Food-borne protozoa

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Pathogenic protozoa are commonly transmitted to food in developing countries, but food-borne outbreaks of infection are relatively rare in developed countries. The main protozoa of concern in developed countries are *Toxoplasma*, *Cryptosporidium* and *Giardia*, and these can be a problem in immunocompromised people. Other protozoa such as *Entamoeba histolytica*, *Cyclospora cayetanensis* and *Sarcocystis* can be a food-borne problem in non-industrialised countries. *C. cayetanensis* has emerged as a food-borne pathogen in foods imported into North America from South America. *Microsporidia* may be food-borne, although evidence for this is not yet available. The measures needed to prevent food-borne protozoa causing disease require clear assessments of the risks of contamination and the effectiveness of processes to inactivate them The globalisation of food production can allow new routes of transmission, and advances in diagnostic detection methods and surveillance systems have extended the range of protozoa that may be linked to food.

Protozoa are a diverse group of organisms that have evolved to occupy a variety of ecological niches. There are over 30 phyla of protozoa, but the enteric ones causing food-borne human disease belong to the phyla Apicomplexa, Rhizopoda, Zoomastigina, Microspora and Ciliophora (Table 1). Most of these have evolved a totally parasitic existence. The enteric protozoa that cause human illness are usually transmitted by the consumption of food and drink, or through environmental contamination and poor hygiene (Table 1). Some of these can cause substantial illness, and have economic consequences[1,2]. Many cause problems in immunocompromised patients, particularly in HIV-infected people and individuals with T-cell deficiencies. The range of parasitic protozoa present in the human population and agricultural animals is greater in non-industrialised countries than in industrialised ones. There is a greater exposure to infection because food and water distribution systems are poor, and microbial contamination of food and water is common. Toilet facilities in non-industrialised countries are often primitive, and food sold in native markets may be contaminated from hands that have not been washed after defaecation or from flies that land on both food and faeces.

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Table 1 Food-borne and water-borne protozoa

Protozoa	Life cycle in a single host	Recognised human pathogen	Host range[a]	Food-borne infections/ outbreaks	Water-borne infections/ outbreaks	Present in animal meat[b]	Present in animal faeces	Present in human faeces
APICOMPLEXA								
Cryptosporidium parvum Type 1	✔	✔	H	✔	✔	✘	✘	✔
Cryptosporidium parvum Type 2	✔	✔	HAW	✔	✔	✘	✔	✔
Cryptosporidium parvum Type 3	✔	S	H	✔[c]	✔[c]	✘	?	✔
Cryptosporidium felis	✔	S	HA	✔[c]	✔[c]	✘	✔	✔
Cryptosporidium spp (canine strain)	✔	S	HA	✔[c]	✔[c]	✘	✔	✔
Cyclospora cayetanensis	✔	✔	H	✔	✔	✘	✘	✔[d]
Isospora belli	✔	✔	HA	✘	✘	✘	✘	✔[d]
Sarcocystis hominis	✘	✔	HAW	✔	✘	✔	✔	✔
Sarcocystis suihominis	✘	✔	HAW	✔	✘	✔	✔	✔
Toxoplasma gondii	✘	✔	HAWB	✔	✔	✔	✔	✘
MASTIGOPHORA								
Chilomastix mesnili	✔	✘	H	✘	✘	✘	✘	✔
Dientamoeba fragilis	✔	✔	H	✘	✘	✘	✘	✔
Enteromonas hominis	✔	✘	H	✘	✘	✘	✘	✔
Giardia lamblia	✔	✔	HAW	✔	✔	✘	✔	✔
Retortomonas intestinalis	✔	✘	H	✘	✘	✘	✘	✔
Trichomonas hominis	✔	✘	H	✘	✘	✘	✘	✔
SARCODINA								
Endolimax nana	✔	✘	H	✘	✘	✘	✘	✔
Entamoeba coli	✔	✘	H	✘	✘	✘	✘	✔
Entamoeba dispar	✔	✘	H	✘	✘	✘	✘	✔
Entamoeba hartmanni	✔	✘	H	✘	✘	✘	✘	✔
Entamoeba histolytica	✔	✔	H	✔	✔	✘	✘	✔
Iodamoeba butschlii	✔	✘	H	✘	✘	✘	✘	✔
Blastocystis hominis	✔	✘	HAWB	?	✔	✘	✔	✔
Acanthamoeba spp	✔	✔	F	✘	✔	✘	✘	✘
Naegleria fowleri	✔	✔	F	✘	✔	✘	✘	✘
MICROSPORA								
Brachiola vesicularum	?	S	Q	✘	✘	✘	✘	✘
Enterocytozoon bieneusi	✔	✔	HAW	✘	✘	✘	✔	✔
Encephalitozoon cuniculi	?	✔	Q	✘	✘	✘	✘	✘
Encephalitozoon hellem	?	S	Q	✘	✘	✘	✘	✘
Encephalitozoon intestinalis	✔	✔	HAW	✘	✘	✘	✔	✔
Nosema connori	?	S	Q	✘	✘	✘	✘	✘
Pleistophora spp	?	S	Q	✘	✘	✘	✘	✘
Trachipleistophora anthropophthera	?	S	Q	✘	✘	✘	✘	✘
Trachipleistophora hominis	?	S	Q	✘	✘	✘	✘	✘
Vittaforma corneae	?	S	Q	✘	✘	✘	✘	✘
CILIOPHORA								
Balantidium coli	✔	✔	HAW	✘	✘	✘	✔	✔

