with standardized technology, providing technical support for emergency decisions related to food-borne diseases.

In the 1960s and 1970s, eradication of snails was considered the focal point of the schistosomiasis control campaign, despite concerns that the molluscicides might lead to environmental pollution. Since the 1980s, the use of praziquantel for mass chemotherapy has become the chief means to control schistosomiasis. In 2004, the State Council established two targets for the National Schistosomiasis Control Program. In 2007, the Chinese Central Government demonstrated its commitment to enhance fundamental research into the food-borne diseases problem by funding a project for basic research relating to the control of schistosomiasis and malaria through the National Basic Research Program of China (i.e., the 973 program). This strengthened the national control program and, when implemented, effectively reduced schistosomiasis infections [67, 68].

In addition, insufficient financial support for research on and control of food-borne parasitic disease from central and local governments has resulted in an increase in the proportion of food-borne parasite infection. For example, when the World Bank Loan Project ended in 2001, a corresponding drop occurred in the funding for schistosomiasis control, which in turn caused an increase in human cases of schistosomiasis in 2003 [7, 69].

9.3.5 Development of a Food-borne Parasitic Disease Vaccine and New Drugs

The research and development of new drugs is an effective way to control the drug resistance of the parasite. Many of the new drug targets are being discovered with much more ease as the genomic sequences of many parasitic organisms are becoming available. A pressing need exists for the identification of compounds that are efficacious in *in vivo* animal studies and can be subjected to clinical trials.

Drug development for *C. parvum* is particularly challenging because of the difficulty of *in vitro* screening. Maximum effort should be directed towards new compounds to treat cryptosporidiosis because of the limited availability of effective drugs [70].

9.3.6 Surveillance of Exotic Diseases

The spread of food-borne parasitic diseases is enhanced by changes in human behaviour, demographics, environment, climate, land use, and trade, among other drivers [71]. Most emerging and re-emerging zoonotic diseases come from wildlife. The globalization of food trade offers new opportunities for dissemination and variations in food preferences and consumption patterns. For example, meat consumption in emerging countries is expected to increase globally over the next 20 years because of the rising tendency to eat meat, fish, or seafood that is raw, undercooked, smoked, pickled or dried, or the demand for exotic foods, such as bush meat or wild game. In China, a large number of wild animals are imported from Africa, South America, Oceania, and other sources every year. To prevent certain exotic diseases that are emerging abroad, the government seeks to strengthen the surveillance and control of cross-border transmission of exotic diseases and has established the Joint Control Mechanism of Trans-boundary Food-borne Parasitic Diseases [72]. Because of increasing international communication, food-borne parasitic infections are becoming more prevalent nationwide. Not only is the number of immigrants infected increasing, but infectious vectors are also entering the region. Therefore, we should strengthen the detection of immigrants. Increasing safety management and supervision in the food industry and strengthening the control of raw food materials are the most effective ways to reduce the occurrence of food-borne parasitic diseases.

9.3.7 Emphasis on Interdisciplinary and International Cooperation

Interdisciplinary and cross-sector collaborations, with communication occurring among human, animal, and environmental health services, reflect the "One Health" strategy to confront emerging food-borne parasitic disease. One Health is a collaborative effort between multiple disciplines working locally, nationally, and globally to attain optimal health for people, animals, and the environment. Moreover, an emphasis should be placed on the construction of international early warning systems through international collaboration and coordination to detect unknown infectious diseases of international public health importance [73].

Enhancement of collaboration among the World Health Organization, the Food and Agriculture Organization of the United Nations (FAO), the Office International Des Epizooties, and the World Bank is needed. For example, the FAO has established a global network of professionals directly involved in food-borne zoonotic diseases, including cysticercosis and echinococcosis, and this network provides a basic framework for the spread of information related to the diagnosis, prevention, and control of major zoonotic diseases [74]. Significant progress has been made in international cooperation on the control of schistosomiasis, cysticercosis, and echinococcosis in China [69, 75].

