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Foodborne protozoan parasites

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Abstract

This report addresses *Cryptosporidium, Giardia, Cyclospora*, and more briefly, *Toxoplasma* as the main parasitic protozoa of concern to food production worldwide. Other parasitic protozoa may be spread in food or water but are not considered as great a risk to food manufacture. The protozoan parasites *Cryptosporidium, Giardia*, and *Cyclospora* have proven potential to cause waterborne and foodborne disease. *Toxoplasma gondii* has been considered a risk in specific cases, but humans are not its primary host. *Cryptosporidium* and *Giardia* are widespread in the environment, particularly the aquatic environment, and major outbreaks of cryptosporidiosis and giardiasis have occurred as a result of contaminated drinking water. Large outbreaks of waterborne cyclosporiasis have not been identified. *Cryptosporidium, Giardia,* and *Cyclospora* have potential significance in the preparation and consumption of fresh produce and in catering practice, in which ready-to-eat foods may be served that have not received heat treatment. None of the three organisms *Cryptosporidium, Giardia*, and *Cyclospora* has been shown to be a problem for heat processed food or tap water that has undergone appropriate treatment at a water treatment works. All three are sensitive to standard pasteurisation techniques. Although humans are not a primary host for *T. gondii*, the potential exists for both waterborne and foodborne toxoplasmosis. Parasitic protozoa do not multiply in foods, but they may survive in or on moist foods for months in cool, damp environments. Their ecology makes control of these parasites difficult. For general control of parasitic protozoa in the food chain, the following steps are necessary:

- Follow good hygienic practice in food service and catering industries.
- Minimise dissemination of cysts and oocysts in the farming environment and via human waste management.
- Include these microorganisms in Hazard Analysis Critical Control Point (HACCP) plans of water suppliers, industries or sectors that use fresh produce, and operations in which contaminated process or ingredient water could end up in the product (e.g., where water supplies may become contaminated).

Keywords: Foodborne protozoan parasites; Cryptosporidium; Giardia; Cyclospora

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1. Introduction

Over recent decades, parasitic protozoa have been recognised as having great potential to cause waterborne and foodborne disease. The organisms of greatest concern in food production worldwide are *Cryptosporidium, Cyclospora, Giardia,* and *Toxoplasma.* Although other parasitic protozoa can be spread by food or water, current epidemiological evidence suggests that these four present the largest risks. The scope of this report is thus different to that of other authors e.g. Orlandi et al. (2002) in selecting these particular organisms.

Toxoplasma differs from the other three organisms because humans are not this organism's primary host and its life cycle is completed only in felines. Although consumption of uncooked or undercooked meat is considered the major risk factor for toxoplasmosis, spread of the organism through environmental routes also occurs and may be an emerging issue in food safety. Given these differences, *Toxoplasma* is considered separately from the other three organisms in this report.

Giardia was the first of these organisms to be widely associated with human disease, and there have been many documented cases of waterborne giardiasis since the 1970s (Craun, 1986).

Cryptosporidium emerged as a threat to water supplies in the 1980s, particularly in the United States and the United Kingdom, and since that time other countries have had recognised outbreaks of waterborne cryptosporidiosis. *Cryptosporidium* caused probably the largest documented outbreak of gastrointestinal disease in a developed country, which occurred in Milwaukee, Wisconsin, USA, in 1993, during which there were an estimated 403,000 cases of illness as a result of a contaminated drinking water supply (Mackenzie et al., 1994). *Giardia* and *Cryptosporidium* both have the potential to cause foodborne disease through transfer from water; outbreaks of foodborne disease have occurred but tended to involve fewer reported cases than those attributed to the drinking water supply.

Cyclospora has only been documented as a significant human pathogen since the early 1990s. It has been recognized in developed countries as the causative agent of a few gastrointestinal outbreaks associated with fresh (unprocessed) food produce, i.e. soft fruits and leafy vegetables. Waterborne disease has also been reported, but not to the same extent as with *Cryptosporidium* and *Giardia*.

This report focuses mainly on *Cryptosporidium, Cyclospora* and *Giardia*, on their potential to cause foodborne (and waterborne) disease, and on the prevention and control of foodborne disease.

2. The organisms

2.1. Giardia

The *Giardia* species infecting humans (and other mammals) and causing giardiasis is *Giardia duodenalis*, sometimes referred to as *Giardia lamblia* or *Giardia intestinalis* (regarded by some as a 'race' of *G. duodenalis*). Other species for example, *Giardia muris* only infect other animals (rodents, birds and reptiles). In developed countries, giardiasis is particularly associated with foreign travel, and a common route of infection is through faecal/oral transmission.

Giardia has a two-stage life cycle: a reproductive trophozoite and an environmentally resistant cyst stage. An ingested cyst passes into the duodenum, where excystation occurs, releasing two trophozoites. These then multiply rapidly via asexual reproduction and colonise the small intestine. It is during the trophozoite stage that clinical symptoms occur, as a result of damage to the mucous membrane. The trophozoite is 9–21 μm long and 5–15 μm wide, has a teardrop shape, contains two nuclei and four flagella, and exhibits a tumbling motility (Smith, 1993). Some trophozoites encyst, completing the cycle. The cyst, which is ovoid and 9–12 μm long, is shed in the faeces. Trophozoites are also shed, but they are not infective (Meyer and Jarroll, 1980).

The infective dose for *Giardia* is between 10 and 100 cysts (Rentdorff, 1954). The incubation period in humans for *Giardia* is typically 1–2 weeks. *Giardia* is the most commonly isolated parasite worldwide. Symptoms typically include diarrhoea, bloating and flatulence. Stools are often fatty and weight loss can be significant. Untreated, the disease lasts for at least 5 days and potentially much longer. It can also reoccur, and asymptomatic carriers are common. Therapy is typically with metronidazole or tinidazole.

Asymptomatic infection is also believed to be common.

Giardia is known to be common in faeces of pets, livestock and wild animals, but it is not generally considered to cause significant animal disease. *Giardia* cysts, as with *Cryptosporidium* oocysts, are commonly found in sewage effluent and surface waters and in shallow springs. However, it is not clear how many of these cysts detected in animal and environmental samples represent strains that are infective to humans (Thompson, 2000). Cysts are infectious when shed in the faeces and can remain infectious for prolonged periods in cool, damp environments, e.g. more than 77 days at less than 10 °C (World Health Organization, 2004).

In some outbreaks of waterborne disease, isolates analysed by molecular typing techniques such as pulsed field gel electrophoresis have implicated the beaver population (Isaac-Renton et al., 1993)—hence the term "beaver fever" is well known in the literature. Genotyping studies have identified assemblages of *G. intestinalis* with different host ranges, and large-scale studies will help to better identify sources and routes of transmission (Thompson et al., 2000).

Giardia poses a risk to water supplies because of its resistance to chlorine. Although *Giardia* is more susceptible than *Cryptosporidium*, both have a greater resistance to chlorine than do bacteria and survive levels routinely used for water treatment (see Table 3). *Giardia* can more readily be removed by filtration than *Cryptosporidium* because of its larger size.

2.2. Cryptosporidium

Cryptosporidium causes diarrhoea in livestock and in humans, and the organism was first recognised in veterinary medicine. Although the first case of human cryptosporidiosis was reported in 1976, full recognition of cryptosporidiosis as a human disease came with its association with AIDS patients in the late 1970s and early 1980s and with improved laboratory testing and diagnosis.

