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Review

Cryptosporidium in foodstuffs—an emerging aetiological route of human foodborne illness

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Human cryptosporidiosis has emerged as an important gastrointestinal infection in the 1990s, due to the ingestion of contaminated water and foodstuffs containing the protozoan parasite, *Cryptosporidium parvum*. This pathogen has particular clinical significance for immunocompromised persons, including AIDS patients and cancer patients receiving toxic chemotherapeutic drug regimens. Employment of contaminated water in the production of foodstuffs may represent an important potential source of entry into food processing. This reviews aims to examine (i) the incidence of *Cryptosporidium parvum* in foods and waters, (ii) the association between ingesting contaminated foodstuffs and subsequent development of infection, (iii) detection methods and (iv) processing controls that may be beneficial to the food industry to help reduce or eliminate this parasite from the human foodchain. In addition, the potential of *Cryptosporidium* as a bioterrorist agent in the foodchain is examined.

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Introduction

Over the past 25 years, the food industry has been challenged by the emergence of novel foodborne microbiological pathogens such as thermophilic *Campylobacter* spp. in the late 1970s and thereafter and *E.coli* O157 in the 1990s. One of the significant reasons for the emergence of such pathogens has been major improvements in detection systems, primarily in clinical microbiology that have identified such organisms as important human causal agents of gastrointestinal disease. Cryptosporidiosis is the most recent and significant microbiological pathogen to emerge, which has consequently caused concern within the food processing sector. This concern is founded on three parameters, (i) that the causal agent of this infectious disease can be transmitted through contaminated water and food, (ii) that when ingested the causal agent is capable of causing a high degree of morbidity in healthy populations and mortality in vulnerable populations, and (iii) that there is no effective antimicrobial treatment to eradicate this agent from the gastrointestinal tract of symptomatic individuals.

Cryptosporidium parvum, an oocyst-forming apicomplexan protozoan, is an obligate intracellular parasite that infects the microvillus border of the epithelium in the gastrointestinal tract of humans and various animal hosts (Clark, 1999). To date, the genus, *Cryptosporidium*, consists of at least 10 recognized species (Fayer, Morgan, & Upton, 2000). Human infection, however, is predominately caused by *C. parvum* (Kosek, Alcantara, Lima, & Guerrant, 2001) and human illness caused by *Cryptosporidium* has now been reported in more than 40

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countries in six continents (Kosek *et al*., 2001). In the US, it is estimated that approximately 20% of the young adult population have been exposed to this protozoan (Kosek *et al*., 2001). However seroprevalence studies in developing countries, namely rural China (Zu *et al*., 1994) and in a Brazilian shanty town (Kosek *et al*., 2001) have demonstrated positives in 75% of 11–13 year old children and in 90% of children by the age of 1 year, respectively. Two distinct genotypes of *C. parvum* exist, namely the Human Genotype (Genotype I), which is mostly found in humans and the Bovine Genotype (Genotype II), which is found in a wide range of animal hosts, including humans (Peng *et al*., 1997). Generally, cryptosporidiosis is a self-limiting disease, with a high degree of morbidity and a low rate of mortality, and is known to be associated with children in endemic areas and all age groups in developed countries. The infection is mainly concerned with the gastrointestinal tract (GIT), but has been reported, albeit infrequently, in other areas of the body, including the respiratory tract (Clark, 1999). Reported GIT-related symptoms in both immunocompromized (Hunter & Nichols, 2002) and immunocompetent (Kosek *et al*., 2001) populations have included severe diarrhoea, dehydration, abdominal cramps, vomiting, weight loss and electrolyte imbalance.

Immunocompromized people are susceptible to different species, as compared with immunocompetent people and it is now estimated that 32% of AIDS patients have at some stage suffered from cryptosporidiosis (Hunter & Nichols, 2002).

This parasite is monoxenous and once ingested, the environmentally resistant oocysts excyst in the intestine releasing four infective sporozoites, which undergo asexual and sexual development (Kosek *et al*., 2001), once they adhere to the microvillus surface of intestinal epithelial cells. The complicated life cycle and autoinfectivity of *C. parvum* results in the infective dose being relatively low, as few as 10 viable oocysts (Okhuysen, Chappell, Crabb, Sterling, & DuPont, 1999). For a comprehensive review of infective dose determination of *C. parvum* in humans, see Kothary and Babu (2001).

