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Emerging parasite zoonoses associated with water and food

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Abstract

The environmental route of transmission is important for many protozoan and helminth parasites, with water, soil and food being particularly significant. Both the potential for producing large numbers of transmissive stages and their environmental robustness, being able to survive in moist microclimates for prolonged periods of time, pose a persistent threat to public and veterinary health. The increased demands on natural resources increase the likelihood of encountering environments and produce contaminated with parasites. For waterborne diseases, the protozoa, *Cryptosporidium*, *Giardia* and *Toxoplasma*, are the most significant causes, yet, with the exception of *Toxoplasma*, the contribution of zoonotic transmission remains unclear due to the absence of 'standardised' methods. The microsporidia have been documented in one waterborne outbreak, but the role of animals as the cause of contamination was not elucidated. In foods, surface contamination is associated with the faecal–oral pathogens, and some data are available to indicate that animal wastes remain an important source of contamination (e.g. cattle faeces and apple cider outbreaks), however, further work should focus on examining the source of contamination on fruit and vegetables. Increasing recognition of the burden of human fascioliasis has occurred; it is now recognised as an emerging zoonosis by the WHO. *Toxoplasma*, *Trichinella* and *Taenia* spp. remain important meatborne parasites, however, others, including *Pleistophora*-like microsporidians may be acquired from raw or lightly cooked fish or crustaceans. With increased international travel, the public health importance of the foodborne trematodiases must also be realised. Global sourcing of food, coupled with changing consumer vogues, including the consumption of raw vegetables and undercooking to retain the natural taste and preserve heat-labile nutrients, can increase the risk of foodborne transmission. A greater awareness of parasite contamination of our environment and its impact on health has precipitated the development of better detection methods. Robust, efficient detection, viability and typing methods are required to assess risks and to further epidemiological understanding. © 2000 Published by Elsevier Science Ltd. on behalf of the Australian Society for Parasitology Inc.

Keywords: Zoonoses; *Cryptosporidium*; *Giardia*; Microsporidia; *Toxoplasma*; *Fasciola*; *Taenia*; *Trichinella*; Waterborne; Foodborne

1. Introduction

Zoonotic diseases are described as those diseases transmitted from animals to humans. Zoonotic parasitic diseases are transmitted to humans either by ingesting environmentally robust transmissive stages (spores, cysts, oocysts, ova, larval and encysted stages) or by eating raw or undercooked 'meat' containing infective tissue stages. Humans can be final, intermediate or paratenic (maintenance) or accidental hosts. While the transmissive stages of some of these zoonoses can be transmitted directly (e.g. by animal human contact or through contact with contaminated faeces, soil and herbage), they can also be transmitted through contaminated water and food. Some parasite zoonoses transmitted by the waterborne and foodborne routes are presented in Table 1.

2. Water and food as sources of infection

The water–food connection for parasite zoonoses is complex (Fig. 1), with faeces as a major vehicle for many environmental transmissive stages. However, the spores of some microsporidia (e.g. *Encephalitozoon cuniculi*) and the ova of *Schistosoma haematobium* contaminate the environment through urine. The transmissible stages can contaminate water or foods directly, voided in faeces, or indirectly. The disposal of animal (and human) wastes remains a significant public health issue that has yet to be assessed or controlled in most countries.

Water is a major conduit for these parasites, and contaminated water is an important source of human infection either by direct consumption or by the use of contaminated water

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Table 1
Some parasite zoonoses transmitted by the waterborne or foodborne routes

Family	Parasite	Transmission route	Contaminated/infected matrix	Final hosts
Microspora				
Enterocytozoonidae	*Enterocytozooan bieneusi*	?Water, food	?Spores in water and on uncooked or undercooked food	Humans, rhesus monkeys
Unikaryonidae	*Encephalitozoon cuniculi*	?Water, food	?Spores in water and on uncooked or undercooked food.	Humans, pets / animals residing in & around human dwellings (e.g. rabbits, canines, mice, pigs, goats, cows
	Encephalitozoon intestinalis			
	Encephalitozoon hellem			Parakeet, parrot
Pleistophoridae	'*Pleistophora*-like organisms'	Food	Uncooked or undercooked fish or crustacea	Humans, fish, crustacea
Protozoa				
Cryptosporidiidae	*Cryptosporidium parvum* (genotype 2)	Water, food	Oocysts in water and on uncooked or undercooked food	Humans and other mammals
Hexamitidae	*Giardia duodenalis*	Water, food	Cysts in water and on uncooked or undercooked food	Humans, other mammals and birds
Sarcocystidae	*Toxoplasma gondii*	Food, water	Oocysts in water and on uncooked or undercooked food. Tissue cysts in uncooked or undercooked meat	Felines
Balantidiidae	*Balantidium coli*	Water, food	Cysts in untreated or minimally treated water and on uncooked or undercooked food	Humans, pigs, non-human primates, cats, rodents
Blastocystidae	*Blastocystis hominis*, *Blastocystis* sp.	Water, food	Cysts in untreated or minimally treated water and on uncooked or undercooked food	Humans and other mammals
Trematodes				
Opisthorchiidae	*Clonorchis* spp., *Opisthorchis* spp.	Meat. Fresh water fish	Metacercariae in musculature	Humans, cats, dogs, etc
Heterophyidae	*Metagonimus yokogawai*	Meat. Freshwater fish (sweetfish)	Metacercariae in musculature	Humans, cats, dogs, etc
	Heterophyes spp.	Brackish water fish		
Echinostomatoidea	*Echinostoma* spp.	Meat. Loach, frogs, snails,	Intestinal submucosa of loach, kidney of frogs, head, mantle and liver of snails	Humans, dogs, rats, birds etc
Fasciolidae	*Fasciola hepatica*	Waterplants (e.g. watercress, rice, dandelion, *Nasturtium* and *Mentha* spp	Metacercariae encysted on leaves (about 10% of metacercariae float in water)	Primarily ruminants
	Fasciolopsis buski	Water chestnut, water caltrop, water hyacinth	Metacercariae encysted on leaves	Humans, pigs
Troglotrematidae	*Paragonimus* spp.	Potamid and other crabs/ crayfish/ shrimp	Metacercariae in lungs and musculature of crabs.	Humans, canines, felines etc.
Schistosomatidae	*Schistosoma* spp.	Water. Skin penetration	Cercariae in water	Humans, non-human primates, bovines, cats, dogs, pigs, rodents, etc
	Schistosome dermatitis	Water. Skin penetration	Cercariae in fresh and marine waters	Birds, non-human mammals
Cestodes				
Diphyllobothriidae	*Diphyllobothrium latum*	Salmonid and other fish	Plerocercoid in musculature, liver, roe	Humans, canines, felines, various land and marine mammals
	Marine diphyllobothriasis	Marine fish. Ceviche (made of raw fish in Peru and Chile)	Plerocercoid in musculature	Marine mammals
Taeniidae	*Taenia saginata*	Meat. Bovine and cervine	Cysticerci in musculature	Humans
	Taenia soilium	Meat. Pig, camel, rabbit, bear, etc	Cysticerci in musculature	Humans
	Echinococcus spp.	Unfiltered water	Ova in water	Canines
Nematodes				
Ascarididiae	*Ascaris suum*	Contaminated vegetables	Infective ova on contaminated vegetables	Pigs, primarily