There are a number of species in the genus *Cryptosporidium,* but the vast majority of human cases of illness are caused by *Cryptosporidium parvum,* which has both human and animal reservoirs. *C. parvum* isolates have been separated into 2 genotypes, based on sequencing of the thrombospondin-related adhesive protein (TRAP-C2). Type I isolates have been recovered exclusively from humans and type 2 from bovines and humans exposed to infected cattle (see Section 5). Although it is well established that the growing immunocompromised population is prone to infection by species other than *C. parvum,* it is increasingly recognised that immunocompetent people can also be infected by other species of *Cryptosporidium.* An example is *C. meleagridis*, a species previously associated with birds that is now known to infect otherwise healthy humans (Chalmers et al., 2002).

The infective dose is believed to be low; in one isolate of *C. parvum,* for example, a dose of fewer than ten oocysts is enough to cause infection (Okhuysen et al., 1999).

The life cycle of *Cryptosporidium* is completed in a single host and culminates in the shedding of mature oocysts in the faeces (Fayer et al., 1990). These are immediately infective for another susceptible host. The oocysts are 4–6 μm in diameter (smaller than many other protozoa), and contain four crescent-shaped infective structures—the sporozoites. After ingestion the oocyst excysts in the small intestine, releasing the sporozoites. The sporozoites attach themselves to the gut epithelium, initiating the infection, which develops through further stages of asexual and sexual multiplication, zygote formation, oocyst formation, and sporulation (Meinhardt et al., 1996). Each of the stages of the organism's life cycle is found within the cell but outside the cytoplasm, and after an incubation period of 2–10 days, the pathogen gives rise to symptoms in humans. No specific toxin is produced.

Diarrhoea is the most common symptom of *C. parvum* infection, followed by abdominal pain and vomiting. The disease has a longer symptomatic period than most bacterial gastrointestinal infections, being commonly 1–2 weeks, and hospitalisation may occur. In people whose immune systems are fully functional, a complete recovery is normally made. In immunocompromised individuals, the disease may be much more severe and persistent, with invasion of other organ systems including the lungs and the bile duct, and it is life threatening (Farthing, 2000). Although some drugs may suppress the parasite, there is no reliably effective drug treatment to clear the infection.

Two types of oocyst are produced in vivo: thin-walled oocysts that cause further (auto-) infection of the host and thick-walled oocysts that can survive in the environment after being shed in the faeces. The faeces of infected animals may contain large numbers of oocysts; for example, infected calves shed up to 10^{10} oocysts daily during acute clinical infection. It is thought that humans shed a similar number during acute infection (Expert Group on Cryptosporidium in Water Supplies, 1998). However, smaller numbers of oocysts are shed during asymptomatic infection in both humans and animals.

C. parvum oocysts are widespread in the environment and are often found in surface waters and shallow springs. Vehicles of environmental contamination include agricultural runoff as well as sewage effluent. Oocysts survive well in cool, damp conditions. Robertson et al. (1992) exposed two isolates of *C. parvum* in semipermeable containers to different environments (tap water; river water; cow faeces) and found viable oocysts in both isolates after 6 months.

Because of its chlorine resistance (see Table 3), *C. parvum* has been a particular threat to otherwise safe drinking water supplies. Public and private water suppliers generally rely on natural purification by geological processes or have employed coagulation and filtration mechanisms, often as the sole barrier to *C. parvum*. But when such measures fail, and the number of organisms in the environment is high enough, sufficient numbers can pass into drinking water supplies to cause sporadic illness or an outbreak of disease.

2.3. Cyclospora

Many species of *Cyclospora* have been identified in animals. *Cyclospora cayetanensis* is the only species found in humans, and it is apparently restricted to this host. The parasite was first recognised as a human pathogen in 1977 (Ashford, 1979).

The complete life cycle of *C. cayetanensis* is not known, although it is established that the parasite multiplies in the cells lining the small intestine of the host (Ortega et al., 1997a). The life cycle culminates in the production of spherical oocysts. The oocysts are 8–10 μm in diameter and thus are larger than those of *Cryptosporidium.* The oocysts are shed in the faeces and are probably as robust as those of *Cryptosporidium*. However, unlike *Cryptosporidium*, some time is needed outside the gut for sporulation to occur. Eventually a mature oocyst develops that contains two ovoid sporocysts, each containing two infective sporozoites. Studies of human and baboon-derived oocysts suggest that optimal sporulation occurs at 20–22 °C within 14 days (Smith et al., 1997).

The incubation time for *Cyclospora*, from ingestion of oocysts to onset of symptoms, is between 2 and 11 days, but typically about 1 week. In immunocompetent individuals the disease typically lasts for 2 weeks. Symptoms include non-bloody diarrhoea, loss of appetite, weight loss, stomach cramps, nausea, vomiting, fatigue and fever. Symptoms may follow a relapsing and remitting course. Cyclosporiasis is effectively treated with trimethoprim-sulphamethoxazole.

The biology of *Cyclospora* is less well understood than those of *Cryptosporidium* and *Giardia*, but to date the largest outbreaks of foodborne disease have been associated with this parasite. They occurred during the late 1990s in North America, and many were associated with the consumption of fresh raspberries imported from Guatemala (Herwaldt, 2000). Unlike *Cryptosporidium* and *Giardia*, direct person-to-person spread of *Cyclospora* is unlikely because of the period required for the sporulation of the oocysts outside the host; thus, a transmission vehicle must be involved.

A significant reservoir of *C. cayetanensis* has yet to be identified in animals. It is likely that the most significant transmission may occur where sewage or water contaminated by human sewage effluent can affect humans or contaminate crops. *Cyclosporiasis* has occurred as a waterborne as well as a foodborne disease (Sterling and Ortega, 1999). Limited disinfection studies have been conducted on *Cyclospora* (see Soave et al., 1998, Table 3).

3. Outbreaks of foodborne and waterborne diseases

Many outbreaks of waterborne disease have been documented, particularly in cases of *Cryptosporidium* and *Giardia* infection. Only a few are cited in Table 1 to illustrate the range of water types and geographical locations. Contaminated drinking water has the