Presently, there are two main recognized modes of transmission of this parasite to humans, namely (i) from infected animals to humans via direct contact, foodstuffs and contaminated water and (ii) from human to human. The main mode of transmission is via the faecal–oral route, through the ingestion of contaminated food or water.

Sources of *C. parvum*

Comprehension of the ecological niche occupied by this organism is of fundamental importance to an understanding of its natural reservoirs and sources. As the primary niche of this organism is the gut of warm-blooded animals, there have been numerous reports in a diverse variety of animals. The parasite has been identified worldwide and is ubiquitous among animals, in particular livestock including both beef and dairy cattle (Xiao & Herd, 1994; de la Fuente *et al*., 1999). It is important to note that the parasite has been described in all the major food animals, including sheep (Chalmers *et al*., 2002) and pigs (Izumiyama *et al*., 2001). For a comprehensive review of animal sources, see Fayer *et al*. (2000).

During the slaughter of cattle and sheep, contamination of the carcass and edible offals with viable oocysts remains a major cause for concern. Unlike poultry processing, the dressing of the carcass in cattle and sheep slaughtering remains a non-automated labour intensive operation. Contamination may occur through faecal spillage onto the hide or fleece, which may then contaminate the carcass during removal. Recently, various guidelines and codes of practice have been introduced to improve the cleanliness of cattle being presented at abattoirs for slaughter (Pennington, 1997), in an attempt to mainly reduce bacterial pathogens, although this may also have a beneficial effect in reducing contamination with *Cryptosporidium* oocysts. Although *Cryptosporidium* has been widely documented in live animals, there has been a relative absence in the literature describing the presence of oocysts from dressed carcasses and fresh meat products. The lack of information on the contamination of fresh meat by this parasite may be related to problems associated with its detection from meat. Due to the fact that oocysts have a high survival rate in the environment (Table 1), it would be unusual for the oocyst not to persist in raw meat systems. Therefore, this is an area that requires urgent attention, in order to help elucidate the possible role of raw meat in the epidemiology of this disease in humans.

Humans are also important sources of this organism, as the parasite is a well-documented emerging pathogen of the GI tract. Consequently, person-to-person spread of the organism is well-described, particularly in secondary cases in outbreak settings (Glaberman *et al*., 2002) and in day care and hospitals (Guerrant, 1997). This is important for the food processing and catering industries, as production personnel and catering staff may act as vehicles of transmission to products and hence to customers (Quiroz *et al*., 2000).

Contaminated water

Water has been recognized as an important vehicle for the transmission of *Cryptosporidium* (Rose, 1990). The widespread practice of disposal of animal manure to land, e.g. by slurry spreading on pasture, may lead to infection directly by aerosol spread or indirectly by contamination of water courses and reservoir feeder streams (Casemore, Wright, & Coop, 1997). The ubiquitous nature of this protozoan parasite and its potential for waterborne transmission is further facilitated by

Table 1. Inactivation of cryptosporidial oocysts in food, water, clinical and environmental matrices

Food/water/clinical/environmental matrix	Survival[a]
Beverages	
Whole milk	>99.9999% inactivation at 71.7°C for 5 s
Cola	>85% inactivation at 4°C for 24 h
Beer [3–4% v/v alcohol]	52–80% inactivation at 4°C for 24 h
Orange juice	35% inactivation
Infant formula	11% inactivation
Tap water	96–99% inactivation at 5–10°C after 176 days
River water	89–99% inactivation at 5–10°C after 176 days
Distilled water	>99.9% inactivation at 60°C for 1 min
Deionized water	>99.9% inactivation at −15°C for 7 days
	>99.9% inactivation at −20°C for 8 days
	>99.9% inactivation at −70°C for 1 h
Cow faeces	60–72% inactivation at 5–10°C for 176 days
Human faeces	41–>99% inactivation at 4°C for 178 days
Sea water[b]	Oocysts viable for minimum of 1 year at 6–8°C

[a] Rose and Slifko (1999).
[b] Tamburrini and Pozio (1999).

the perpetual infectivity of the oocysts, their small size (3.5–6.0 μm) and their low sedimentation rate (0.5 μm/s) (Rose, Lisle, & Le Chevallier, 1997).