A = agricultural animals, H = humans and primates, W = wild animals, B = birds, F = free-living organisms, Q = infects immunocompromised humans rarely, but the main host is unknown, S = has been demonstrated in a small number of patients with disease, ? = not known;

[a]Host range as currently known, the true host range may be greater [b]It is likely that some of the microsporidia infect man through consuming the infected meat from inadequately cooked birds, mammals or fish but there is no evidence for this

[c]Information extrapolated from similar species, [d]Oocysts/sporocysts need to mature in the environment before they are infectious

Table 1 *(continued)* Food-borne and water-borne protozoa

Protozoa	Transmission stage	Pathogen grows in the environment	Endemic in the UK	Geographic distribution
APICOMPLEXA				
Cryptosporidium parvum Type 1	Oo	✘	✔	World-wide
Cryptosporidium parvum Type 2	Oo	✘	✔	World-wide
Cryptosporidium parvum Type 3	Oo	✘	✔	?
Cryptosporidium felis	Oo	✘	?	?
Cryptosporidium spp (canine strain)	Oo	✘	?	?
Cyclospora cayetanensis	Oo/Sc	✘	✘	Non-industrialised countries
Isospora belli	Oo/Sc	✘	✘	Non-industrialised countries
Sarcocystis hominis	Oo/Sc/Bz	✘	✘	World-wide
Sarcocystis suihominis	Oo/Sc/Bz	✘	✘	World-wide
Toxoplasma gondii	Oo/Bz	✘	✔	World-wide
MASTIGOPHORA				
Chilomastix mesnili	Cy	✘	✘	Non-industrialised countries
Dientamoeba fragilis	Tr	✘	✘	Non-industrialised countries
Enteromonas hominis	Tr	✘	✘	Non-industrialised countries
Giardia lamblia	Cy	✘	✔	World-wide
Retortomonas intestinalis	Tr	✘	✘	Non-industrialised countries
Trichomonas hominis	Tr	✘	✘	Non-industrialised countries
SARCODINA				
Endolimax nana	Cy	✘	✘	World-wide
Entamoeba coli	Cy	✘	✔	World-wide
Entamoeba dispar	Cy	✘	✘	World-wide
Entamoeba hartmanni	Cy	✘	✘	Non-industrialised countries
Entamoeba histolytica	Cy	✘	✘	Non-industrialised countries
Iodamoeba butschlii	Cy	✘	✘	World-wide
Blastocystis hominis	Cy/Tr	?	✔	World-wide
Acanthamoeba spp	Cy/Tr	✔	✔	World-wide
Naegleria fowleri	Cy/Tr	✔	✔	World-wide
MICROSPORA				
Brachiola vesicularum	Sp			
Enterocytozoon bieneusi	Sp	✘	✔	?
Encephalitozoon cuniculi	Sp	✘	?	World-wide
Encephalitozoon hellem	Sp	✘	?	?
Encephalitozoon intestinalis	Sp	✘	✔	World-wide
Nosema connori	Sp	✘	?	?
Pleistophora spp	Sp	✘	?	?
Trachipleistophora anthropophthera	Sp	✘	?	?
Trachipleistophora hominis	Sp	✘	?	?
Vittaforma corneae	Sp	✘	?	?
CILIOPHORA				
Balantidium coli	Cy	✘	✘	Non-industrialised countries

? = not known, Oo = oocyst, Sc = sporocyst, Bz = bradyzoite, Tz = tachyzoite, Cy = cyst, Tr = trophozoite, Sp = spore

Vegetables and fruit can also be affected by washing with contaminated water.

The protozoa that are of most concern in industrialised countries are *Cryptosporidium*, *Giardia* and *Toxoplasma*, although *Cyclospora* has been identified in a number of outbreaks in the US and Canada in recent years. Food-borne outbreaks of infection with protozoa are not common. This is partly because surveillance systems for detecting outbreaks of protozoan infections are poorly developed in most countries. Pathogens, like *Cryptosporidium* and *Giardia*, can be transmitted by a variety of routes other than food. Some protozoan pathogens, like *Toxoplasma gondii*, cause only mild disease in most people and outbreaks are difficult to detect without mass antibody screening. In industrialised countries, testing for enteric protozoa is often done on patients returning from non-industrialised countries but not on other patients. This results in indigenous infections not being detected. Some protozoa (*Sarcocystis* spp. and *T. gondii*) can be present within meat as part of their normal life-cycle. Others get into food through faecal contamination of the raw materials and inadequate treatment of the food before eating it, or through post-treatment contamination. This is true for the oocysts or sporocysts of *Cryptosporidium* spp., *Cyclospora cayetanensis*, *T. gondii* and *Sarcocystis* spp., and for many of the cysts or spores of other protozoa.

Apicomplexa

Cryptosporidium spp.