Table 1 (*continued*)

Family	Parasite	Transmission route	Contaminated/infected matrix	Final hosts
	Toxocara canis	Contaminated vegetables; liver, paratenic hosts such as snails	Infective ova on contaminated vegetables, infective larvae in tissues	Canines
	Toxascaris leonina	Contaminated vegetables	Infective ova on contaminated vegetables	Canines
	Toxocara cati	Contaminated vegetables	Infective ova on contaminated vegetables	Felines
	Lagochilascaris minor	Contaminated vegetables	Infective ova on contaminated vegetables	Felines, racoons
Anisakidae	*Anisakis simplex* and *Pseudoterranova decipiens*	Intestine and musculature of marine fish, squid	Third stage larvae in tissues of marine fish and squid	Dolphins and toothed whales
Metastrongylidae	*Angiostrongylus* spp.	Contaminated vegetables. Infected frogs, prawns, crabs, etc	3rd stage larvae on vegetables. 3rd stage larvae in frogs, prawns, crabs, etc.	Rodents, especially rats
Gnathostomatidae	*Gnathostoma spinigerum* (and other species)	Meat. Fresh water fish	3rd stage larvae in musculature	Canines and felines
Trichinellidae	*Trichinella* spp.	Meat	Infective larvae in musculature	Humans, pigs, bears, wild boar, warthog, walrus, seal
Others				
Acanthocephalans	*Marcoacanthrhynchus hirudinaceus*	Beetles (as food/folk remedy)	Cystacanth in body cavity	Pigs
Pentastomids	*Armillifer armillatus* and *Armillifer moniliformis*	Contaminated water or food. Snake meat contaminated with eggs	Eggs in water or on vegetables. Nymphs in snake meat	Python and other snakes
	Linguatula serrata	Organs (esp. liver) of infected herbivores	Nymphs in organs/tissues of herbivores (halzoun/marrara)	Canines

in food processing or preparation. Water transports transmissible stages into drinking water supplies, recreational sites, including fresh and marine waters, and irrigation waters, which, in turn, can contaminate the food supply through agricultural and food industry practices from the farm to the fork. In addition to the use of water for irrigating crops, the food industry uses large volumes of water for its manufacturing and ancillary processes. Contamination can

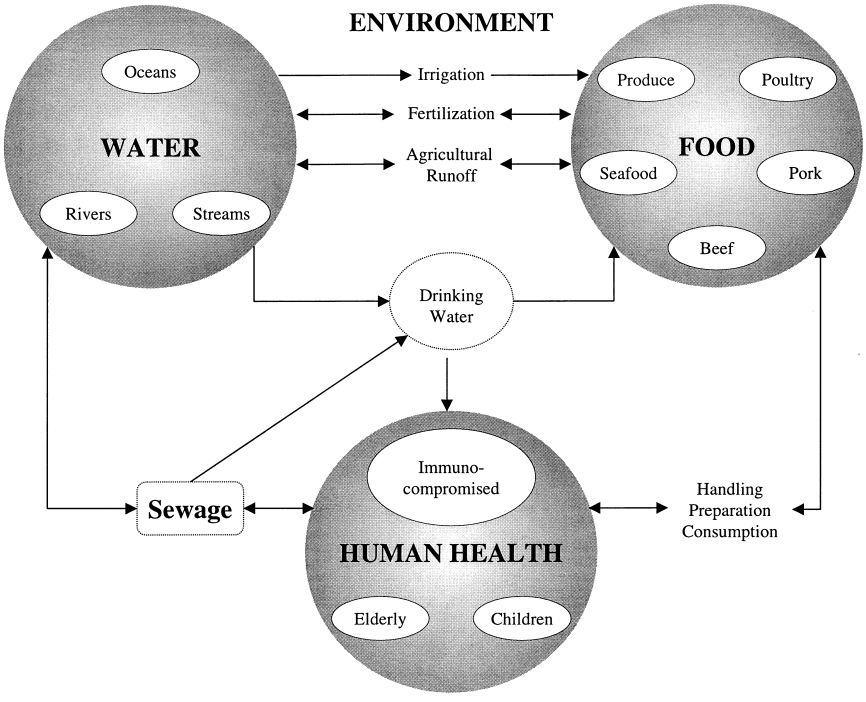

Fig. 1. Food–water connection between human health and the environment.

also occur when foods, particularly salad vegetables and fruit, are rinsed in parasite-contaminated potable water in the household. Furthermore, consumer vogues, such as the consumption of raw vegetables and undercooking to retain the natural taste and preserve heat-labile nutrients, can increase the risk of foodborne transmission.