Table 1
Some examples of outbreaks of waterborne and foodborne disease

Waterborne outbreaks	Location	Water Type	Cases	Reference
Cryptosporidium				
1987	Carrolton, Georgia (USA)	Surface water	13,000 (estimate)	Expert Group on Cryptosporidium in Water Supplies, 1990
1989	Oxford/Swindon (UK)	Surface water	5,000 cases of which 500 laboratory confirmed cases	Expert Group on Cryptosporidium in Water Supplies, 1990
1993	Milwaukee, Wisconsin (USA)	Surface water	403,000 estimated	Mackenzie et al., 1994
1996	Ogose (Japan)		9000 estimated	*Cryptosporidium Capsule* (newsletter) (1996a)
1997	North London (UK)	Borehole water	345 laboratory confirmed cases	Gray, 1998
Giardia				
1985	Bristol (UK)	Treated reservoir	108 laboratory confirmed cases	Browning and Ives (1987)
1992	Sweden	Drinking water at ski resort	More than 3000 cases estimated	Hunter (1997)
1985–1986	Massachusetts (USA)	Unfiltered water supply	703 reported cases	Hunter (1997)
Cyclospora				
1990	Chicago (USA)	Storage tank of drinking water	21	Huang et al. (1995)
1994	Nepal	Storage tank of drinking water	12	Rabold et al. (1994)
Foodborne outbreaks	**Location**	**Food or beverage**	**Cases**	**Reference**
Cryptosporidium				
1993	Maine (USA)	Unpasteurised apple juice	154	Millard et al. (1994)
1996	New York (USA)	Unpasteurised apple juice	31	*Cryptosporidium Capsule* (newsletter) (1996b)
1997	Washington (USA)	Unwashed salad onions	54	*Cryptosporidium Capsule* (newsletter) (1998)
Giardia				
1979	Minnesota (USA)	Prepared salmon	29	Rose and Slifko (1999)
1985	Connecticut (USA)	Noodle salad at picnic	13	Rose and Slifko (1999)
1986	New Jersey (USA)	Fruit salad at party	10	Rose and Slifko (1999)
1986	Minnesota (USA)	Sandwiches (nursing home)	88	Rose and Slifko (1999)
Cyclospora				
1996	United States (many states)	Raspberries	725 cases (55 clusters)	Chalmers et al. (2000), Herwaldt (2000)
1997	United States (various states)	Raspberries	762 cases (41 clusters)	Chalmers et al. (2000), Herwaldt (2000)
1997	United States	Mixed baby lettuce	Number of cases unknown	Chalmers et al. (2000), Herwaldt (2000)
1997	United States	Basil	Number of cases unknown	Chalmers et al. (2000), Herwaldt (2000)
1998	Canada	Raspberries	192 cases (13 clusters)	Chalmers et al. (2000), Herwaldt (2000)
2000	Germany	Salad	26 approximately	Brockmann et al. (2001)
2004	United States	Salad	95 (two outbreaks)	Anonymous (2004)

potential to cause large disease outbreaks, whereas outbreaks of foodborne illness caused by protozoan parasites have generally been smaller, with the exception of the outbreaks of cyclosporiasis associated with raspberries in North America. It may be that foodborne outbreaks are hard to detect when they involve retail purchases of produce over a large geographical area.

In the Western world, outbreaks of foodborne parasitic disease have been traced to fresh foods, typically those that are difficult to clean thoroughly and are consumed without further processing that can inactivate or remove protozoan parasites.

3.1. Giardia-related outbreaks

Waterborne giardiasis was first documented in Aspen, Colorado, USA, in 1965/1966, and the Center for Disease Control and Prevention (CDC) began waterborne disease surveillance in 1971. Between 1979 and 1988, *Giardia* was the most frequently implicated organism in waterborne disease. Between 1965 and 1984, some 90 outbreaks with a total of 23,776 cases were reported in the United States (Flanagan, 1992). Between 1992 and 1997, surveillance carried out by 43 states in the United States indicated that as many as 2.5 million cases of giardiasis occur annually in that country (Furness et al., 2000). Waterborne giardiasis is well known among travellers to countries in Eastern Europe and the former Soviet Union.

A number of outbreaks of foodborne giardiasis related to food preparation have been documented, probably caused by infected food handlers or contact by food handlers with infected people, particularly children. For example, an outbreak of giardiasis occurred after a family party for 25 people. Nine people who ate fruit salad at the party became ill. The person who prepared the fruit salad had a diapered child and a pet rabbit at home who were both positive for *G. lamblia* (Porter et al., 1990). In 1990 an outbreak among insurance company employees resulted in 18 laboratory-confirmed and nine suspected cases of giardiasis. Raw sliced vegetables served in the employee cafeteria and prepared by a food handler infected with *G. lamblia* were the probable cause of the outbreak (Mintz et al., 1993). No outbreaks of foodborne giardiasis related to industrially manufactured foods have been reported.

3.2. Cryptosporidium-related outbreaks

Waterborne cryptosporidiosis was first identified in the United States and the United Kingdom in the mid-1980s, and many outbreaks have been recognised since then, particularly in these two countries. The Milwaukee waterborne outbreak, caused by spring runoff into the intake of two waterworks combined with their inefficient operation, was the largest on record (Mackenzie et al., 1994). Both treatment works extracted water from Lake Michigan and treated it by the addition of chlorine and polyaluminium chlorine coagulant, rapid mixing, mechanical flocculation, sedimentation, and rapid sand filtration. The filters were cleaned by backwashing the water, which was reused. Although no specific failure was identified, it was noted that unusually high turbidity readings were recorded in the treated water at the start of the outbreak. The estimation of cases was based on a random telephone survey and was adjusted for background gastrointestinal disease numbers (see Table 2). In other incidents, cases have been identified in different ways, including routine or enhanced surveillance or special studies. Molecular typing of *Cryptosporidium* has implicated both human and animal strains.

Numerous outbreaks have been linked to contaminated water from various sources. For example, an outbreak in the North Thames area of the United Kingdom in 1997 was linked to a contaminated filtered borehole-derived water supply, which infected 345 people (Gray, 1998). In the United States, an outbreak of cryptosporidiosis in 1994 that was linked to contaminated recreational lake water infected an estimated 2070 people (Kramer et al., 1998). Several outbreaks have been linked to contaminated swimming pool water. One of these occurred in Sydney, Australia, in 1994, when 70 people contracted cryptosporidiosis (Lemmon et al., 1996). A sequence of incidents with drinking water that occurred at Sydney Water (Sydney's waterworks) in 1998 potentially exposed some 3.7 million to *Cryptosporidium* and *Giardia*, although illnesses were not recorded, and therefore a true outbreak did not occur (McClellan, 1998 and Table 2). Several

Table 2
Summaries of potable water supply incident in Milwaukee and Sydney

Supplying company	Milwaukee Water Works, USA	Sydney Water, Australia
Date	March–April 1993	1st incident: 30 July–4 August 1998 2nd incident: 25 August–4 September 1998 3rd incident: 5–19 September 1998
Nature of incident	Contamination of potable water supply with *Cryptosporidium*	Contamination of potable water supply with *Cryptosporidium* and *Giardia*
Persons exposed	800,000	3.7 million
Persons sick	403,000 estimated cases of watery diarrhoea 600 confirmed cases of cryptosporidiosis 104 deaths (all HIV positive)	None reported. It is not known why no illnesses were reported despite repeated exposure of the population. Possible explanations are that the organism was a noninfective strain of *Cryptosporidium* or that the organisms were dead or dying. Methodological issues were also involved.
Cause	Contamination of source water from Lake Michigan Inadequate removal of oocysts by coagulation/filtration process	Heavy rains washed animal faeces containing parasites into source water. Inadequate removal of oocysts by coagulation/filtration process
Actions taken by water company	Issued public warning to boil water for 1 min Large-scale investigation into cause and treatment of outbreak	Issued public warning to boil water for 1 min Tried to flush the parasites from the water system using clean water
Actions taken by food and beverage industry	More than 100 food and beverage products were recalled or withheld, including cottage cheese, pickled herring, fresh-cut produce, and various types of chilled salad products, seafood, coleslaw, strawberry fruit gelatine, and flavoured fruit drinks	Soft drink companies warned outlets not to use dispensers until the water supply was cleared by the authorities. Food manufacturing companies urgently assessed the implication of these parasites for their products and processes.
Cost	Huge costs to food and beverage industry for products recalled or withheld	Water company to pay $10–$100 million in compensation to residents and businesses Two senior managers dismissed
Official bodies involved	Milwaukee and Wisconsin Departments of Health, Wisconsin Department of Natural Resources	New South Wales Health Department, Australian Consumers Association, Australian legal system
Lessons for food and beverage industry	–Food companies cannot assume that municipal water supplies are free from contamination; they must treat water companies as they would any other supplier. –Food companies should anticipate the possibility of such contamination happening anywhere and must prepare contingency plans. –Waterborne *Cryptosporidium* and *Giardia* should be included in HACCP plans and the control measures identified; for example, pasteurisation significantly reduces numbers of *Cryptosporidium* and *Giardia*. –Because of the vast quantities of potable water used by the food and beverage industry, the potential for large-scale contamination of products is high.	