Cryptosporidium parvum is a well-recognised cause of large waterborne outbreaks of gastroenteritis[3–7], but can also cause food-borne outbreaks[8–13]. These organisms can cause a chronic life-threatening infection with watery diarrhoea in people with a compromised T-cell condition such as acquired immune deficiency syndrome (AIDS) or severe combined immunodeficiency (SCID). However, in most people, a diarrhoeal episode that can last from a few days to a few weeks is followed by remission of symptoms.

Infection can derive from children, dogs, cats, farm animals and wild animals. Birds are not thought to be infected by human strains, although the oocysts of *C. parvum* can remain viable after passing through their intestines. Water sources are commonly contaminated with oocysts from animal and human faeces, and infection can occur in farmers and veterinarians working with animals.

Work over the last few years[14–24] is indicating that what we currently call *C. parvum* is composed of three or more types that are infectious to

humans, and additional isolates from cats and dogs that are regarded as separate species are also infectious to humans. *C. parvum* type 1 is infectious to humans and other primates, but will not infect most agricultural and laboratory animals tested. *C. parvum* type 2 has a wider host range and is infectious to humans, sheep, cattle and laboratory animals. *C. parvum* type 3 has been found in humans, but the animal host range is not known. *C. felis* is infectious to cattle, cats and humans. The main *Cryptosporidium* strains associated with human disease in the UK are *C. parvum* types 1 and 2.

The oocysts of *Cryptosporidium* are infectious when excreted in faeces, and these can pass into rivers and lakes. They are resistant to chlorine and can pass into drinking water when there are failures in filtration or contamination of apparently secure source waters. Food can become contaminated through drinking water at the time of a water contamination incident, and raw products can be contaminated through irrigation or spraying with non-potable water. Outbreaks have been associated with inadequately pasteurised milk[25], apple juice[13], uncooked green onions in salads[8], and chicken salad[26]. Incidents have also been linked to raw milk[27], inadequately pasteurised milk[25], sausage and frozen tripe[9]. *Cryptosporidium* oocysts have a low infectious dose and individual strains have been found to differ in their infectivity, with an LD_{50} for human volunteers varying from 10 to 1000[28]. *Cryptosporidium* oocysts have been found in 14% of raw vegetables in Peru[29]. Foods that are consumed without heat treatment represent an important potential source of infection. Food-handlers who are infected, or are the parents of infected children, can also be a source of infection.

The major identifiable source of human cryptosporidiosis in England and Wales is water supplies that have become contaminated with animal faeces or sewage. During water-borne outbreaks, there is the potential for contaminated water to contaminate food. Special arrangements need to be made by food producers and retailers when the public water supply is thought to be contaminated with *Cryptosporidium* oocysts. Assessments of the risk of oocysts in the water causing infection following food processing need to be made, and depends on the extent of processing. *C. parvum* oocysts are sensitive to drying[30], to moderate heat treatment[31], and are killed by pasteurisation[32]. Oocysts are otherwise quite resistant to most chemical disinfectants and food preservatives, although the biocidal effect of combinations of pH, a_w, temperature, *etc.* have not been fully evaluated. (Water activity value, a_w, is a term that is used to describe the availability of water in a product rather than the total water content. It is taken by measuring the vapour pressure created by a food sample in a head-space of air and values range from 0 to 1 a_w.) Oocysts can survive in water at pH 3–10, and may survive (although in reduced numbers), for more than 24 h in beer, carbonated beverages and orange juice[4].

Cyclospora cayetanensis

Cyclospora is a coccidian parasite that causes protracted watery diarrhoea. It occurs world-wide but is common only in non-industrialised countries. Several recent reviews have summarised the life cycle, clinical manifestations and epidemiology of the parasite[33–40]. In endemic countries, the disease is seasonal with the highest incidence recorded in the late spring and summer months. The incubation period is 7–14 days and the duration of illness is around 7 weeks[34]. In the UK it is normally associated with travel to non-industrialised countries, several cases of cyclosporiosis have been reported in non-travellers in the US and Canada, and imported fruits and vegetables and drinking water have been implicated as vehicles of infection. Person-to-person spread is not thought to occur, because the oocysts need to mature (sporulate) under environmental conditions outside the host for 1–2 weeks before they become infectious[34].

The life cycle of *Cyclospora* is not fully known, but is believed to involve both asexual and sexual stages of proliferation[34]. It appears that *C. cayetanensis* requires only a single host to complete its entire life cycle. The morphology of *Cyclospora* in the intestine is similar to that of *Isospora*. Light microscopy and electron microscopy have been used to identify the asexual stages of *C. cayetanensis* in enterocytes seen in intestinal biopsies, including the sporozoite, trophozoite, schizont, and merozoite[41]. The sexual cycle also takes place in the human host, producing oocysts that are excreted in the faeces. The lamina propria and submucosa are not involved. The oocysts have been reported to be relatively resistant to chlorine[34 42].