In addition to the foodborne parasite zoonoses transmitted by 'meat', those transmitted through the surface contamination of produce (often fruit and vegetables) either at source or during food processing (see above) must also be included. Both source contamination of produce and contamination from water used in food preparation are transmission routes that are significant to the food industry. Surface contamination can be direct, following contamination by the infected host, or indirect, following contamination by transport (birds, flies, etc.) hosts, the use of manure and contaminated water for irrigation, fumigation and pesticide application, etc. Whether seasonal variation occurs in the surface contamination of foods requires further investigation, however, seasonal peaks in parasitism will influence when water and foods become surface-contaminated. The rapid transportation of foods acquired from global markets and their chilling and wetting can enhance parasite survival. Water and food enhance the survival of environmental stages by preventing their desiccation.

A variety of infective tissue parasite stages are responsible for transmitting meat and fishborne zoonoses (Table 1). Here, the preparation of the foodstuff is the key to the risk of transmission. Eating raw, undercooked, cured, smoked, salted, pickled or air-dried meat and offal can increase the risk of contracting foodborne parasite zoonoses, especially when the preservation treatment is inadequate. As well as transmitting infective stages, some filter feeders also act as transport hosts. For example, bivalves act as transport hosts by concentrating viable *Cryptosporidium* oocysts and *Giardia* cysts (and probably other zoonotic transmissive stages found in faecally-contaminated fresh, estuarine and marine waters) from their environment and have been suggested as reservoirs for zoonotic transmission [1–5].

Previously, the consumption of raw or undercooked meat and fish was associated with specific cultures and practices, but with shifting consumer vogues, increased international travel, globalisation of food supply and cosmopolitan eating habits, what were once regarded as rare diseases are now becoming increasingly more recognised. While there are no adequate estimates of the numbers of foodborne and waterborne disease world-wide, agencies, such as the United States Centers for Disease Control and Prevention (CDC), collect and report the incidence of notifiable diseases such as giardiasis and cryptosporidiosis. A recent review of food related illness and death in the United States reported that an estimated 2.5 million (7%) foodborne illnesses were caused by parasitic diseases (300 000; 2 000 000; 225 000; and 52 for *Cryptosporidium parvum*, *Giardia lamblia*, *Toxoplasma gondii* and *Trichinella spiralis*, respectively), all with zoonotic implications [6]. Some of the primary human zoonotic diseases, together with their association with food, are presented in Table 2. From 1993 to 1997, 19 foodborne outbreaks of parasitic origin occurred in the United States, with a total of 2325 cases reported.

For many parasites that appear in Table 1, only one life cycle stage is responsible for transmission, primarily following ingestion by a susceptible host. However, for some parasites, more than one life cycle stage can be responsible for transmitting the infection (e.g. oocysts and tissues cysts of *T. gondii*, ova and second stage larvae of *Toxocara canis*); these span both waterborne and foodborne transmission routes. Some transmissible stages require a period for external maturation before they become infective in the environment, and in these instances, contact with recently voided faeces is not a risk.

2.1. Current limitations

Some parasite zoonoses complete their life cycles in the human host (e.g. cryptosporidiosis, giardiasis, microsporidiosis, trichinellosis, etc.), while others do not (e.g. toxoplasmosis, toxocariasis, anisakiasis). The attribution of zoonotic status for those parasites where humans are intermediate, paratenic or accidental hosts is clear-cut, as is the attribution where humans are one of many final hosts to 'uncommon' parasites, particularly where information on person to person transmission is wanting. However, for those zoonoses where person to person transmission is a

Table 2
Primary human zoonotic diseases associated with food[a]

Parasite	Disease	Foodborne transmission (%)
Microsporidia (*Encephalitozoon*, *Enterocytozoon*)	Microsporidiosis	ND
Giardia lamblia	Giardiasis	10
Cryptosporidium parvum (genotype 2)	Cryptosporidiosis	10
Toxoplasma gondii	Toxoplasmosis	50
Taenia spp. (*T. solium*, *T. saginata*)	Cysticercosis, taeniasis	100
Fasciola hepatica	Fascioliasis	ND
Trichinella spiralis	Trichinellosis	100
Toxocara cati or *Toxocara canis*	Toxocariasis	ND
Anisakis simplex	Anisakiasis	100

[a] Adapted from Ref. [6]. ND, no data.

major route, the lack of effective typing and subtyping systems limits our knowledge of the significance of zoonotic waterborne and foodborne transmission, although descriptive epidemiology incriminates these routes. Currently, this is a particular problem with *C. parvum* and *Giardia duodenalis*. While direct zoonotic transmission has been documented for *Cryptosporidium* and *Giardia*, it remains in doubt whether zoonotic waterborne and foodborne transmissions of *Giardia* occur commonly. The widespread distribution of infection in a variety of livestock, wild animals and household pets indicates the potential for this route of transmission. That outbreaks of waterborne giardiasis do occur with relative frequency from supplies considered to be pristine (i.e. not receiving contributions from *Giardia*-infected humans) is support that the zoonotic contribution may be important. Increased demands made on natural resources increase the likelihood of encountering both environments and produce contaminated with these parasites.

3. Environmental contamination

The potential for environmental contamination depends upon a variety of factors, including the number of infected non-human hosts, the number of transmissive stages excreted, agricultural practices, host behaviour and activity, socio-economic and ethnic differences in human behaviour, geographic distribution, sanitation, safety of drinking water and food sources and supplies, and the climate and hydrogeology of the area.