9.3.8 Health Education

Education and increasing awareness were identified as important components of food-borne parasite control and, in some cases, may be the only feasible options available. Education should be directed at participants throughout the food chain, from farm and abattoir workers to food handlers (consumers and food retail outlets), and should address good animal husbandry practices, and hygiene and sanitation measures. In terms of consumer education, a need may exist to address specific high-risk population groups. For consumers, especially those who are pregnant or immunocompromised (e.g., individuals with HIV/AIDS), advice on the preparation and consumption of high-risk foods, such as fresh produce and tubers, carrots, and so on, adequate cooking of meat and fish prior to consumption, and the importance of hygiene, for example, hand-washing, is critical. Pets may be another important source of zoonotic diseases in China, including rabies and toxoplasmosis. The proper care of pets may prevent the transmission of pet-borne zoonotic diseases to humans; care would include the compulsory vaccination of pets, keeping pet living areas clean, and washing hands thoroughly after handling pets [76].

Educational campaigns utilize various media, in particular TV and radio, to promote awareness of the significance of and control strategies for food-borne parasitic diseases. The change of people's unhealthy eating habits is crucial to the successful control of food-borne parasitic diseases in some parts of China, particularly for some ethnic groups [7]. Popularizing health knowledge, striving to improve people's self-protection awareness, gradually changing irrational cooking and eating habits, and controlling the sources of infection, are conducive to the protection of vulnerable populations.

In conclusion, at present, research progress on food-borne parasitic diseases has many disadvantages in China, which shows a large gap with developed countries. We should actively learn from the experiences of the developed countries to improve the prevention and control programs of food-borne parasitic diseases.

References

1 LiHua, X., Ryan, U., YaoYu, F.(2015) *Biology of Foodborne Parasites*, CRC Press, Taylor&Francis Group.
2 Food and Agriculture Organization of the United Nations and World Health Organization. (2012) *Multicriteria-based ranking for risk management of food-borne parasites. Microbiological Risk Assessment Series No. 23*. Report of a Joint FAO/WHO Expert Meeting, September 3–7, 2012, Rome, Italy.
3 Zengshan, L. (2007) *Food Pathogenic Microbiology*, China Light Industry Press. [In Chinese].
4 Kruse, H., Kirkemo, A.M., Handeland, K. (2004) Wildlife as source of zoonotic infections. *Emerging Infectious Diseases*, 10, 2067–2072.
5 Li, T., He, S., Zhao, H. *et al*. (2010) Major trends in human parasitic diseases in China. *Trends in Parasitology*, 26(5), 264–270.
6 Rose, J. B., Slifko, T. R. (1999) *Giardia*, *Cryptosporidium*, and *Cyclospora* and their impact on foods: A review. *Journal of Food Protection*, 62(9), 1059–1070.
7 Zhou, P., Chen, N., Zhang, R.L. *et al*. (2008) Food-borne parasitic zoonoses in China: perspective for control. *Trends in Parasitology*, 24(4), 190–196.
8 Xu, L.Q., Yu, S.H., Jiang, Z. X. *et al*. (1995) Soil-transmitted helminthiases: nationwide survey in China. *Bulletin of the World Health Organization*, 73(4), 507–513.
9 YingDan, C., LinHua, T., LongQi, X.(2008) Current status of soil-transmitted nematode infection in China. *Biomedical and Environmental Sciences*, 21(2), 173–179.
10 Coordinating Office of the National Survey on the Important Human Parasitic Diseases. (2005) A national survey on current status of the important parasitic diseases in human