Outbreaks of cyclosporiosis in the US and Canada have been associated with raspberries and salad items imported from South America[43–48]. The incidence of this parasite in the population of the UK is thought to be low. There are no known non-primate animal hosts[49], and the *Cyclospora* isolated from baboons differs from human isolates[50]. The numbers of oocysts getting into sewage is likely to be small, and it is unlikely that significant numbers reach source waters. As a consequence, the risk of *Cyclospora* being transmitted via treated mains water in the UK is considered to be low. In non-industrialised countries, transmission is likely to be through sewage contaminated water and the contamination of fruit and vegetables with sewage contaminated water used for irrigation or pesticide application[34].

Although protocols for the detection of *Cyclospora* in food have been used, they are not very sensitive[34]. They involve the use of microscopy or PCR to detect oocysts in washings from foods.

Isospora belli

Human intestinal isosporiasis is caused by *Isospora belli*. Members of the genus *Isospora* cause intestinal disease in several mammalian host

species[51]. The symptoms of *I. belli* infection in immunocompetent patients include diarrhoea, vomiting, abdominal pain, dehydration, weight loss, steatorrhoea, headache, fever and malaise. The disease is often chronic, recurrences are common and infections can continue for months to years. Symptoms are more severe in AIDS patients, with the diarrhoea being more watery. In the US, AIDS patients' isosporiasis was more common in people who had travelled abroad and in indigenous Hispanic populations than in the rest of the population[52]. Extra-intestinal stages of *I. belli* have been observed in AIDS' patients but not immunocompetent patients. Asexual and sexual stages grow within intestinal cells of their hosts and produce an environmentally resistant oocyst. Infections are thought to be acquired by the ingestion of sporulated oocysts in contaminated food or water, although good evidence for the source of infection in most infected patients is limited.

Toxoplasma gondii

Toxoplasma gondii and *Sarcocystis* spp. have life cycles involving a sexual cycle with oocyst production in a carnivorous host (*e.g.* cats with *T. gondii*) and an asexual life cycle in other mammals and birds. The parasites form cysts within the secondary host's tissues and the life cycle is completed when the carnivorous primary host consumes the secondary host. Man is infected through consuming inadequately cooked meat from infected secondary host species such as agricultural animals, or from oocysts contaminating food or water.

Toxoplasmosis is common within many countries of the world and is usually a sub-clinical condition. In pregnant women, infection can lead to mental retardation and loss of vision in their congenitally infected children. Intestinal and hepatic toxoplasmosis[53-58], pneumonia[59], disseminated infection[55], cerebral and ocular infection[60] and death can occur in immunosuppressed or immunocompromised patients.

T. gondii is found in the tissues of food animals and is an important cause of abortion and mortality in sheep and goats throughout the world. A live vaccine, using a non-persistent strain of *T. gondii*, is available in New Zealand, the UK and Europe which prevents *T. gondii* abortion in sheep. A live vaccine using a mutant strain of *T. gondii* (T-263) is being developed in the US to reduce oocyst shedding by cats[61]. As yet, there are no drugs that are effective at killing *T. gondii* tissue cysts in human or animal tissues.

Outbreaks of infection have been associated with food[62,63], milk[64–66], water[67,68] and environmental contamination with cat faeces[69,70]. Food-borne infections can arise through the consumption of tissue cysts or trophozoites within meat, offal or unpasteurised milk, or from oocyst

contamination. Waterborne infections arise only from the consumption of oocysts[67,68,71,72]. Demonstrating outbreaks is difficult, but common source outbreaks seem to be frequent in the families of patients with acute lymphadenopathic toxoplasmosis[73]. There is some evidence that infections derived from oocysts are more severe than those from tissue cysts[74].

Freezing to −12°C, cooking to an internal temperature of 67°C, or gamma irradiation (0.5 kGy) can kill tissue cysts in meat. The effect of heat on the infectivity of *T. gondii* tissue cysts has been examined using a homogenate of infected mouse brains and pork[75].

A prospective case-control study designed to identify preventable risk factors for *T. gondii* infection in pregnancy was conducted in Norway[76]. A total of 63 of 37,000 women tested in a screening programme for pregnant women had serological evidence of recent primary *T. gondii* infection and 128 seronegative control women were matched by age, stage of pregnancy, expected date of delivery, and geographic area. The factors found to be independently associated with an increased risk of maternal infection included eating raw or under-cooked minced meat products, eating unwashed raw vegetables or fruits, eating raw or under-cooked pork or mutton, cleaning a cat litter box and inadequate washing of kitchen utensils after raw meat preparation.

Recommendations for primary prevention are chiefly designed for 'sero-negative' pregnant women without specific anti-*T. gondii*-IgG and for persons with continuous or temporary immune deficiencies[77,78]. Prevention in this group should focus on meat, and cats. Meat should only be eaten when well cooked or when it has been frozen prior to preparation. There should be no mouth–finger contact while handling raw meat. Raw food that is to be eaten without cooking, including fruit and vegetables, should be carefully washed before consumption. Food should not be prepared in the same place and with the same utensils as raw meat. Household cats should be fed with canned food rather than with raw meat. Contact with cats' faeces, must be strictly avoided (use plastic gloves), and cats' toilets should be disinfected daily with boiling water, and litter discarded daily.

Sarcocystis spp.