3.1. Sources, contributions and survival

Some aspects of the biology of the intestinal parasites *C. parvum*, *G. duodenalis* and *T. canis* can be used to demonstrate the potential for environmental contamination. For *Cryptosporidium* and *Giardia*, the contribution from livestock and farming practices is difficult to assess. Infection can be clinical in calves, but subclinical in adult cattle. A clinically ill neonate can excrete $\leq 10^9$ oocysts daily during the course of infection, whereas a clinically-well, infected cow can excrete between 7.6×10^5 and 7.2×10^8 oocysts daily [7]. The sum total of oocysts contributed into the environment over a year is similar for both ill and well animals, given that immunity prevents the acquisition of further infection in the neonatal host. Enumerating contributions from agricultural practices, such as the storage and spread of farmyard manure and slurry, on-farm discharge of oocyst-contaminated dirty water to land or to water courses, pasturing of livestock in land adjoining water sources, and from the the disposal of faecally-contaminated waste from abattoirs provides data on the potential for livestock to contribute to *C. parvum* oocysts present in water courses. On an UK dairy farm, with a history of cryptosporidiosis, over 550 oocysts l^{-1} were discharged into watercourses. The practices which contributed high densities of oocysts into water courses included hosing down calf rearing pens and sluices (180 oocysts l^{-1}) and the contamination of farm drains with slurry and farm yard manure applied onto land (c. 370 oocysts l^{-1}) [7]. Practices such as hosing down calf rearing pens and sluices release recently excreted oocysts into an aquatic environment where survival is prolonged. Such oocysts are likely to have a higher viability than those excreted onto grazing land, which take time to percolate through substrata into watercourses. Overall contamination rates with *Cryptosporidium*, for a pristine watershed, have been estimated to be between $0.5–32 \times 10^5$ oocysts/ha per day and $0.12–2 \times 10^5$ cysts/ha per day for *Giardia* [8] from different reaches of another watershed, with uses ranging from recreation only to dairy farming [9].

T. canis has a high prevalence rate in adult dogs and foxes, with approximately 20% of adult dogs having patent intestinal infections [10], and is particularly abundant (90% plus) in puppies [11]. Gravid females produce up to 200 000 fertile eggs/day [12], which are voided in faeces, and embryonate to infectivity in the environment. Kennelled dogs excrete between 100 and 2000 eggs/g [13], and adult foxes up to 2145 eggs/g [14]. Environmental contamination with *T. canis* ova can be high: 66% of parks [15], 38% of gardens [16] and 56% of sandpits [17], with 11% of parks containing viable ova [18]. Ova can leach through soils or be washed into combined sewer overflows during periods of heavy rain. *T. canis* ova survive composting for at least 1 year [19] and survive in the environment for up to 4 years [20]. The ova remain dormant but viable if covered by snow at -11.5 to 10°C or protected in faeces [21], but are killed when unprotected at -15°C [22].

The transmissible stages can also be redistributed to other uncontaminated matrices by coprophagous transport hosts, including pigs, dogs, chickens, gulls and flies [23–29]. Flies ingest 1–3 mg faeces over 2–3 h [27], and can transmit *Giardia* cysts [30], *Cryptosporidium* oocysts [23–24] and *Toxocara* ova [28]. Filth flies transmit *C. parvum* oocysts both in excreta and on their external surfaces (experimentally, up to eight oocysts in the adult digestive tract; 150–320 oocysts on maggot and pupal surfaces) and wild-caught flies harboured a mean of 73 oocysts/fly [23]. *Toxocara* ova were detected on 2.4 and in 2.1% of wild-caught naturally infected flies in Nigeria [29]. *Toxocara* ova require a period of embryonation before being infective, although flies could deposit ova in/on food which could be ingested later.

4. Waterborne parasite zoonoses

Waterborne outbreaks of protozoan parasites are far more common than outbreaks due to helminths because of the smaller sizes of their transmissible stages. *Giardia* and *Cryptosporidium* have become significant waterborne pathogens in the developed world for three reasons. Firstly, giardiasis and cryptosporidiosis are indigenous infections in many animals; secondly, the densities of environmental

contamination with infective cysts and oocysts are sufficient to pollute the aquatic environment; and thirdly, the cysts and oocysts which penetrate water treatment processes are insensitive to the disinfectants commonly used in water treatment. Giardia cysts and *Cryptosporidium* oocysts are also small enough to pose a threat to groundwaters [31,32].

Toxoplasma and microsporidia have been associated with waterborne diseases on rare occasions. *T. gondii* oocysts are resistant to disinfection. The spores of the microsporidia are small (1–5 μm) and less is known regarding their resistance to water treatment. Community water systems are not regarded as a major route of transmission for the helminth zoonoses. Filtration, as a minimum, is an effective barrier to helminth ova (>20 μm) and the larger protozoan cysts, although ova can be found in the air, in dust and soil, and can be transferred to uncovered water sources.

4.1. Giardia *and* Cryptosporidium

The waterborne transmission of the intestinal protozoan parasites *G. duodenalis* and *C. parvum* has been well documented [33–37]; over 160 waterborne outbreaks of giardiasis and cryptosporidiosis have been reported, with the greatest documentation in the US and UK. Within the last 12 years, 39 documented outbreaks of waterborne cryptosporidiosis have occurred in the USA, Canada, UK and Japan [38]. Activities associated with cattle farming, particularly muck spreading, slurry spraying and run off from contaminated grazing land, have been proposed as causes of many of these outbreaks, but, in the absence of definitive information in many instances, the number attributed to the zoonotic route has to remain speculative. The search for both the contributors and causes has driven method development (Table 3) [26,39–41].

Developments in molecular and genetic analyses of waterborne protozoan parasites, including the determination of species identity and subtyping species, will help in determining the contributors of environmental contamination. Considerable research is currently ongoing in this area, and several genotyping techniques have been developed for *Cryptosporidium*, *Giardia*, microsporidia spp. and *Toxoplasma* [42–46]. In British Columbia, a waterborne outbreak of giardiasis was considered to have originated from an infected beaver, not only because beavers were epidemiologically-linked to the outbreak and were found to inhabit lodges close to the water supply, but also because typing studies (isoenzyme analysis and pulsed-field gel electrophoresis) indicated that *Giardia* isolated from the individuals affected by the outbreak were found to be of the same zymodeme and karyotype as *Giardia* isolated from the epidemiologically-linked beavers [47,48]. A comparison of 11 previously described species differentiation and genotyping protocols for *Cryptosporidium* determined that two were not *Cryptosporidium* specific [42]. While these molecular methods have great potential for tracking the source of contamination [49], comprehensive comparisons are necessary to validate the efficiency of the protocols, particularly with respect to environmental contamination [26].