The first recorded outbreak due to oocysts in potable water occurred in Texas, USA, in 1984, and resulted in 117 people becoming infected. Oocysts were detected in the patients' stools and the incidence of diarrhoea was found to be 12 times greater than in the neighbouring community; it was suggested that the oocysts had percolated through the ground, from nearby sewage, to a chlorinated well (D'Antanio *et al*., 1985). In 1987, a large outbreak of waterborne cryptosporidiosis in Carrollton, Georgia, USA, infected 13,000 people (Hayes *et al*., 1989). An increased incidence of student illness at a university clinic enabled authorities to identify the public water system as the most possible source of the infection. Cryptosporidial oocysts were detected in the treated water source which had undergone conventional treatment (coagulation, sedimentation, filtration, disinfection) and met coliform (1 cfu/100ml) and turbidity (1 ntu) standards. Free chlorine residuals were as high as 1.5 mg/l at the treatment plant (Rose, 1988). The first recorded outbreak of waterborne cryptosporidiosis in the UK occurred in Ayshire, Scotland, in 1988. The outbreak affected 27 people and occurred in two towns that shared the same source of potable water (Smith *et al*., 1989).

In early April 1993, the largest recorded waterborne outbreak occurred in Milwaukee, Wisconsin, USA. Based on epidemiological data collected through telephone surveys, it was estimated that 419,000 people in the Greater Milwaukee area suffered watery diarrhoea during the survey period, compared with a baseline of 16,000 gastroenteritis cases that would be expected during a similar, non-outbreak, time period. It was thus concluded that the cryptosporidiosis outbreak affected 403,000 people (MacKenzie *et al*., 1994).

Contaminated foodstuffs, links with foodborne outbreaks and modes of transmission

Cryptosporidium oocysts have been isolated from several foodstuffs (Table 2) and these have mainly been associated with fruit, vegetables and shellfish. The association of oocyst contamination with these produce is particularly important from a public health viewpoint, as these products are frequently consumed raw without any thermal processing to inactivate contaminating oocyts. Mollusc filter feeders, such as oysters, mussels and clams, pose a risk because they can concentrate pathogens which are removed from large volumes of potentially contaminated water. Such waters may be polluted with sewage, industrial and agricultural run-off, and storm run-off water, on a regular basis (Fayer *et al*., 1999). In addition, *Cryptosporidium* has been implicated in several cases and outbreaks of human gastrointestinal disease (Table 3), either by direct isolation of oocyts from the suspected foodstuff or by epidemiological association.

The main mode of transmission of this parasite is water and some foodborne outbreaks can be linked to the use of contaminated water. Many outbreaks of waterborne cryptosporidiosis have been documented in the US, Canada, UK and Japan (Fayer *et al*., 2000). Surface water may become contaminated through the entry of human or animal faeces by either a direct or indirect route. Direct contamination of water may be due to entry of faeces from agricultural run-off from adjacent farm animals, or indirectly through accidental contamination from human sewage (Peng *et al*., 1997). In addition, marine water may also be contaminated

Table 2. High risk foods in which *Cryptosporidium parvum* oocysts have been detected but which did not knowingly contribute to human cryptosporidiosis