Sarcocystis is a tissue coccidian with an obligatory two-host life-cycle, and there are more than 100 *Sarcocystis* species that have life-cycles involving diverse avian, mammalian and reptilian hosts[79]. All have a distinctive life-cycle involving a definitive (usually a carnivore) and an intermediate host (usually a herbivore). The sexual generations of gametogony and sporogony occur in the lamina propria of the small intestine of definitive hosts which shed infective sporocysts in their

stools and present with intestinal sarcocystosis[80,81]. Asexual multiplication occurs in the skeletal and cardiac muscles of intermediate hosts which harbour *Sarcocystis* cysts (sarcocysts) in their muscles and present with muscular sarcocystosis. Sarcocysts are long sinuous cylindrical objects and they can be classified by their three dimensional appearance[82]. Humans can get intestinal sarcocystosis through the consumption of raw meat containing sarcocysts and muscular sarcocystosis through the consumption of water or food contaminated with sporocysts. The main species are *S. hominis* acquired from infected beef and *S. suihominis* from pork. Water-borne infection in man has not been reported, but may occur in the same way as water-borne toxoplasmosis (*i.e.* through sporocysts contaminating drinking water). Animal muscular infection is common throughout the world and follows ingestion of food or water contaminated with sporocysts. Monoclonal antibodies against *S. muris* have been used to differentiate different *Sarcocystis* species[81,83]. Experimental studies on human intestinal sarcocystosis showed that a calf could be infected with *S. hominis* sporocysts and developed sarcocysts in cardiac and skeletal muscles. When meat from the calf was fed to rhesus monkeys, they developed intestinal sarcocystosis with sporocyst production[84]. Clinical sarcocystosis is less commonly diagnosed than toxoplasmosis and is not normally associated with fetal infection or abortion in man and only occasionally in animals.

Human *Sarcocystis* infection is probably under-diagnosed, particularly in non-industrialised countries[85–87]. Enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody technique (IFAT) have been used to diagnose extra-intestinal infection[88,89] and muscle biopsy can be used for demonstrating the sarcocysts[90]. In farm labourers in Thailand, where consumption of raw meat is common, intestinal infection with sarcocystis is also common[91]. *Sarcocystis*-like organisms have been demonstrated in immunocompromised patients in Egypt[92]. In non-industrial countries, *Sarcocystis* spp. can be commonly found in the muscles of a range of livestock using haematoxylin-eosin (HE) stained muscle tissue samples[93]. Histological analysis of the tongues of routine autopsy subjects has been used to assess the extent of human infection in non-industrialised countries[86]. Human intestinal infection can be chronic and involve other bacterial pathogens[94,95].

In animals, clinical signs include fever, anaemia, loss of appetite and weight loss or reduced weight gain. Central nervous system signs include hind limb weakness, unsteadiness and partial paralysis, and acute myopathy and death may occur. Diagnosis can be difficult in countries where infection is common because clinical signs can be absent, mild or non-specific. Serology may be useful in some situations and histopathology/immunohistochemistry is valuable for confirming the cause of

death. Control of *Sarcocystis* infection in farm animals relies on preventing the contamination of pasture and water with dog and fox faeces and preventing the access of young stock to contaminated land.

Other coccidia

Many coccidia are host specific whereas others have a wide host range. Other coccidia, including *Neospora caninum*, which causes paralysis and abortion in dogs and abortion in cattle[96–98], have not been associated with human disease.

Mastigophora

Giardia lamblia

Giardia spp. are flagellated protozoans that parasitize the small intestines of mammals, birds, reptiles, and amphibians and giardiasis is a common cause of diarrhoea world-wide[99]. Clinical manifestations of *G. lamblia* infection range from asymptomatic to a transient or persistent acute stage, with steatorrhoea, intermittent diarrhoea, and weight loss, or to a subacute or chronic stage that can mimic gallbladder or peptic ulcer disease. Sources of infection in addition to humans include beavers and other wild[100] and domestic animals[101], and carriage in these can be long-term[102]. Experimental inoculation of beavers identified that a dose of 50–500 cysts was required to cause infection with a human strain[103] and similar infectivity studies have been done in gerbils[104]. Experimental human infections have been conducted[105] and a low infecting dose (10–25 cysts) is reported to be sufficient to produce human infection[106].

Giardia species and types have been differentiated using isoenzyme electrophoresis[107–112], phospholipid analysis[113], immunoblotting[114], DNA probes[112,115], RAPD (random amplified polymorphic deoxyribonucleic acid)[111], karyotyping[116,117], DNA fingerprinting with hypervariable minisatellite sequences[118,119] and PCR[120]. *Giardia* species will grow in culture[121] and this makes the application of a variety of typing techniques possible. The antigenic makeup of isolates can change during infection[122].

Outbreaks of infection related to drinking water[123–127], recreational water[128,129] and food[130–133] have been described. Food-borne infections commonly implicate food-handlers in the contamination of prepared foods, often following contact with the faeces of infected young children. The implicated foods have included canned salmon, sandwiches, noodle salad, fruit salad, salad items, raw vegetables and ice. The cysts of *G.*

lamblia are resistant to chlorine, although less resistant than *Cryptosporidium* oocysts. Water-borne infection can occur and, although outbreaks have mostly been associated with recreational water use, drinking water related outbreaks can occur[124–126]. The cysts can remain viable in cold water for months.