population. *Chinese Journal of Parasitology and Parasitic Diseases.* 23(5 Suppl), 332. [In Chinese].
11 Robertson, L. J., Sprong, H., Ortega, Y. R. *et al.*(2014) Impacts of globalisation on foodborne parasites. *Trends in Parasitology*, 30(1), 37–52.
12 Liu, M., Boireau, P.(2002) Trichinellosis in China: epidemiology and control. *Trends in Parasitology*, 18(12), 553–556.
13 Lin, W., Wang, X. (2004) Epidemiology of *Angiostrongylus cantonensis* in mainland. *Chinese Journal of Zoonoses*, 20(11), 1004–1007. [In Chinese].
14 Bart, J., Abdukader, M., Zhang, Y. *et al.* (2006) Genotyping of human cystic echinococcosis in Xinjiang, PR China. *Parasitology*, 133(5), 571–579.
15 Lun, Z.-R., Gasser, R. B., Lai, D.H. *et al.* (2005) Clonorchiasis: a key foodborne zoonosis in China. *The Lancet Infectious Diseases*, 5(1), 31–41.
16 Yang, X. (2005) Epidemic characteristics of human cryptosporidiosis. *Parasitic Disease and Infectious Disease*, 3(3), 135–137. [In Chinese].
17 Wang, Z., Cui, J. (2001) The epidemiology of human trichinellosis in China during 1964-1999. *Parasite*, 8, S63–S66.
18 Tang, J., Lin, R., Zhu, X. (2007) Clonorchis sinensis infection in animals in China. *Chinese Journal of Zoonoses*, 23(2), 177–179. [In Chinese].
19 Tang, Y., Sun,Q., Peng, P. *et al.* (2012) Research advances of Anisakiasis in China. *Journal of Anhui Agricultural Sciences*, 40(24), 12079–12081. [In Chinese].
20 Carabin, H., Budke, C. M., Cowan, L. D. *et al.* (2005) Methods for assessing the burden of parasitic zoonoses: echinococcosis and cysticercosis. *Trends in Parasitology*, 21(7), 327–333.
21 Wu, W., Qian, X., Huang, Y. *et al.* (2012) A review of the control of clonorchiasis sinensis and Taenia solium taeniasis/cysticercosis in China. *Parasitology Research*, 111(5), 1879–1884.
22 World Health Organization.(2011) *Report of the WHO informal working group on cystic and alveolar echninococcosis surveillance, prevention and control, with the participation of the Food and Agriculture Organization of the United Nations and the World Organisation for Animal Health*, 22–23 June 2011, Department of Control of Neglected Tropical Diseases, WHO, Geneva, Switzerland.
23 Xia, D., Yan, L.N., Li, B. *et al.* (2005) Orthotopic liver transplantation for incurable alveolar echinococcosis: report of five cases from west China. *Transplantation Proceedings,* 37(5), 2181–2184.
24 Capo, V., Despommier, D. D.(1996) Clinical aspects of infection with *Trichinella* spp. *Clinical Microbiology Reviews*, 9(1), 47–54.
25 Bruschi, F., Murrell, K.(2002) New aspects of human trichinellosis: the impact of new Trichinella species. *Postgraduate Medical Journal*, 78(915), 15–22.
26 Carabin, H., Ndimubanzi, P. C., Budke, C. M. *et al.* (2011) Clinical manifestations associated with neurocysticercosis: a systematic review. *PLoS Neglected Tropical Diseases*, 5(5), 1–13.
27 Hill, D., Dubey, J.(2002) Toxoplasma gondii: transmission, diagnosis and prevention. *Clinical Microbiology and Infection*, 8(10), 634–640.
28 Olariu, T. R., Remington, J. S., McLeod, R. *et al.* (2011) Severe congenital toxoplasmosis in the United States: clinical and serologic findings in untreated infants. *Pediatric Infectious Disease Journal*, 30(12), 1056–1061.
29 Feng, Y., Xiao, L. (2011) Zoonotic potential and molecular epidemiology of Giardia species and giardiasis. *Clinical Microbiology Reviews*, 24(1), 110–140.

30 Audicana, M. T., Kennedy, M. W. (2008) *Anisakis simplex*: from obscure infectious worm to inducer of immune hypersensitivity. *Clinical Microbiology Reviews*, 21(2), 360–379.

31 McCarthy, J. S., Lustigman, S., Yang, G.J. *et al.* (2012) A research agenda for helminth diseases of humans: diagnostics for control and elimination programmes. *PLoS Neglected Tropical Diseases*, 6(4), e1601.

32 Huang, S.Y., Zhao, G.-H., Fu, B.Q. *et al.* (2012) Genomics and molecular genetics of *Clonorchis sinensis*: Current status and perspectives. *Parasitology International*, 61(1), 71–76.

33 Gorski, L., Csordas, A. (2009) *Molecular Detection of Foodborne Pathogens*, CRC Press.

34 Hoorfar, J. (2011) Rapid detection, characterization, and enumeration of foodborne pathogens. *Apmis*, 119(s133), 1–24.

35 Patrascu, I., Gamble, H., Sofronic-Milosavljevic, L. *et al.* (2001) The lateral flow card test: an alternative method for the detection of *Trichinella* infection in swine. *Parasite*, 8 (2 Suppl), S240–S242.

36 Thiruppathiraja, C., Kamatchiammal, S., Adaikkappan, P. *et al.* (2011) An advanced dual labeled gold nanoparticles probe to detect *Cryptosporidium parvum* using rapid immuno-dot blot assay. *Biosensors and Bioelectronics*, 26(11), 4624–4627.

37 Xu, X., Sui, J., Cao, L. *et al.* (2010) Direct competitive enzyme-linked immunosorbent assay (ELISA) for rapid screening of anisakid larvae in seafood. *Journal of the Science of Food and Agriculture*, 90(5), 877–881.