Food type	Country	Comments	Reference
Vegetables	Costa Rica	Cilantro leaves 5.2% (4/8), Cilantro roots 8.7% (7/80), lettuce 2.5%	Monge and Arias (1996)
		Radish (1.2%0, carrot (1.2%), tomato (1.2%), cucumber (1.2%)	Monge and Chinchilla (1996)
	Peru	14.5% of vegetables examined contained *C. parvum* oocysts	Ortega *et al.* (1997)
	Norway	19/475 (4%) fruits and vegetables examined positive—5 lettuces, 14 mung bean sprouts oocyst density low [3 oocysts/ 100 g food]	Robertson and Gjerde (2001a)
Shellfish			
Clams	Spain	*Dosinia exoleta, Venerupis pullastra, Venerupis rhomboideus, Venus verrocosa*	Freire-Santos *et al.* (2000)
	Italy	*Ruditapes philippinarum*	Freire-Santos *et al.* (2000)
Mussels	Spain	*Mytilus galloprovincialis*, genotype 2	Gomez-Bautista, Ortega-Mora, Tabares, Lopez-Rodas, and Costas (2000)
	Northern Ireland	*Mytilus edulis*, genotype 1	Lowery *et al.* (2001a)
	Canada	Zebra mussel (*Dreissena ploymorpha*), 220 oocysts/g tissue of genotype 1	Graczyk *et al.* (2001)
	USA	Bent mussel (*Ischadium recurvum*)	Graczyk, Fayer, Lewis, Trout, and Farley (1999)
	Ireland	Marine mussel (*Mytilus edulis)*	Chalmers *et al.* (1997)
Oysters	USA	*Crassostrea virginica* (Chesapeake Bay) Genotype I and genotype II	Fayer *et al.* (1999)
	Spain	*Ostrea edulis*	Freire-Santos *et al.* (2000)
	UK	*Ostrea edulis*	Freire-Santos *et al.*, 2000
Cockles	Spain	Cerastoderma edule genotype 2	Gomez-Bautista *et al.* (2000)
Meat and meat products	Europe	Association shown between meat from small ruminants, including sheep and goats and *C. parvum*	Pepin, Russo, and Pardon (1997)

due to sewage disposal at sea or from rivers entering, carrying contaminated agricultural run-off.

The main cause for concern in the food processing and catering industries is the risk of raw water that is positive for this organism entering the premises. As untreated surface waters are frequently contaminated, employment of such supplies should not be used without adoption of control mechanisms to eliminate viable oocyts from such supplies. Chlorination alone has not been successful for eliminating waterborne *Cryptosporidium* oocysts, as they are resistant to the biocidal activity of free chlorine. Furthermore, intake of mains water from the public supply should be carefully monitored, as there have been several reports of such supplies being positive (Rose *et al.*, 1997). Consequently, employment of contaminated water in food processing, as either an ingredient or in cleaning/rinse systems, without adequate controls for the elimination of *Cryptosporidium*, may thus lead to the production of contaminated foodstuffs.

Cryptosporidium survival in food, water, clinical and environmental matrices

Cryptosporidium parvum is an extremely robust organism, with the capability of long-term survival in a variety of natural environments. Unlike the majority of Gram-negative and Gram-positive bacteria, with the exception of *Bacillus* and *Clostridium* spp., this parasite has developed a specialized and physiologically distinct resting stage, as part of its complex life cycle, in the form of the oocyst. This oocyst stage allows the organism to remain viable but dormant, awaiting the opportunity to infect a new susceptible host and to perpetuate the life cycle in this host. Unlike bacterial spore composition, relatively little is presently known about the chemical composition of oocysts and how this composition is associated with survival ability. Table 1 summarizes the survival of cryptosporidial oocysts in a variety of matrices under controlled conditions. The survival rates of oocysts under a variety of environmental conditions indicates that even after initial contamination, natural environments may remain positive for long periods of time, where such environments are still a potential source of cryptosporidial infectivity. The prolonged survival of oocysts in seawater has important implications to coastal recreational waters and shellfish-rearing waters, contaminated with human and animal sewage. Tamburrini and Pozio (1999) reported that oocysts remain infective in seawater for up to one year and can be filtered out by