Dientamoeba fragilis

Dientamoeba fragilis is a protozoan that shares a common evolutionary history with the trichomonads[134]. *D. fragilis* is commonly found among patients with diarrhoea lasting longer than one week[135,136], particularly children[137], and may masquerade as chronic allergic colitis in children[138]. It is common in some non-industrialised countries[139,140] and industrialised ones[135,141]. The importance of stool fixation and staining in diagnosing *D. fragilis* has been emphasised as this pathogen does not produce cysts[142]. The absence of a cyst suggests that it is less likely to survive in the environment than many other protozoa, and its common presentation in children rather than adults suggests a person-to-person mode of transmission. There have been no reports of food or water related infections or outbreaks of *D. fragilis* and infections appear to be sporadic. If food-borne infection does occur, it is likely to be through an infected food-handler.

Other flagellates

Other flagellated organisms that are occasionally demonstrated in the faeces of people with diarrhoea include *Chilomastix mesnili*, *Trichomonas hominis*, *Retortomonas intestinalis* and *Enteromonas hominis*. These organisms are usually found in non-industrialised countries, but there is no good evidence that any of them cause gastrointestinal disease.

Sarcodina

Entamoeba histolytica

Entamoeba histolytica causes amoebic dysentery and abscesses, particularly in the liver. The motile trophozoites of *E. histolytica* phagocytose erythrocytes and these are diagnostic when seen in fresh faeces. Its cysts cannot be differentiated from those of the non-pathogenic *E. dispar*[143] using conventional microscopic identification

and, as a consequence, much of the scientific literature may relate to *E. dispar*. Modern molecular methods can readily differentiate these organisms, but this may not be done routinely[143–146]. Because endemic infection in the UK does not seem to occur, the infection risks are mostly associated with consuming contaminated food or water in countries where it is endemic.

Blastocystis hominis

The significance of *Blastocystis hominis* in diarrhoeal disease has been the subject of much debate. The organism occurs world-wide and appears in both immunocompetent and immunodeficient individuals. The symptoms generally attributed to *B. hominis* infection are non-specific, and the need for treatment is debated[147]. *B. hominis* was detected by faecal examination in 34 of 6,476 healthy people in Japan who visited a health screening centre for a routine medical check-up[148]. *B. hominis* has been associated with development of diarrhoea in travellers to tropical destinations, and concurrent infections with other organisms are common[149]. It occurs as commonly in asymptomatic control populations as in patients with diarrhoea[150]. Serum antibody was detected by fluorescent antibody test in patients with symptomatic *B. hominis* infection[151], and invasive disease has been reported[152]. However, in a group of symptomatic patients with *B. hominis* infection, endoscopy typically did not show evidence of significant intestinal inflammation or impaired intestinal permeability[153]. A study of *B. hominis* in AIDS patients found no association with clinical symptoms[154]. The isolation of *B. hominis* does not justify treatment even in symptomatic, severely immunocompromised patients. Most patients will either have spontaneous resolution of symptoms or successful identification of other infectious or non-infectious aetiologies. *B. hominis* is unlikely to be an important enteric pathogen, and transient symptomatic infection, if it occurs at all, resolves quickly.

Isoenzyme patterns show that *B. hominis* is highly polymorphic, but there is no correlation between isoenzyme patterns and disease[155]. Faecal samples from mammals, birds, reptiles, amphibians, fish, and snails were isolated by culture, put into axenic culture and serogrouped[156]. Most cultures belonged to the four serogroups. Human isolates were mainly serogroups I and II, pigs harboured serogroups III and IV. DNA polymorphisms in *Blastocystis* showed similarities between human and chicken isolates[157]. It was suggested that the genus *Blastocystis* may consist of more than one species.

Colonisation of people with *B. hominis* could well involve transmission via contaminated food or water[158] and it has been isolated from sewage[158,159].

Other amoebae

A number of other amoebae can be found in the faeces of patients with diarrhoea including *Entamoeba coli*, *Iodamoeba butschlii* and *Endolimax nana,* but there is no evidence that these organisms cause diarrhoea in humans. *Acanthamoeba* spp. and *Naegleria fowlerii* can cause water-borne disease but do not infect humans via food.

Microspora

Microsporidia

Microsporidia are a diverse, distinctive and ubiquitous group of protozoa with characteristics including a lack if mitochondria and a distinctive coiled polar tube in the spores[160]. An increasing number of species of microsporidia are being recognised in immunocompromised patients, particularly those with AIDS. Diarrhoea, malabsorption and weight loss are the most common clinical problems, but several other clinical syndromes can affect the eye, kidney, sinuses, lungs, brain, liver, bone and muscle[161]. Even in AIDS patients some infections may be asymptomatic[162]. Their relatively recent emergence as human pathogens and the difficulties in diagnosis mean that food-borne associations have not yet been demonstrated although spores have been demonstrated in water.