38 Xu, S., Mutharasan, R. (2010) Rapid and sensitive detection of *Giardia lamblia* using a piezoelectric cantilever biosensor in finished and source waters. *Environmental Science and Technology*, 44(5), 1736–1741.

39 Zarlenga, D. S., Chute, M. B., Martin, A. *et al.* (1999) A multiplex PCR for unequivocal differentiation of all encapsulated and non-encapsulated genotypes of Trichinella. *International Journal of Parasitology*, 29(11), 1859–1867.

40 Chen, J., Tang, J., Liu, J. *et al.* (2012) Development and evaluation of a multiplex PCR for simultaneous detection of five foodborne pathogens. *Journal of Applied Microbiology*, 112(4), 823–830.

41 de Freitas, C. G., Santana, Â. P., da Silva, P. H. C. *et al.* (2010) PCR multiplex for detection of *Salmonella enteritidis*, *Typhi* and *Typhimurium* and occurrence in poultry meat. *International Journal of Food Microbiology*, 139(1), 15–22.

42 Wilson, I. G.(1997) Inhibition and facilitation of nucleic acid amplification. *Applied and Environmental Microbiology*, 63(10), 3741–3751.

43 Powell, H., Gooding, C., Garrett, S. *et al.* (1994) Proteinase inhibition of the detection of Listeria monocytogenes in milk using the polymerase chain reaction. *Letters in Applied Microbiology*, 18(1), 59–61.

44 Rossen, L., Nørskov, P., Holmstrøm, K. *et al.* (1992) Inhibition of PCR by components of food samples, microbial diagnostic assays and DNA-extraction solutions. *International Journal of Food Microbiology*, 17(1), 37–45.

45 Skotarczak, B. (2009) Methods for parasitic protozoans detection in the environmental samples. *Parasite*, 16(3), 183–190.

46 Schwab, K. J., McDevitt, J. J. (2003) Development of a PCR-enzyme immunoassay oligoprobe detection method for *Toxoplasma gondii* oocysts, incorporating PCR controls. *Applied and Environmental Microbiology*, 69(10), 5819–5825.

47 Higuchi, R., Dollinger, G., Walsh, P. S. *et al.* (1992) Simultaneous amplification and detection of specific DNA sequences. *Biotechnology*, 10(4), 413–417.
48 Kerambrun, E., Palos Ladeiro, M., Bigot-Clivot, A. *et al.* (2015) Zebra mussel as a new tool to show evidence of freshwater contamination by waterborne Toxoplasma gondii. *Journal of Applied Microbiology*, 120(2), 498–508.
49 Atterby, H., Learmount, J., Conyers, C. *et al.* (2009) Development of a real-time PCR assay for the detection of *Trichinella spiralis* in situ. *Veterinary Parasitology*, 161(1), 92–98.
50 Lopez, I., Pardo, M. A. (2010) Evaluation of a real-time polymerase chain reaction (PCR) assay for detection of *Anisakis simplex* parasite as a food-borne allergen source in seafood products. *Journal of Agricultural and Food Chemistry*, 58(3), 1469–1477.
51 Ramirez, N. E., Sreevatsan, S. (2006) Development of a sensitive detection system for *Cryptosporidium* in environmental samples. *Veterinary Parasitology*, 136(3), 201–213.
52 Notomi, T., Okayama, H., Masubuchi, H. *et al.* (2000) Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research*, 28(12), e63–e63.
53 Karanis, P., Ongerth, J. (2009) LAMP: A powerful and flexible tool for monitoring microbial pathogens. *Update*, 25(11), 498–499.
54 Arimatsu, Y., Kaewkes, S., Laha, T. *et al.* (2012) Rapid detection of *Opisthorchis viverrini* copro-DNA using loop-mediated isothermal amplification (LAMP). *Parasitology International*, 61(1), 178–182.
55 Sanpool, O., Intapan, P. M., Thanchomnang, T. *et al.* (2012) Rapid detection and differentiation of *Clonorchis sinensis* and *Opisthorchis viverrini* eggs in human fecal samples using a duplex real-time fluorescence resonance energy transfer PCR and melting curve analysis. *Parasitology Research*, 111(1), 89–96.
56 Traub, R. J., Macaranas, J., Mungthin, M. *et al.* (2009) A new PCR-based approach indicates the range of *Clonorchis sinensis* now extends to Central Thailand. *PLoS Neglected Tropical Diseases*, 3(1), 1–7.
57 Cai, X., Xu, M., Wang, Y. *et al.* (2010) Sensitive and rapid detection of *Clonorchis sinensis* infection in fish by loop-mediated isothermal amplification (LAMP). *Parasitology research*, 106(6), 1379–1383.
58 Chen, Y., Wen, T., Lai, D.H. *et al.* (2013) Development and evaluation of loop-mediated isothermal amplification (LAMP) for rapid detection of *Clonorchis sinensis* from its first intermediate hosts, freshwater snails. *Parasitology*, 140(11), 1377–1383.
59 Wang, Z., Vora, G. J., Stenger, D. A. (2004) Detection and genotyping of *Entamoeba histolytica*, *Entamoeba dispar*, *Giardia lamblia*, and *Cryptosporidium parvum* by oligonucleotide microarray. *Journal of Clinical Microbiology*, 42(7), 3262–3271.
60 Brinkman, N., Francisco, R., Nichols, T. *et al.* (2013) Detection of multiple waterborne pathogens using microsequencing arrays. *Journal of Applied Microbiology*, 114(2), 564–573.
61 Chen, J.X., Chen, M.X., Ai, L. *et al.* (2013) An outbreak of human *Fascioliasis gigantica* in southwest China. *PloS One*, 8(8), e71520.
62 Choi, S., Lam, P. (2009) Enhancing legal preparedness for the prevention and control of infectious diseases: Experience from severe acute respiratory syndrome in Hong Kong. *Public Health*, 123(3), 242–246.
63 Zhang, L., Wilson, D. P. (2012) Trends in notifiable infectious diseases in China: implications for surveillance and population health policy. *Plos One*, 7(2), 1–12.