4.2. Microsporidia

The microsporidia are obligate, intracellular spore-forming protozoa that belong to the phylum Microspora. About 1000 species of microsporidia are recognised [50], being, primarily, ubiquitous parasites of invertebrates and fish [50,51]. Largely unknown as causes of human disease before the HIV pandemic [51,52], human microsporidial infections have been found predominantly in HIV-infected immunocompromised individuals, although some infections in immunocompetent individuals have also been identified [53]. Currently, their role as emerging pathogens is being increasingly recognised. The prevalence of microsporidiosis in studies of patients with chronic diarrhoea ranges from 7 to 50%, world-wide [54], although it is unclear whether this broad range represents a geographic variation, differences in diagnostic capabilities or differences in risk factors for exposure to microsporidia.

In the summer of 1995, a waterborne outbreak of microsporidiosis occurred in France, with approximately 200 cases, primarily in the immunocompromised (chronic diarrhoea, dehydration and significant weight loss (>10% body weight), and low CD4 counts) [55]. While faecal contamination of the drinking water was never demonstrated, contamination from a nearby lake was suspected, but the source of that contamination (animal or human) was not suggested.

Microsporidial spores are stable in the environment and remain infective for days to weeks outside their hosts [56–58]. Their small size (1–5 μm) makes them difficult to remove using conventional water filtration techniques and there is concern that they may possess increased resistance to chlorine disinfection; similar to *Cryptosporidium*. Initial studies using cell culture suggest that the spores may be susceptible to disinfection [59].

4.3. Toxoplasma

Two outbreaks of toxoplasmosis, associated with the consumption of oocyst-contaminated water, have also been documented [60,61]. The first outbreak occurred in Panama in British troops, and epidemiological evidence indicated that the most likely vehicle for transmission was the ingestion of creek water, contaminated with oocysts excreted by jungle cats, consumed during manoeuvres in the jungle. The second outbreak occurred in British Columbia, Canada in 1995, and 110 acute *Toxoplasma* infections were identified. Fifty-five were in non-pregnant individuals and 42 women were pregnant at the time of infection. Eleven infants became infected. The epidemiological evidence was consistent with a waterborne source and implicated the municipal drinking water [61] whose raw

Table 3
Methods for detecting some waterborne and meatborne parasite zoonoses

Detection in water			
Method[a]	Giardiasis	Cryptosporidiosis	Toxoplasmosis/microsporidiosis
Regulatory methods for drinking water	USEPA method 1623 [106]]	USEPA method 1623 [106] UK Statutory Instruments 1999 no. 1524 [107]	None
Monitoring methods for raw and treated waters	Large and small volume filtration, flocculation, flow cytometry, immunomagnetisable separation, immunofluorescence detection with monoclonal antibodies, morphology, morphometry, PCR, fluorescence in situ hybridisation, electrorotation [26,39–41]	Large and small volume filtration, flocculation, flow cytometry, immunomagnetisable separation, immunofluorescence detection with monoclonal antibodies, morphology, morphometry, PCR, fluorescence in situ hybridisation, electrorotation [26,39–41]	Large and small volume filtration, ?flocculation, immunomagnetisable separation (for some microsporidia), brightfield detection, morphology, morphometry, PCR [112]
Viability determination	In vitro excystation, animal infectivity, fluorogenic vital dyes, PCR of inducible heat shock protein 70, reverse transcription PCR, fluorescence in situ hybridisation [26,39–41]	In vitro excystation, animal infectivity, in vitro infectivity of cell culture, fluorogenic vital dyes, PCR of inducible heat shock protein 70, reverse transcription PCR, fluorescence in situ hybridisation [26,39–41]	Animal infectivity [112], in vitro infectivity of cell cultures
Detection in 'meat'			
Method[b]	Toxoplasmosis	Cysticercosis	Trichinellosis
Direct detection in meat	Impractical due to microscopic nature of tissue cysts	Visual and invasive: incision of muscle tissue and palpation of other tissues. Accuracy for meatborne cysticerci not high [113–115]	Compression or digestion of muscle tissue. Compression: time consuming and relatively insensitive; not recommended for use in routine meat inspection; sensitivity for a 1 g sample, infection level of >3 larvae/g of tissue [116–118]; sensitivity for a 5 g sample, approximately 1 larva/g of tissue. Digestion: sensitivity for a 1 g sample, >3 larvae/g of tissue [116–118]; sensitivity for a 5 g sample, >3 larvae/g of tissue
Mouse bioassay	Not suitable for inspection of animals or meat at slaughter. Results take up to 4 weeks to obtain	Not used. Organoleptic method available	Not used. Organoleptic method available
Antibody detection. Sabin–Feldman dye test, indirect haemagglutination (IHA), latex agglutination (LA) and modified agglutination (MAT)	Unsuitable for routine testing. High degree of technical skill required in performing dye test. MAT, using formalised tachyzoites superior to other agglutination methods [119]. MAT superior to LA, IHA and bioassay in 1000 naturally exposed pigs: sensitivity, 82.9%; specificity, 90.3% [120]. No cross-reactivity with sera from pigs infected with *Sarcocystis miescheriana*, *Ascaris suum*, *Trichuris suis*, *Trichinella spiralis* and a number of swine viruses using MAT [121]. MAT unsuitable for slaughterhouse or field use as it requires large numbers of intact tachyzoites	Not applicable	Not applicable