Two species, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, commonly cause diarrhoea in AIDS patients throughout the world. Other species cause organ specific or systemic infections and these include *Nosema connori*, *Encephalitozoon hellem*, *E. cuniculi*, *Vittaforma corneae*, *Microsporidium ceylonensis*, *Brachiola vesicularum*, *Pleistophora* spp., *Trachipleistophora anthropophthera* and *T. hominis*.

Enterocytozoon bieneusi

E. bieneusi causes chronic diarrhoea in immunocompromised people and has occasionally been found in people with diarrhoea in the absence of any apparent immune deficiency[163,164]. It is the most common microsporidial cause of intestinal disease. Multi-organ involvement can occur through local extension of the infection to the hepatobiliary tract[165]. Some patients have had respiratory involvement[166,167]. *E. intestinalis* and *E. bieneusi* have been found in stools of more than 40% of AIDS patients with diarrhoea. PCR has been used to examine faecal samples for *E. bieneusi*[168,169]. A study of microsporidiosis in AIDS

patients in Tanzania demonstrated *E. bieneusi* in 18% of faeces' samples using modified Trichrome stain and 51% using PCR[170]. Another study, using light microscopy and fluorochrome staining with Uvitex 2B, found 8/104 samples positive compared to 10 positive by PCR[171]. A synthetic, labelled oligonucleotide has been used for the detection and identification of *E. bieneusi* in clinical samples[172].

A variety of methods have been used to detect *E. bieneusi* in water, and PCR approaches seem to be suitable[173,174], and have been used to detect the organism in surface waters[175]. Detection in food remains problematic. This work indicates that *E. bieneusi* may be present in water sources, although there is no evidence to indicate whether the organisms detected are viable.

PCR has been used to detect[176–180] and type[181,182] *E. bieneusi*. *E. bieneusi* has been detected by PCR in 35% of 109 pigs, and the four pig genotypes identified were different from the three human ones from Swiss patients[183]. Isolates have also been detected in cats and dogs[184], and a rhesus monkey[185]. Isolates from humans and macaques with AIDS have been used to infect immunosuppressed gnotobiotic piglets that remained asymptomatic but colonised for up to 50 days[186]. Attempts to culture *E. bieneusi* in tissue culture have so far proved difficult[187,188].

Combination antiretroviral therapy including a protease inhibitor have been shown to restore immunity to *E. bieneusi* and *C. parvum* in HIV-1 infected individuals[189].

Encephalitozoon intestinalis

A second enteric microsporidian, *E. intestinalis* (originally named *Septata intestinalis*) is associated with disseminated as well as acute and chronic intestinal disease[190]. Clinical features of disseminated infection include chronic diarrhoea, fever, cholangitis, sinusitis, bronchitis, or mild bilateral conjunctivitis[191]. The spores of this organism have been differentiated from those of *E. bieneusi* by their smaller size and fluorescence using a specific polyclonal rabbit antiserum[192], but PCR is more definitive[171,178–180,193–195].

E. intestinalis spores have been demonstrated in the faeces of a donkey, dog, pig, cow, and goat using PCR and polyclonal antibody immunofluorescence[196]. These organisms can be grown in tissue culture[197,198]. *E. intestinalis* has been detected in tertiary sewage effluent, surface water, and ground-water using PCR[175].

E. intestinalis was found in two patients who had no obvious immunodeficiency[199]. The pathogenic role of *E. intestinalis* in immunocompetent individuals remains to be demonstrated.

Pleistophora and Trachipleistophora spp. and other microsporidia

These organisms cause disease in immunocompromised patients and are rare. *Pleistophora* spp. have been demonstrated in the muscles of a few patients with myositis, fever and progressive weakness[200,201]. A *Pleistophora* spp.-like microsporidian infection was identified in a patient with progressive severe myosotis associated with fever and weight loss[202]. The organism was demonstrated by light microscopy and electron microscopy in corneal scrapings, skeletal muscle, and nasal discharge and named *Trachipleistophora hominis*. A similar organism *Trachipleistophora anthropophthera* was found at autopsy in the brain of one patient and in the brain, kidneys, pancreas, thyroid, parathyroid, heart, liver, spleen, lymph nodes, and bone marrow of a second patient with AIDS[203]. Two ocular infectious disorders attributed to *Microsporidia* have been observed[204]. One infection involves the corneal stroma leading to corneal ulceration and suppurative keratitis and is caused by *Vittaforma corneae* (synonymous with *Nosema corneum*)[205]. The other infection involves the conjunctival and corneal epithelium and is caused by *Encephalitozoon hellem*[206]. *E. hellem* also causes urogenital and respiratory infections[207]. Identical genotypes of *E. hellem*, determined from the sequence of the rDNA internal transcribed spacer, have been identified from human and bird sources[208]. *Microsporidium ceylonensis* has also caused corneal microsporidiosis[209], as has *E. intestinalis*[191].A nosema-like microsporidian, *Brachiola vesicularum*, has been identified in biopsied muscle tissue, examined by light and electron microscopy in an AIDS patient with myositis[210]. The organisms develop in direct contact with the muscle cell cytoplasm and fibres.