64 USEPA. (Long Term 2 Enhanced Surface Water Treatment Rule (LT2). https://www.epa.gov/dwreginfo/long-term-2-enhanced-surface-water-treatment-rule-documents (access October 2016).

65 Takeuchi, T., Nozaki, S., Crump, A. (2007) Past Japanese successes show the way to accomplish future goals. *Trends in Parasitology*, 23(6), 260–267.

66 Zhou, X.N., Lin, J.J., Cao, J.P. *et al.* (2006) Theory and practices of the infrastructure establishment on parasite germplasm resources in China. *Chinese Journal of Parasitology and Parasitic Diseases*, 35, S1.

67 Wang, L.D., Chen, H.G., Guo, J.G. *et al.* (2009) A strategy to control transmission of Schistosoma japonicum in China. *New England Journal of Medicine*, 360(2), 121–128.

68 Wang, L., Utzinger, J., Zhou, X.N. (2008) Schistosomiasis control: experiences and lessons from China. *The Lancet*, 372(9652), 1793–1795.

69 Utzinger, J., Zhou, X.N., Chen, M.G. *et al.* (2005) Conquering schistosomiasis in China: the long march. *Acta Tropica*, 96(2–3), 69–96.

70 Azam, A., Peerzada, M. N., Ahmad, K. (2015) Parasitic diarrheal disease: drug development and targets. *Frontiers in Microbiology*, 6, 1183.

71 Broglia, A., Kapel, C. (2011) Changing dietary habits in a changing world: emerging drivers for the transmission of foodborne parasitic zoonoses. *Veterinary Parasitology*, 182(1), 2–13.

72 Burke, R., Kronmann, K., Daniels, C. *et al.* (2012) A review of zoonotic disease surveillance supported by the Armed Forces Health Surveillance Center. *Zoonoses and Public Health*, 59(3), 164–175.

73 Angelos, J., Arens, A., Johnson, H. *et al.* (2016) One Health in food safety and security education: A curricular framework. *Comparative Immunology, Microbiology and Infectious Diseases*, 44, 29–33.

74 Eddi, C., Juan, L., William, A. *et al.* (2006) Veterinary public health activities at FAO: cysticercosis and echinococcosis. *Parasitology International*, 55, S305–S308.

75 Ito, A., Urbani, C., Jiamin, Q. *et al.* (2003) Control of echinococcosis and cysticercosis: a public health challenge to international cooperation in China. *Acta Tropica*, 86(1), 3–17.

76 Ryan, C. P. (2007) Where do pets fit into human quarantines? *Journal of Public Health*, 29(1), 70–71.