Table 3 *(continued)*

Detection in 'meat'			
Method[b]	Toxoplasmosis	Cysticercosis	Trichinellosis
Antibody detection. ELISA/ Western blotting	ELISA in pigs has specificity of 85.9% and sensitivity of 72.9%, compared with bioassay [120]. Anti-*Toxoplasma* IgG ELISA, using a crude tachyzoite lysate, is reliable for identifying infected animals and correlates well with dye test results in experimentally infected pigs. Cross-reactivity with pigs harbouring *Sarcocystis* infection [122]. Useful for detecting both acute and chronic infections in humans [123]. Recombinant antigens: compared with the native antigen ELISA, the recombinant antigens (H4 and H11) have a sensitivity of 79% and a specificity of 100% using sera from naturally exposed sheep. Do not recognise antibodies sera from chronically infected pigs [124]	Western blotting (WB) more sensitive than ELISA and, with affinity purified glycoprotein antigens, is the method of choice for the serodiagnosis. Antigen strips commercially available. WB sensitivity for pigs with one detectable cyst, 60–80% [125]. Heterologous antigens: antibodies from *Taenia saginata*-infected cattle react with lipoprotein antigens from *Taenia hydatigena* cyst fluid (ThFAS) [126]. ThFAS has low reactivity with anti-*Fasciola hepatica* antibodies. ThFAS can detect 'Taiwan *Taenia*' pig infections [127]. In pigs naturally infected with *Taenia solium*, *Taenia crassiceps* antigens achieved 97% specificity and 100% sensitivity [128]. Recombinant antigens: Tc A2-MBP (recombinant from a *T. crassiceps* cDNA sequence) is specific for *T. saginata* and can detect infections in cattle [129]	ELISA is the best method for ante-mortem diagnosis. Comparable in sensitivity to best direct methods with infection levels as low as 1 larva/100 g of tissue detected [117,130]. Only short-term ES antigen or biochemically purified antigens can be used currently [131–133]. Overall estimates of ELISA efficacy, 93.1–99.3% sensitivity and 90.6–99.0% specificity [134–138]. Recombinant antigens: the major problem is inability to reproduce the glycoprotein structure of immunodominant antigens. Synthesised neoglycan [139] with the antigenic structure found on *Trichinella* glycoproteins used in ELISA [138] and performed as well as native ES antigens when testing sera from experimentally and naturally infected pigs
Circulating antigen detection. ELISA	Sufficient antigen available for detection only for a short time in human sera and mouse tissues, during acute phase of infection	Monoclonal antibodies against metacestode extracts can detect circulating antigens in the sera of 79% infected pigs; specificity, 97% [140]	Antigen detection is unreliable for routine diagnosis as antigenaemia detected in only 56% of animals tested [141]
Molecular methods (PCR and DNA probes)	PCR of repetitive gene fragment (B1) has sensitivity of 10 tachyzoites/10^5 leukocytes Burg et al. [142]. PCR of ribosomal DNA for specific identification of *Toxoplasma* [143]. Highly sensitive and specific when combined with P30 gene PCR	Combined use of DNA probes for *T. saginata* and *T. solium* provides positive identification of *T. saginata* proglottids from faecal samples [144]	Important to identify infecting species and types as this can assist in identifying the source of infection. Different species/types produce differing pathology in humans. Randomly primed PCR reactions (RAPD) used to differentiate the eight accepted groups of *Trichinella* [145,146]

[a] Adapted from Ref. [26].
[b] Adapted from Ref. [95].

water source was probably contaminated with oocysts from domestic and feral cats and cougars.

5. Recreational water

Giardia and *Cryptosporidium* are the most commonly recognised cause of recreational waterborne disease. Most recreational water outbreaks are the result of faecal accidents or cross-connections in swimming pools, and the contamination of recreational waters with animal wastes is not well documented or recognised [62], although defecation by infected livestock and feral animals into lakes, canals, other outdoor recreational water bodies or receiving waters must be borne in mind. A statistically significant association was identified between the drinking of untreated surface water and illness in New Mexico [63]. An increased risk of infection was also related to swimming in surface water, as well as attending a day-care centre, camping and having a pet that was ill or young. In 1997 and 1998, in the most recent reports on recreational outbreaks in the USA, *Cryptosporidium* was responsible for nine of 18, and all but one occurred in swimming pools, with one occurring in an interactive fountain [64]. *Giardia* was not reported. The source of the contamination that occurred in a lake at a State park was not identified. In 1999, the second interactive fountain outbreak of gastroenteritis occurred at a beachside park [65], however, no source of contamination was determined.

While outbreaks can be seen as extreme consequences of zoonotic transmission, it is likely that numerous cases of disease associated with recreational exposure go unrecognised, and hence, are not reported. The swimming in waters influenced by the wastes of animals is of concern, yet assessment of the risk requires further quantification. Increased utilisation of outdoor recreational waters for immersion water sports is likely to precipitate increased reporting of water-associated zoonoses. Of interest is the increased reporting of periodic clusters of swimmers' itch, a dermatitis caused by cercariae of avian schistosomes that penetrate into human skin, but which are unable to complete their life cycles in the human host.

6. Foodborne parasite zoonoses

6.1. Surface contamination

The increased demand, global sourcing and rapid transport of foods, especially soft fruit and salad vegetables, enhance both the likelihood of surface contamination and survival of the transmissive stages of parasites pathogenic to man. Food normally becomes a potential source of human infection by contamination, during production, collection, transport and preparation (e.g. milk, fruit, vegetables, soft drinks, etc.) or during processing, and the sources of zoonotic contamination are usually faeces, faecally-contaminated soil or water. The number of contaminating organisms will vary depending upon the route or vehicle of contamination, and therefore, the sensitivity of the methods developed will have to address the detection of the smallest numbers of contaminants, practicable (1–100). Given the low infectious doses of many parasites, surface contamination with low numbers of viable parasites, in produce that receives minimal washing prior to ingestion, poses a threat to public health. It is often difficult to associate an outbreak with a particular food item and furthermore, if the foodborne route is suspected, to identify how the food implicated became contaminated. Due to these difficulties, the acquisition of parasitic infections via the foodborne route is almost certainly under-detected. Casemore [66], in reviewing foodborne protozoal infection, suggested that the degree of under-detection might be by a factor of 10 or more.

With these current limitations, it not surprising to realise that documented zoonotic foodborne outbreaks are rare, although some foods can be important vehicles of transmission, especially in situations of poor hygiene and endemnicity of infection [28,67]. Currently, foodborne giardiasis and cryptosporidiosis are of significance because of both the low infectious doses and the robustness and disinfection insensitivity of their transmissive stages [68–72], and modifications of the methods based on their detection in water are being developed [71,72].