Source: Unpublished information provided by Unilever.

outbreaks have occurred in close social groups such as households, nurseries, and hospital or nursing home settings. *Cryptosporidium* transmission occurs frequently in nurseries, where infants are clustered within classrooms, and share toilets and play areas. Employees can also become infected through careless diaper changing. In an outbreak at a day-care centre in the United States, 49% of the centre's 79 children and 13% of its 23 staff became infected with *Cryptosporidium* through person-to-person spread (Tangermann et al., 1991). An outbreak was reported in a bone marrow transplant unit when five

patients developed cryptosporidiosis after an infected patient was admitted to the unit (Casemore et al., 1994).

The association between human *Cryptosporidium* infection and cattle is well established. Outbreaks have been associated with farm animal contact, e.g. in a family after a visit to a dairy farm (Ribeiro and Palmer, 1986), among small groups of veterinary students (Reif et al., 1989), and in schoolchildren who had visited a farm (Shield et al., 1990). There is also evidence that many sporadic cases of cryptosporidiosis are due to animal contact. A large study carried out in England and Wales showed that 31% of 480 children aged 1–4 years who had cryptosporidiosis and 37% of 210 children aged 5–14 years reported exposure to farm animals or raw milk (Public Health Laboratory Service Study Group, 1990). In a study of sporadic cryptosporidiosis in the Unites States, eating of raw vegetables was found to be protective against the disease (Roy et al., 2004).

Few outbreaks of foodborne cryptosporidiosis have been recorded, and those that have were probably due to environmental contamination. For example, an outbreak of cryptosporidiosis in 1993 in the United States was associated with drinking unpasteurised fresh-pressed apple juice. The apples used for the juice were most likely contaminated by cattle faeces when they fell to the ground in a cow pasture (Millard et al., 1994). In 1996, an outbreak occurred in Maine (USA) in which children developed cryptosporidiosis after drinking apple juice prepared from apples that were probably contaminated when they were washed with well water (CDC, 1997). These outbreaks imply that the low pH of these products (usually between 3.4–4.2) is not sufficient to effect complete inactivation of contaminating oocyts. An outbreak in the United States in 1995 was associated with eating chicken salad that may have been contaminated by a food worker who operated a day-care facility in her home (CDC, 1996).

3.3. *Cyclospora*-related outbreaks

A number of small outbreaks of waterborne cyclosporiasis have been identified, but large outbreaks similar to those caused by *Cryptosporidium* and *Giardia* have not occurred. This may be because the organism has been less associated with human diarrhoeal illness and the potential reservoir of infection is smaller. In 1990, the first documented outbreak in the United States occurred in Chicago, where 23 cases were associated with a hospital water supply (Huang et al., 1995). In Nepal in 1994, an outbreak occurred among British soldiers and their dependants stationed at a small military detachment. Twelve (92%) people were infected. *Cyclospora* organisms were detected in the drinking water used by the camp, which consisted of a mixture of river and municipal water that was treated by chlorination. The water had also been filtered, but particles of the size of *Cyclospora* were not sufficiently removed by the process (Rabold et al., 1994).

Cyclospora has been associated with a number of high-profile outbreaks of foodborne illness from contaminated raspberries in the United States and Canada. The contamination of the raspberries may have been via water that was used in application of pesticides, or through transfer from the hands of pickers (Sterling and Ortega, 1999; Sathyanarayanan and Ortega, 2004). More recently, two outbreaks have occurred in the Unites States linked to salads served at separate restaurants (Anonymous, 2004).

4. Disease surveillance in relation to the food chain and water supplies

When outbreaks of infectious intestinal disease are described, cases reported may be calculated in a number of different ways. Cases may be identified by laboratory tests and confirmed by the detection of cysts or oocysts in stool samples from patients. The number of cases can also include people who have symptoms of the disease even if they have not submitted stool samples. If the numbers quoted rely only on individuals who visited the doctor, they will probably represent a significant underestimation of the true number of cases. Handysides (1999) estimated that in England and Wales, for every 136 cases of infectious intestinal disease in the community, only 23 people saw a community doctor, six gave a stool sample, and only one was identified by national laboratory surveillance. The number of cases identified in communities is related to both the true incidence of disease in the population and the proportion and types of cases detected by health surveillance.

In the health profession, understanding and knowledge of these three organisms is variable. Of the three, *Giardia* has the longest association with human illness and hence a greater familiarity among health professionals, and as a result it is more likely than *Cryptosporidium* and *Cyclospora* to be identified as a cause of gastrointestinal illness. However, it can be difficult to obtain a diagnosis from a single specimen. The advent of AIDS and of cryptosporidiosis in AIDS patients as well as improved diagnosis through staining of faecal smears led to more familiarity with *Cryptosporidium.* This familiarity grew as numerous outbreaks of waterborne disease were recognised from the mid-1980s onwards and the number of publications on the organism increased dramatically.

Cyclosporiasis is particularly difficult to diagnose in many countries because of poor awareness both in primary care practice and in the laboratory. Cann et al. (2000) in a study of cyclosporiasis in England and Wales between 1993 and 1998 concluded that ascertainment may be poor due to use of inappropriate laboratory methods. In North America, improved laboratory ascertainment and increased disease surveillance in recent years have resulted in the identification of outbreaks of both waterborne and foodborne cyclosporiasis. For example, in 1995 a community outbreak of cyclosporiasis was detected in Florida because of the routine screening of all faecal samples at a hospital (Chalmers et al., 2000).

Both waterborne and foodborne outbreaks of disease due to protozoans may be hard to detect for a number of other reasons, including the geographical spread of exposed populations (as in the case of food from retail outlets) and low attack rates (mostly in the case of waterborne disease). Greater awareness of specific protozoan parasites in certain countries and increased familiarity with them or with the symptoms of the illnesses they cause, along with improved surveillance programmes for these organisms, increase the likelihood that future outbreaks will be identified.

Cryptosporidiosis and giardiasis are perceived more as waterborne than as foodborne diseases, whereas *Cyclospora* has been associated with higher-profile outbreaks related to fresh produce. It is possible that *Cryptosporidium* and *Giardia* also cause illness through contamination of fresh produce, but existing health surveillance programmes, together with difficulties in detection, may have missed identification of such an association. Moreover, collection of samples of the foods that were eaten before the illness became apparent may be hampered by the relatively long incubation periods of these parasites.

5. Environmental occurrence and transfer

We know that *Cryptosporidium* and *Giardia* are widespread in the environment, particularly in aquatic environments, as surveys of waters in both temperate and tropical countries have shown. Both organisms can be found in effluent from sewage treatment plants, which passes into rivers and water reservoirs, but dissemination into the environment of organisms from agricultural sources (manure and slurry) and wild animals is also significant. A study of irrigation water used for production of crops traditionally eaten raw has shown widespread presence of protozoan parasites in the United States and Central America (Thurston-Enriquez et al., 2002) (Fig. 1).