It is not clear where all these different infections originate from and whether food or water are important in transmission. Most of the microsporidial infections have come to light through the intensive investigation of patients with syndromes associated with an immune deficiency. In most cases, the source of their infections is not known. Limited information on *E. bieneusi* and *E. intestinalis* suggests that agricultural animals may be a source of infection. As viable spores are passed by infected patients, person-to-person transmission and contamination of food and water with human waste remain possible transmission routes. The demonstration of *E. intestinalis* in tertiary sewage effluent, surface water, and ground-water, *E. bieneusi* in surface water and *Vittaforma corneae* in tertiary effluent[175] suggests sewage may be a source of contamination of the environment. Spores of the other microsporidian species have not been found in human faeces. A case-control study of HIV-infected individuals determined risk factors for microsporidiosis[211]. Cases were more likely than controls to have low CD4 cell counts, to be homosexual and to have swum in a pool in the

previous 12 months. This suggests faecal–oral transmission through water is possible, but no link was found with treated mains drinking water. The findings were corroborated by a study of HIV positive patients in California[212]. There was no seasonal variation in the prevalence of microsporidiosis. Although a water-borne route of infection with microsporidiosis is possible[213], there is no direct evidence of infection being acquired through the consumption of potable mains water or food.

Ciliophora

Balantidium coli

Balantidium coli causes an ulcerative dysentery in humans. Human infection is sporadic in non-industrialised countries, and very rare in industrialised ones, and seems to occur when there is close contact between people and pigs. Food-borne and water-borne infection have not been well documented, but remain possible. Cysts of *B. coli* from the faeces of infected patients are infectious to piglets and hydrocortisone-treated rhesus monkeys[214]. *B. coli* occurs naturally in wild and domesticated pigs[215], monkeys and apes[216,217], but was not found in wild rodents, dogs or cats[217].

Identifying and managing the risks of protozoan contamination of foods

Within the UK, the main food risks to human health are from *Toxoplasma*, *Cryptosporidium* and *Giardia*. Each protozoan has a different epidemiology and the risks of food-borne transmission are outlined in Table 2. The way foods are produced and distributed can have an important impact on the potential health risks from protozoa. There are specific potential problems associated with the globalization of food production and the import of foods from countries where diarrhoeal disease is more common in the community. This is exemplified by the *Cyclospora* outbreaks in Canada and the US. There are hygiene issues for preventing the contamination of soft fruits and salad items by people employed to pick these crops, by wildlife and from contaminated water used in sprays. There are also potential problems associated with the contamination of potable water with *Cryptosporidium*, and the control measures that are necessary for individual food production processes that use this water. The water industry is tightening its risk assessment and monitoring of drinking water treatment works to satisfy new legislation[218].

Table 2 Identifying and managing the risks of protozoan contamination of foods

Food type	Protozoan risk	Risk management
Raw meat	Intrinsic contamination with *Toxoplasma* or *Sarcocystis* tissue cysts	1 Control the access of cats, foxes and dogs onto pasture 2 Freeze meat 3 Cook meat 4 Determine whether any curing process being used will kill *Toxoplasma* or *Sarcocystis*
	Surface contamination with oocysts and cysts of *Cryptosporidium*, *Toxoplasma* or other protozoa	1 Good abattoir and post processing hygiene 2 Cook meat
Raw fruit and vegetables sold at retail	Surface contamination with oocysts and cysts of *Cryptosporidium*, *Cyclospora*, *Toxoplasma* or other protozoa in foods that are eaten without cooking	1 Prevent faecal contamination by using potable water for spraying, irrigation, *etc* 2 Wash with potable water containing chlorine 3 Prevent agricultural animals grazing in the vicinity 4 Provide toilet and washing facilities for fruit/vegetable pickers 5 Educate fruit/vegetable pickers 6 Use mechanical picking 7 Control flies and other insects 8 National/international controls
Processed foods	*Cryptosporidium*/other parasites in the water	1 Use a secure supply (deep borehole or surface water with good water treatment process) 2 Determine the risks of water contamination with *Cryptosporidium* from the water provider 3 Determine whether processing will kill the parasites 4 Decide what to do in the event of a boil water notice 5 Install additional water filtration or other treatment if necessary
Retail ready-to-eat foods	*Cryptosporidium*/other parasites causing contamination from food handlers	1 Hygiene training for food handling staff 2 Good washing and toilet facilities 3 Preventing staff with diarrhoea from working
	Cryptosporidium/other parasites causing contamination from pets and other animals	1 Restrict the access of cats, dogs and other pets from the cooking and serving areas
	Cryptosporidium/other parasites in the water	1 Decide what to do in the event of a boil water notice
Unpasteurised milk	*Toxoplasma* or *Cryptosporidium* in milk	1 Pasteurise milk to be used for babies, pregnant women and immunocompromised people 2 Freeze milk

Key points for clinical practice

- Protozoan infections linked to food are not commonly detected
- The main food risks in the UK are *Toxoplasma*, *Cryptosporidium* and *Giardia*
- Many of the protozoa are a particular problem in AIDS patients
- A majority of food-borne protozoan infections are probably acquired abroad
- Protozoa can be transmitted to food through contaminated water
- Imported soft fruit and salad vegetables are a potential risk

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