6.1.1. Giardia *and* Cryptosporidium

The foodborne transmission of giardiasis was suggested in the 1920s [73,74], and anecdotal evidence from other outbreaks has frequently implicated food handlers and contaminated fruit and vegetables [75]. The first foodborne outbreak of giardiasis in the US was described in 1979 [75,76]. Of eight outbreaks of foodborne giardiasis documented, only one reports the possibility of food (i.e. tripe) being intrinsically infected. The other outbreaks, affecting 217 individuals, between 1979 and 1990, are associated with contamination by food handlers, and include foods such as salmon, fruit salad, raw vegetables, lettuce, onions and tomatoes. In two outbreaks, the original source of infection was traced to the infected infant of the food handler [72].

Suspected outbreaks of foodborne cryptosporidiosis have been reported from travellers visiting Mexico, in the UK and Australia, the suspect foods including salads, raw milk, sausages and tripe [76]. An outbreak following the consumption of apple cider was the first associated with the zoonotic transmission route [77]. The fresh pressed cider was squeezed from apples collected from an orchard in which an infected calf grazed. Some apples had fallen onto the ground and had probably been contaminated with infectious oocysts [77]. While three other outbreaks have been reported since 1993, none implicated zoonotic transmission [78,79]. Again, the absence of standardised detection and subtyping methods limits our understanding of the zoonotic route of infection.

6.1.2. Fasciola

For many, the perception of human fascioliasis, caused by *Fasciola hepatica* or *Fasciola gigantica*, is that it is a sporadic disease of low economic importance, but Chen and Mott [80] highlighted the importance of this zoonosis, identifying 2594 cases from 42 different countries between 1970 and 1990. Current estimations indicate between 2.4 and 17 million human infections world-wide [81,82] and the WHO recognises fascioliasis as an emerging disease of humans [83]. Estimates based on faecal egg counts, will be underestimates, as they will not include those individuals with prepatent or ectopic infections, or those with low grade infections excreting intermittently or at very low egg densities.

The distribution of the disease is predominantly rural, being associated with cattle and sheep breeding [84–86], although high prevalences in humans are not necessarily associated with areas where fascioliasis is a significant veterinary problem [86]. The incidence appears to be concentrated within families, as they are all likely to eat the same contaminated product(s) [87]. Interestingly, Mas-Coma et al. [86] state that in hyperendemic areas, the parasite is better adapted to the human host, presumably leading to reduced liver pathology, increased adult numbers and egg production. Further information on human infection can be found in the reviews of Chen and Mott [80], Mas-Coma et al. [86] and Esteban et al. [88].

The most common transmission route is the ingestion of watercress (*Nasturtium* and *Roripa* species; Table 2) contaminated with encysted metacercariae, although, depending upon the geographical location, a variety of edible aquatic plants can be vehicles of transmission. Water containing floating metacercariae has also been implicated in disease transmission [89], as have salads contaminated with metacercaria-contaminated irrigation water [90]. In Iran, the risk factors include the use of animal manure as fertiliser and wastewater effluent for irrigating aquatic or semi-aquatic vegetable crops [83]. Recently, transmission following consumption of fresh, raw liver dishes containing immature flukes was suggested [91].

6.2. Meatborne infection

Meatborne parasitic zoonoses remain an important cause of illness and economic loss, globally [92–95]. Of known importance are toxoplasmosis, cysticercosis and trichinosis, while fishborne parasites remain a problem in certain regions of the world. Foodborne trematode infections also exert a significant economic impact, with more than 40 million people infected with one or more different species [82,83]. Efforts to control these zoonoses continue, yet the overall progress is unsatisfactory [96]. In addition to eating infected meat bought over the counter, eating inadequately cooked game (e.g. bear, boar) and fish, during or after hunting, fishing and shooting expeditions also contributes to the increased reporting of zoonotic meatborne infections.

6.2.1. Toxoplasma, Taenia spp. and Trichinella

In terms of illness and death, *Toxoplasma*, *Listeria* and *Salmonella* are the three most important pathogens transmitted by food in the USA, and possibly Europe [97]. Pork, lamb and mutton are the most important sources of meatborne infections of *Toxoplasma*, together with game such as bear and feral swine meat [6]. In a European multicentre case-controlled study, Cook et al. [98] identified that eating undercooked lamb, beef or game, contact with soil, and travel outside Europe, the USA and Canada were the risk factors most strongly predictive of acute *Toxoplasma* infection in pregnant women. The infection of livestock with larval stages (cysticercosis) of *Taenia saginata* (beef tapeworm) and *Taenia solium* (pork tapeworm), which develop into adults in the human intestine, is also of great public health concern [93,99,100]. Clinically, *T. solium* is of greater concern because, unlike *T. saginata*, humans also serve as the intermediate host for the cysticercus stage, following autoinfection or if ova are ingested accidentally from the environment or in folk remedies. Human trichinellosis, contracted by eating raw or undercooked meat containing infective larvae (trichinae) of the nematode parasite *Trichinella* spp., is most commonly associated with eating pork, bear meat and horse meat [95]. *T. spiralis* is the species of greater concern, as it the species most commonly found in pigs. High priority is placed on the inspection of swine and horse carcasses for trichinae at slaughter in many countries: the European Union spends $570 million each year on *Trichinella* testing [101]. Of the anisakine parasites, *Anisakis simplex* and *Pseudoterranova decipiens* are of major significance [83], with more than 80% of Pacific salmon and red snapper infected with larvae of these species [102].