Cryptosporidium oocysts in the environment are derived from human sewage as well as from animals such as cattle and sheep; the disease is one of veterinary significance and can cause, for example, diarrhoea in young calves. *Giardia* cysts may be of similar origin, although there is some evidence that wild animals may have a greater role in spreading the organism. Much less information is available on *Cyclospora* in the environment and in animal populations. *Cryptosporidium, Cyclospora* and *Giardia* have been detected on fresh vegetables (Amahmid et al., 1999; Beuchat, 1996; Monge and Chinchilla, 1996; Ortega et al., 1997b). This is not unexpected, given the presence of these organisms in the environment and in faeces. However, because of the lack of development in laboratory methods in this area and problems of detection sensitivity, available data are at best questionable, although methodological development is under way (see below). The relationship between numbers of organisms found on fresh produce and numbers in the environment in which crops for retail sale were grown is unclear.

Studies of the isolates of *Cryptosporidium* and *Giardia* that cause human disease have led to recognition of different genotypes, which in turn has improved our understanding of the epidemiology of

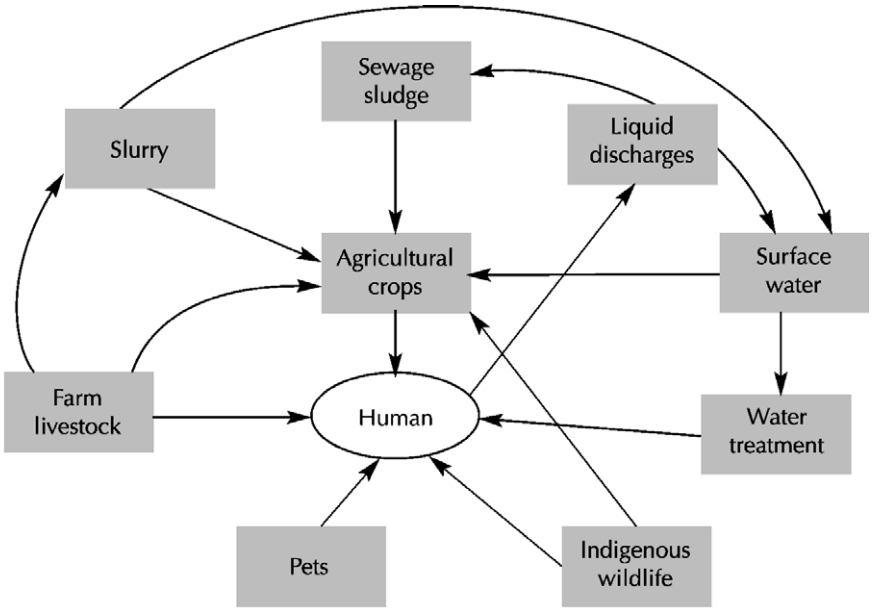

Fig. 1. Some potential routes of transfer in the environment. Transfer from animal sources is not thought to occur in the case of *Cyclospora*.

cryptosporidiosis and giardiasis. In the case of *Cryptosporidium,* a common distinction is now made between a type 1 human isolate and a type 2 animal/human isolate. It has recently been proposed that type 1 oocysts represent a separate species named *C. hominis* (Morgan-Ryan et al., 2002). The relative importance of the two types in causing human disease is not known, although typing of isolates from the more recent outbreaks of waterborne disease indicate that at least some were caused exclusively by the type 1 human isolate (Expert Group on Cryptosporidium in Water Supplies, 1998). Other outbreaks have been attributed to contamination from farm and wild livestock.

In the case of *Giardia,* many waterborne outbreaks have been attributed to contamination of water sources by wild animals, including beaver, muskrat, gerbil and rat. Molecular epidemiology suggests now that cattle are not as significant a reservoir for human infections as was once believed (Olson et al., 2004). In the case of food transmission, the primary route recognised for *Giardia* is contamination from infected food handlers. Outbreaks of foodborne and waterborne cyclosporiasis are due mainly to human faecal contamination in the environment. Since there is little or no monitoring for *Cyclospora* in the environment, it is not known whether the organism is widespread.

6. Methodology for analysis or identification

Analytical methods for identifying the various protozoan parasites have been developed as their public health significance has been realised. Initially, much of the work in this area was done in North America and then in the United Kingdom. In recent years, the Joint Research Centre of the European Union has become more involved with the development of laboratory methods. Detection in stool samples during acute infection is relatively easy because of the high concentration of parasites, although mistakes in identification can be made. Also, intermittent shedding can occur, in which case the organism may escape detection.

Direct examination of stained or unstained stool preparations is generally used for diagnosis in the clinical laboratory. Isolation from food and water, on the other hand, requires more complex methods involving concentration and purification. A complicating matter is that parasites are not evenly distributed, and so large samples are required. Because of

the generally small number of organisms which may be present relative to the amount or volume of interfering material, and the fact that the organisms do not grow on laboratory culture media, the methods are inherently more difficult than many bacteriological methods.

Early techniques for detection of *Cryptosporidium* and *Cyclospora* were based on those developed for *Giardia.* Methods for detecting *Cryptosporidium* and *Giardia* in water have been better developed than methods for detecting these organisms in food. Methods for detection in food, notably in fresh produce, are increasingly being developed and published (for instance, by Robertson and Gjerde, 2000, 2001).

The relevant laboratory methods can be divided into several stages: elution, concentration, purification, and detection of organisms. Mechanical elution from a filter into an appropriate wash buffer containing detergent is followed by concentration, usually by centrifugation. Purification of target organisms from non-target organisms and other debris is increasingly done with paramagnetic beads coated with antibody to the target organisms (Standing Committee of Analysts, 1999); these are commercially available for both *Cryptosporidium* and *Giardia.* The paramagnetic bead/organism complex is then removed from the sample matrix with a magnet, and the beads are disassociated from the organisms, providing a purified concentrate. A number of different detection mechanisms may be applied to purified concentrates. Epifluorescence microscopy is currently the method of choice for food, water, and environmental samples; monoclonal antibodies conjugated to fluorescein isothiocyanate (FITC) are available for *Cryptosporidium* and *Giardia.* There are no commercial paramagnetic beads or labelled antibodies for the detection of *Cyclospora,* which can be identified by its fluorescent appearance under ultraviolet light and, less reliably, by acid-fast staining. Quintero-Betancourt et al. (2002) have reviewed methods for detection of *Cryptosporidium* and *Cyclospora.*

The size, shape, and morphology of cysts or oocysts are important in distinguishing these organisms from other organisms that may be co-purified with the target. Specialised microscopic techniques such as differential interference contrast (DIC), which elucidates internal features, are useful in identification. Sporozoite nuclei within *Cryptosporidium* oocysts may be stained with a DNA binding dye such as 4′6-diamidino-2-phenylindole (DAPI) and viewed with fluorescence microscopy.

Genetic amplification detection techniques, particularly polymerase chain reaction (PCR), have been applied to all three organisms for both detection and characterisation, but they are not in widespread use for routine testing. Orlandi and Lampel (2000) have published a promising PCR method that reportedly is able to detect 10–30 *C. cayetanensis* oocysts per 100 g raspberries. Tanriverdi et al. (2002) and Fontaine and Guillot (2002) have published real-time PCR methods for detecting *Cryptosporidium.* Methods for *Giardia* detection in environmental waters using antibody capture and PCR have been developed by Mahbubani et al. (1998). A real-time PCR method for *Giardia* has been developed and compared with immunofluorescent techniques (Bertrand et al., 2004). With molecular techniques, cysts or oocysts containing genetic material are detected, whereas immunofluorescence will detect empty cyst or oocyst shells. Notably, neither reliably distinguishes live from dead organisms.

Molecular characterisation of isolates in environmental matrices can greatly improve our understanding of contamination routes. In the case of *Cryptosporidium,* particular genetic markers, such as polymorphisms in the organism's outer wall protein gene, have been identified that discriminate those organisms found only in humans (type 1) from those that infect both humans and animals (type 2) (Chalmers and Elwin, 2000). Using similar techniques, discrimination of genotypes is possible within *G. duodenalis* (Read et al., 2004).

7. Controls in the food chain

Protozoan parasites enter the food production process via three main routes:

- through contamination of food ingredients or raw materials on the farm;
- through contaminated water included in the final product for product processing or washing, or used for cleaning processing equipment;

- through transfer or spread via infected food handlers or food preparers in production, food service or domestic settings.

Preventive or control methods should therefore be devised to cover these three potential routes whenever they could be of significance to the final product consumed. Food producers must undertake a hazard identification within the frame of a Hazard Analysis Critical Control Point (HACCP) plan for the food operation to determine whether protozoan parasites are a significant hazard, which they would be if viable organisms potentially occur in high enough numbers (i.e. at infectious dose levels) in the final product. To generally ensure that protozoan parasites are not a significant hazard, prerequisite systems to HACCP (e.g. GMP, GHP) should include appropriate preventive or control measures. Where relevant and possible, preventive and control measures could be included in Good Agricultural Practice (GAP) programs used in the primary production of ingredients or raw materials. More information on GAP guidelines can be found on the UK Food Standards Agency website: http://www.food.gov.uk.

7.1. Primary production

Parasites can contaminate crops through various routes, for example, via water contaminated by faeces that is used for irrigation or spraying of crops, by poor personal hygiene practices among pickers or handlers of crops, by contact with contaminated soil or by contact with faeces of wild animals. The relative importance of these routes is unknown, although contamination by wild animals is not likely for *Cyclospora.* Even with the well-studied outbreaks of cyclosporiasis that have been traced to Guatemalan raspberries, the exact route of the contamination remains a matter of speculation; spraying and irrigation with contaminated water are unlikely to have been the source of contamination in this case, although it is possible that insecticides and fungicides made with contaminated water have been used to spray crops (Sterling and Ortega, 1999).

A directive of the European Commission (1986) limits the use of sludge particularly in relation to "ground intended for cultivation of fruit and vegetable crops which are normally in contact with the soil and normally eaten raw, for a period of 10 months preceding the harvest of the crops and during the harvest itself." More detailed codes of practice on the use of sludge and the treatment of sludge have been developed at the national level, such as the Safe Sludge Matrix in the United Kingdom (ADAS, 1999). European retailers have drawn up the EUREPGAP Protocol (EUREPGAP, 2001) which covers in outline aspects such as the use of treated sewage sludge on land intended for agricultural production, the nature of irrigation water, and hygienic aspects associated with harvesting.

Our understanding of the survival of these organisms in the environment is developing, but the risk of contamination posed by growing crops under different conditions is not understood. No clear legislation or even widely recognised guidelines have been formulated on the quality of irrigation water; this is unfortunate, because irrigation water can spread significant numbers of pathogens, although the extent of its role in such contamination is unknown.

7.2. Processing and production

If control in primary production is not adequate or if the water used in production or processing has been contaminated, a different set of issues arises concerning control in the factory. If a food business is told that its water supply may be contaminated, this will have certain legal and commercial implications and the risk to public health must be addressed. Examples of issues with a contaminated water supply are given in Table 2. In the Milwaukee outbreak, because a boil water notice (see Glossary) was issued, more than 100 food and beverage products were recalled or withheld, including cottage cheese, pickled herring, fresh-cut produce, and various types of chilled salad products, seafood, coleslaw, and flavoured fruit drinks. This represented a huge cost to the food industry. A similar situation arose in Australia in 1998 due to a perceived risk of cryptosporidiosis and the issue of boil water notices following detection of *Cryptosporidium* in the water supply. By contrast, in this incident illness was never detected in the population but there were significant repercussions for the food and beverage and catering industries.

In order to minimise the impact on human health of contaminated raw materials entering a factory or of

contamination of food products in a factory by the water supply, it is necessary to establish preventive or control measures in the production and processing operation (see Tables 3 and 4 for relevant information). Such measures are best established before any actual incident occurs. This approach was applied by Campden and Chorleywood Food Research Association, UK (CCFRA) for water supplies that had potentially been contaminated with *Cryptosporidium* (Dawson, 2000). The principles can be applied to products contaminated in other ways.

For primary products that have been contaminated, there is evidence that washing–for example, in potable or chlorinated water, which is applied as a standard for decontamination of fresh produce–can reduce levels of microbial contamination, although this is dependent on a number of factors, including the surface properties of the product Contamination of fruits and vegetables by parasites is discussed by the World Health Organization (Beuchat, 1998) and the Codex Alimentarius Commission (2002).

Another possible route of contamination in the factory is through reused water that has not been subjected to adequate treatment and could contain protozoan cysts or oocysts. The Codex Alimentarius Commission is currently considering guidelines to be incorporated into the *Recommended International Code of Practice-General Principles of Food Hygiene* as an annex to that document (Codex Alimentarius Commission, 2001). The draft guidelines include the statement that the water used for food products should meet the microbiological specifications required for potable water and highlights potential contamination by protozoan parasites.

7.3. Spread via infected food handlers

Given the requirement for a latent period in the environment for sporulation to occur, person-to-person transfer of *Cyclospora* is unlikely, whereas it readily occurs with *Cryptosporidium* and *Giardia.* Handling of food by infected personnel or by personnel who have been in contact with infected people can result in contamination of food. Transfer by this route is likely to occur through faecal contamination, which can be prevented through strict adherence to personal hygiene and other good hygienic practices. In some cases, it may be possible to block release of product if there is a risk of contamination (e.g. when a food handler reports gastro-intestinal illness).

7.4. Waste management

To minimise the dissemination of oocysts of *Cryptosporidium* in the environment, appropriate guidelines should be followed. In the United Kingdom guidelines have been produced that cover areas such as management of livestock wastes as well as their storage and treatment (MAFF and Welsh Office, 1991). For example, oocyst viability can be reduced rapidly by aeration of stored slurry because of elevated temperatures and free ammonia levels. Control of the spread of human waste is a means of reducing the risk of contamination by *Cyclospora* and *Giardia.*

8. Information on *Toxoplasma gondii*

The protozoan parasite *T. gondii* may cause the zoonotic infection toxoplasmosis. Members of the cat family (Felidae) are the only known primary hosts of *T. gondii.* Cats can become infected by eating the tissues of infected prey, and this results in the completion of the life cycle via a sexual phase. After a cat ingests *T. gondii,* the tissue cyst wall is lysed by the cat's digestive enzymes and bradyzoites are released. These then penetrate the epithelial cells of the small intestine, where they undergo initial asexual multiplication. This is followed by the sexual cycle, in which male and female gametes migrate into the gut and combine to form a zygote, which then develops into an immature oocyst. Final sporulation of the oocyst usually takes place 1 to 5 days after shedding in the cat faeces. Final sporulation is dependent on environmental conditions and does not usually occur in temperatures below 4 °C or above 37 °C. The mature oocyst measures approximately 12 μm by 11 μm and is potentially infective to any warm-blooded animal that might ingest it. Cats often shed oocysts over a period of 2–3 weeks and peak levels of shedding may reach over a million per day.