6.2.2. Microsporidia

In addition to its waterborne route of transmission, microsporidiosis is also a potential emerging meatborne zoonosis, given that natural hosts of human infective microsporidia can be part of the human food chain. *Pleistophora*-like microsporidians, initially found in muscle, may be acquired from raw or lightly cooked fish or crustaceans. Some evidence for the foodborne route comes from the incidental finding of microsporidial spores in a human stool sample from an AIDS patient with diarrhoea [103], which also contained muscle fibres (meat) infected with microsporidia. The suggested transmission route was as follows: after eating the fish, spores from the infected musculature remained largely intact during passage through the patient's gut, with some of these viable spores initiating the infection. The relationship between microsporidial parasites of fish and crustacean muscle and those found in human cases requires further elucidation.

6.2.3. Foodborne trematodes

Foodborne trematode infections, acquired through eating raw, improperly cooked or processed freshwater fish, shell-

fish, crabs, or unwashed or inadequately washed vegetables (Table 1), were recognised as a public health problem in 1991 by the Southeast Asian Ministries of Education Organisation Regional Tropical Medicine and Public Health Project (SEAMEO-TROPMED). Clonorchiasis, paragonimiasis, fascioliasis, fasciolopsiasis and other intestinal trematodiases are the most important diseases contracted, and strategies to control foodborne trematode infections were identified in 1995 [83]. Among these strategies, health education programmes featured greatly; the awareness of both hazards and risks being fundamental goals, as was the generation of baseline epidemiological data and the development of food safety programmes and hazard assessment at critical control points (HACCP) approaches. The application of international codes of practice (e.g. FAO/WHO Codex for fish and fisheries products; legislation controlling disposal of excreta) could also reduce environmental contamination, as could specific legislation for agriculture and aquaculture.

The identification of strategies to control foodborne transmission in 1995 indicates that the trematode zoonoses are well recognised, yet, for many, particularly in developed countries, these are emerging zoonoses. Have the foodborne zoonoses been ignored, and if so, can they be addressed currently? The World Health Organisation/Pan American Health Organisation informal consultation document on intestinal protozoa [104,105] offers a way ahead. In addition to the strategies identified above, new immunological and molecular technologies were deemed to have applications in the environment, especially where waterborne (and foodborne) transmissions are known to occur. It was concluded that the development of molecular biological tools for diagnostic and epidemiological purposes should be encouraged [104].

7. Trends: current and future

Recent advances in immunology and molecular biology have enabled us to develop more sensitive, specific and rapid tests that could supersede current methods. For waterborne zoonoses, particularly *Giardia* and *Cryptosporidium*, great interest exists in developing both effective detection (Table 3) and typing systems which have public health pertinence. Immunomagnetisable separation, followed by antibody detection or PCR (for intact cysts and oocysts) appear to be effective test formats (Table 3), while for some meatborne zoonoses, antibody detection, in serum or meat juices, as a reflection of exposure, and PCR or nucleic acid probes for determining the presence of the parasite (*Toxoplasma*, cysticercosis and *Trichinella*) appear effective (Table 3). Similarly, the development of new chemotherapeutic agents and alternative vaccine strategies in livestock offer new opportunities to improve the control of some waterborne and meatborne zoonoses, yet, for meatborne zoonoses infecting livestock, testing at slaughter or prior to processing remains necessary to protect public health.

The sample matrix plays a significant role in test development, and once optimised for the matrices and parasites of current significance, the same formats should prove useful for detecting other emerging and re-emerging zoonoses in similar matrices. For example, given the levels of environmental contamination described for *T. canis* ova, our close association with dogs, the large range of intermediate and paratenic hosts which form part of the human food chain, and the recognised foodborne outbreaks (e.g. surface contamination of vegetables with ova; infective larvae in raw liver, edible snails and raw or undercooked meat), might foodborne transmission of toxocariasis be more prevalent that we think?

Two issues will determine the adoption of new methods: whether they can be adapted to, and will be suitable for on line testing; and whether sufficient testing has been undertaken to provide confidence in their use.

8. Epilogue

The under-diagnoses and reporting of these important zoonotic parasites undermines our ability to bring the diseases to the attention of industries, governments and communities, and to implement controls. The multiple routes of transmission complicate the understanding and the ability to estimate the magnitude of contaminated water and food in the overall burden of disease for many of these pathogens. Environmental monitoring of water and food utilising new technologies, along with molecular epidemiology will be one of the best approaches for the identification of the risks in the future, and new approaches for recovery of the parasites from water and food are now available.

Many countries have implemented regulations addressing the control of the spread of waterborne and foodborne diseases, particularly through water reuse programmes. Despite these efforts, the potential for contaminated food and water to cause extensive outbreaks still remains, due to the breadth of populations served. Several problems exist with identifying outbreaks associated with parasitic zoonoses. Often, the foremost problem occurs with detecting and reporting the contaminated water or food. Some countries have adopted regulations to minimise protozoan parasite contamination of potable water [106,107] and some of the meatborne zoonoses, e.g. [83,108–111], however, regulations for other foodborne parasitic zoonoses are fewer, although the livestock and food industries have adopted the best practice by developing effective HACCP programmes. Interestingly, the rise in general public concern over food safety has helped to focus more attention on zoonotic parasites. For many of the zoonotic parasites, the system for routine monitoring or reporting is inadequate, thus the incidence of human disease and parasite occurrence

in water and food is undoubtedly underestimated. Particularly for the foodborne zoonoses, parasitic infections have a lower impact than prokaryotic pathogens, which, again, contributes to an underestimation of the number of identified cases/outbreaks.

With the new international codes regarding food safety, risk assessment methodologies are being seen as the scientific process to address public health, globally. Both epidemiological and risk assessment approaches are dependent upon an evaluation of the occurrence and survival of the transmissive stage(s) of the parasite in question. For example, for many parasitic infections transmitted through the environment, exposure assessment is perhaps the most difficult parameter to measure. The understanding of this variable is dependent not only upon the detection of organisms in the environment, but also on an understanding of the occurrence, transport, survival and fate through various matrices [28]. Here, sensitive, robust and reproducible detection, viability, typing and subtyping methods, with suitability for the matrix in question, will be the arbiters. Only by developing such methods can we attempt to determine the impact of the parasite zoonoses transmitted by water and food, and their public health significance.

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