Secondary hosts for *T. gondii* include many vertebrates, from rodents to domestic farm animals to humans. After *T. gondii* oocysts have been ingested by a secondary host and have released sporozoites, asexual reproduction and invasion of body tissues occur through the circulatory and lymphatic systems,

Table 3
Survival of parasites (cysts/oocysts) in food processes

Parasite	Factors affecting survival						
	Environment	Heat	pH	Freezing	Disinfectant	Drying	UV light
Cryptosporidium	Life cycle complete within a single host. Oocysts (containing infective sporozoites) are resistant to cool damp conditions and can remain viable long periods. One year has been reported in sea water (Tamburrini and Pozio, 1999). Some viability of oocysts in river water and cow faeces after 6 months (Robertson et al., 1992).	No infectivity of oocysts treated at 71.7 °C for 5, 10 and 15 s in either water or milk and calculated a reduction of at least 3 log orders (Harp et al., 1996). Heating to 64.2 °C for 2 min reduces infectivity to 0% in water or buffer (Fayer, 1994).	Low pH in carbonated drinks; oocysts lose >85% viability at either 4 °C or 22 °C in orange juice at pH 3.9, or in carbonated beer or cola both at pH 3.9 (Friedman et al., 1997). Malic, citric and tartaric acids have been found to reduce infectivity by up to 88% (Kniel et al., 2003). Ethanol and low water activity also reduce infectivity (Dawson et al., 2004).	Freezing at −70 °C is enough to render oocysts non-infective. Over a 7-day trial, infectivity declined but viable oocysts remained from batches at −15 and −20 °C (Fayer and Nerad, 1996).	Very resistant to disinfectants; routine chlorination of water has no effect. Ozone highly effective (Casemore, 1995).	Desiccation affects oocysts' viability dramatically: 95% died within 4 h at room temperature (Deng and Cliver, 1999). In a another study, 10^6 oocysts died within 4 h when dried in air at 18–22 °C (Robertson et al., 1992).	UV light effective at current levels used in water treatment (Hargy et al., 2000).
Giardia	Infective cysts (containing trophozoites) survive >2 weeks in a cool moist environment. Vegetative trophozoite (responsible for symptoms) does not survive outside the host (Casemore, 1995; Smith, 1993; Meyer and Jarroll, 1980).	Heating regime of 71.7 °C for 15 s will destroy sufficient numbers of cysts (WHO, 2004).	No information.	Cysts killed at minus 18 °C after 1 h (Mahbubani et al., 1991).	Resistant to chlorine-routine chlorination of water usually has no effect (depends on time and temperature). Susceptible to phenol-based commercially available disinfectants (Smith, 1993; Lee, 1992).	No reliable data available	Work with *Giardia muris* infectivity suggest that UV doses of 2–3 mJ cm^{-2} can inactivate 4 log10 of oocysts (Hayes et al., 2003).
Cyclospora	Life cycle not fully characterised. Oocysts survive in water at 4 °C for 2 months and at 37 °C for 7 days (Smith et al., 1997; Ortega et al., 1998).	After heating at 60 °C for 1 h, oocysts cannot be induced to sporulate (Sterling and Ortega, 1999).	No information	Minus 18 °C for 24 h oocysts cannot be induced to sporulate (Sterling and Ortega, 1999).	Resistant to many disinfectants including chlorine at levels used in water treatment (Soave et al., 1998).	Very sensitive to desiccation (after 15 min, oocyst wall ruptures) (Long et al., 1991).	No information available.

leading to the formation of tissue cysts. Tissue cysts containing bradyzoites can typically vary in size from 12 μm in diameter to 100 μm. Ingestion of infected tissues by another mammal may result in the infection being transmitted by a mechanism similar to that of ingestion of oocysts.

Humans may become infected by a number of routes, for example by ingestion of oocysts from soil or water contaminated with cat faeces, ingestion of viable tissue cysts in raw or undercooked meat and even by transplacental transmission from a mother who is acutely infected.

In immunocompetent individuals, infection with *T. gondii* is usually asymptomatic. When symptoms occur, they are often mild and self-limiting. They may include flu-like symptoms, swollen lymph glands, fatigue, and joint and muscle pains.

In immunosuppressed individuals, infection is life threatening. When a pregnant woman acquires infection shortly before or after conception, there is a significant risk of transmission of the parasite to the foetus. Transplacental infection of the foetus is estimated to occur in about 45% of such cases (Desmonts and Couvreur, 1974). When infection occurs early in pregnancy, it can cause gross foetal abnormalities or spontaneous abortion. When infection occurs later in pregnancy, symptoms may be less severe, and the child may appear asymptomatic at birth, although further complications of congenital toxoplasmosis may occur later in life.

Because the oocysts are resistant, they are likely to be found in water contaminated by the faeces of infected animals or humans. An outbreak of toxoplasmosis in humans due to contaminated drinking water occurred over a 9-month period in British Columbia, Canada, in 1995, in which 110 acute cases of toxoplasmosis were recorded. Among them were 42 pregnant women and 11 neonates identified through a pregnancy-related screening program. It is suspected that the outbreak was caused by contamination of a reservoir and its feeder streams by the faeces of domestic, feralised or wild cats (Isaac-Renton et al., 1998; Aramini et al., 1999). This outbreak together with an outbreak in Brazil in 2002 related to drinking water has focused attention on detection methods (Villena et al., 2004).

Exposure to oocysts can result from consumption of contaminated food such as unwashed vegetables, or via unwashed hands after contact with pet cats, contaminated soil, or other material. Children can become infected by playing near cat litter trays. Exposure to tissue cysts via raw or undercooked meat from an infected host is a possible source of exposure. Two outbreaks of acute toxoplasmosis involving eight adults in Korea were linked to eating undercooked pork. In the first outbreak, three persons were infected after eating a meal consisting of raw spleen and liver from a wild pig. In the second outbreak, five of eleven soldiers were infected after eating a meal of raw liver from a domestic pig (Choi et al., 1997).

Toxoplasmosis can be diagnosed by serology or isolation and microscopic demonstration of organisms in a faecal smear.

Various drugs are available for the treatment of immunocompromised and pregnant patients who have toxoplasmosis. For others, usually no treatment is required.

9. Short-term outlook

In the short term, it is likely that there will be continued concern about the quality of water used in

Notes to Table 3:
The estimates in this table should be regarded as indicative only. The data presented here cannot be used to calculate risks. For precise process calculations or predictions on food manufacturing processes, additional experimental information is needed. The data quoted here are gathered from various sources and represent an attempt to summarise the likely survival of the organisms under a range of conditions. Such data must be treated with caution, because they represent work using different strains of organisms with different assessments of viability being used. Work with *Cryptosporidium* has revealed that the different ways of assessing viability (in vitro excystation, vital dye staining, tissue culture infectivity, and neonatal mouse infectivity) may give different results, depending on the way the organism has been treated. All methods are intended to serve as surrogates for human infectivity, but full comparison has not been made between methods, and their value as surrogate indicators has not been assessed. They provide the best estimates of the effectiveness of control measures used in the food industry, but more comprehensive data are needed.

food processing, production and preparation. In particular, fresh vegetables and fruits may be associated with protozoan pathogens such as *Cryptosporidium, Giardia*, and *Cyclospora*. The food industry will need to continue to prevent contamination of the food chain and control any sources of contamination. A complicating factor in prevention and control is an increasing globalisation of the fresh produce market, greater international trade, and a trend towards more out-of-home consumption. Additional pressure on food producers and food services will result in more refined prevention and control measures, including

Table 4
Food processes and products at risk from *Cryptosporidium* in the event of a boil water notice (BWN) (based on the best available data and assuming that a HACCP plan is in place where *Cryptosporidium* has been included in the hazard analysis)

Process (for ready-to-eat foods or beverages)[a]	Risk to Public Health[b]	Examples of Food Products[c]
Thermal treatment in hermetically sealed containers to achieve microbiological stability	Negligible	All canned foods
Pasteurisation and sealing in hermetic packs (for solid foods, 70 °C for 2 min or equivalent)	Negligible	Paté and other cooked meats
Pasteurisation of liquids and immediate packing (e.g. HTST 71.7 °C for 15 s)	Negligible	Milk
UHT and aseptic filling	Negligible	Long-life milk and other dairy products
Heating (70 °C/2 min) and immediate dispensing	Negligible	Hot drinks from machines
Commercial drying (spray and freeze drying)	Negligible	Dried milk, instant dried soups, dessert mixes, chocolate
Freezing (where the process water and other ingredients have been pasteurised)	Negligible	Ice-cream and frozen desserts
Freezing (where the process water and ingredients have not been pasteurised)	Low	Some frozen fruit juices
Chilled spreads (dairy- or oil-based, pasteurised, but not pasteurised in pack)	Negligible	Butter, margarine
Fermentation	Negligible	Cheese, yoghurt
Acidification and carbonation	Negligible	Lemonade, cola
Acidification and pasteurisation	Negligible	Fruit juices, pizza toppings
Acidification (no pasteurisation or carbonation)	Low	Fruit juices, still fruit drinks
Rinsing of equipment after cleaning (where the rinse water is not part of the product)	Negligible	All equipment and utensils used in food processing or catering
Washing/rinsing (where rinse water can readily be removed due to relatively low surface area of product) and the food is to be eaten without further cooking	Negligible	Apples, carrots
Washing/rinsing (where rinse water may be retained at a level of 1–2% even after water removal, due to surface area of product) and the food is to be eaten without further cooking	Low	Lettuces, spring onions
Freezing of drinking water to prepare ice (in case of BWN)	High (use boiled, filtered or bottled water until BWN lifted)	Ice for drinks or for cold (ready-to-eat) food
Chilling of drinking water (in case of BWN)	High (use boiled, filtered or bottled water until BWN lifted)	Water fountains in factory; vending machines which dilute and dispense cold drinks
No process, i.e. use of mains water from the tap without any treatment (in case of BWN)	High (use boiled, filtered or bottled water until BWN lifted)	Water for drinking
Confectionery paste	Low	Icings

application of HACCP and its prerequisite programs and possibly conformation to GAP.

10. Long-term outlook

Currently we lack a clear quantitative understanding of the relative importance of the various sources and transmission routes of parasitic protozoa as well as of their survival, viability and virulence. Increasing amounts of research will be needed to obtain a better understanding of the significance to human health of these organisms in the environment. Further developments in the methods and systems of epidemiology and identification of organisms will allow a better assessment of the actual risks presented by these pathogens and a more effective design and installation of the necessary control measures.

It is expected that the proportion of immunocompromised people is increasing globally, which makes these foodborne and waterborne pathogens an even greater potential issue.

Water shortages globally may necessitate more water recycling in agriculture, food manufacturing and service operations. To avoid compromising human health, careful management of water supplies and use will be required.

11. Conclusions

- The biology and ecology of the three most significant parasitic protozoa, i.e. *Cryptosporidium, Giardia* and *Cyclospora,* make their prevention and control in food manufacturing and food service operations difficult.
- Cryptosporidium and Giardia are a primary hazard for the water supply industry. Large-scale outbreaks of disease and smaller incidents have occurred in recent years.
- All three organisms are a potential problem for fresh produce and for food prepared in catering practices. Outbreaks of foodborne disease have occurred, but generally on a smaller scale than with waterborne parasites. Contaminated process water or ingredient water is a particular concern, as is handling by infected personnel.
- None of the three organisms has been shown to be a significant problem for industrially manufactured foods or foods that are sufficiently heat-treated before consumption. The organisms do not survive standard heating processes, and their survival may also be reduced using other processing conditions generally applied in food manufacture and preparation (e.g. ethanol and reduced water activity, see Table 3).

Notes to Table 4:
Reproduced by permission of CCFRA (Dawson, 2000).
Risk Categories
"**negligible risk**" means that it is highly unlikely the products will contain viable parasites. There will be no need to recall products manufactured since the BWN and no need to close down the production line.
"**low risk**" means that parasites may be present in very low numbers or may die within a few days. Recall of products manufactured since the BWN will not be necessary and there may be no technical need to close down the production line.
Products are at "**high risk**" where an infectious dose of parasites could be present. Product manufactured since the BWN was issued must be recalled. Further production will depend on the corrective actions.

[a] Foods to be cooked to achieve 70 °C/2 min throughout (or the thermal equivalent) by the consumer or by a food service will have a "**negligible** risk" (see Risk Categories below).

[b] In the event of a BWN, there will be a need to consider whether the foods or beverages could contain viable parasites and, if so, whether to **recall** products from the marketplace (see "Risk Categories" below). There will be a public health risk only where a product is likely to contain an infectious dose of parasites. The table ranks the risks in terms of "**negligible**", "**low**", or "**high**" as described below. Because contaminated water may have been in circulation for quite a long time before the BWN is issued, food and beverage products in the distribution chain before the BWN was issued could carry a risk (albeit a small one in most cases). In practical terms it is hard to define this period, and the key time for the food company is when they are informed of a BWN and their action plan is put into place: recall should only be considered for highrisk products.

[c] This list gives examples only: it is not intended to be an exhaustive list. Individual examples should be assessed by the food company *together with the relevant authority* in line with the principles described. Some flexibility is also allowed where there are additional factors to take into consideration. For example, a washed salad that is low risk which is then acidified in a dressing becomes negligible risk. Similarly, a lettuce which is normally low risk after washing becomes negligible risk if the wash water is filtered to give 99% removal of oocysts.

– *T. gondii* may be an emerging issue for foods other than undercooked or raw meats, with which it has mainly been associated.

12. Recommendations

Enhanced disease surveillance and epidemiological systems and techniques are needed to improve our current understanding of the significance of these organisms in primary production and throughout the various food chains. It is advisable that certain sectors include the organisms in their HACCP plans and in prerequisite programmes to HACCP. These include sectors or industries in which fresh fruit or vegetables appear in ready-to-eat products that are not further heat-processed and not cooked by the consumer. Additionally, for any final product that incorporates water as an ingredient, producers should consider the organisms in their food safety management systems.

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