Table 3. High risk foods associated with outbreaks of human cryptosporidiosis

Suspected contaminated foods	Number of cases	Country	Suspected mode of transmission	Reference
Apple Cider (unpasteurized)	154	Maine, USA	Cider made from dropped apples on ground grazed by livestock. Attack rate 154/284.	Millard *et al.* (1994)
			Subsequent genotyping of oocysts from infected persons demonstrated the presence of the bovine genotype	Peng *et al.* (1997)
Apple Cider (unpasteurized)	31	Connecticut and New York, USA	Apples were washed and brushed with well water, prior to pressing,. This water later was found to contain *E. coli.*	Morbidity and Mortality Weekly Reports (1997a)
Bovine Milk (pasteurized)	50	UK	On-farm pasteuriser faulty leading to outbreak at local school.	Gelletlie, Stuart, Soltanpoor, Armstrong, and Nichols (1997)
Milk	22	Mexico	Canadians travelled to Mexico resulting in possible milk borne outbreak.	Elsser, Moricz, and Proctor (1986)
Raw Goat Milk	2	Australia	Consumption of unpasteurised milk	WHO (1984)
Salad	1	Mexico	Obtained from street vendor	Sterling, Seegar, and Sinclair (1986)
Frozen Tripe	1	UK	Consumption of contaminated food. Oocysts were detected in tripe.	Canada Diseases Weekly Report (1985)
Sausage	19	Wales, UK	Positive correlation between sausage consumption and illness	Casemore, Jessop, Douce, and Jackson (1986)
Chicken Salad	15	Minnesota, USA	Food handler of social event also ran day-care centre. Attack rate 15/26.	Morbidity and Mortality Weekly Reports (1996)
Green Onions	54	Washington, USA	Green onions had not been washed. Attack rate 54/62. Human genotype outbreak	Morbidity and Mortality Weekly Reports (1997b)
Fruit/Vegetables	148	Washington D.C., USA	Food contaminated by handler. Human genotype outbreak	Quiroz *et al.* (2000)
Unknown	18	Wisconsin, USA	Outbreak in company	US Foodborne Disease Outbreaks (2002)
Unknown	6	Wisconsin, USA	Outbreak in private home	US Foodborne Disease Outbreaks (2002)
Unknown	88	Washington, D.C., USA		US Foodborne Disease Outbreaks (2002)

benthic mussels in which they retain their infectivity. Accidental ingestion of seawater contaminated with sewage or the consumption of contaminated shellfish, which act as biological concentrators of these pathogens, may increase the public's risk of acquiring *C. parvum* via this route.

Detection methods

Detection methods for cryptosporidial oocysts can be subdivided into an isolation and detection component and each of these components may consist of several techniques, as shown (Table 4).

Introduced in 1985, immunofluorescent antibody detection has been shown to be a specific and sensitive way to detect *Cryptosporidium* oocysts in faecal smears (Garcia, Brewer, & Bruckner, 1987). This methodology has also been applied successfully to the sensitive detection of *Cryptosporidium* oocysts in environmental water samples (Ongerth & Stibbs, 1987). Both polyclonal and monoclonal antibodies (MAbs) are raised against surface exposed epitopes on purified oocysts. The use of immunofluorescent assays (IFAs) has been shown to be highly sensitive when compared with other conventional staining methods (Arrowood & Sterling, 1989). It appears that oocyst-reactive MAbs do not cross react with the other endogenous stages of the *Cryptosporidium* life-cycle (Barer & Wright, 1990). A drawback of MAb specificity, however, is that one must ensure the antibody-antigen complex stability is maintained under a variety of environmental conditions—if the target epitope is readily stripped from the organism by some mild environmental perturbation, for example, no reaction will ensue and a false negative result will be obtained. This is of particular relevance to *Cryptosporidium* oocysts because disintegration of oocysts may occur in water through time, although the relationship between disintegration and potential infectivity is not known (Rose, 1990).

Ongerth and Stibbs (1987) used a polyclonal antibody specific to the oocyst wall in an indirect IFA. Rabbit antiserum against *Cryptosporidium* was prepared and applied to polycarbonate filters on which river particulates were depositied. The antibodies in the antiserum reacted with antigenic determinants on oocysts present on the filter. After washing, the filters were further

Table 4. Isolation and enumeration techniques for *Cryptosporidium* oocysts from foods and drinking water

Product	Isolation method	Detection method	Recovery efficiency/limit of detection	Reference
Fruit				
Strawberries	Detergent elution, agitation, sonication, centrifugation & IMS	IFA	12–35% recovery (5 min wash) 27–54% recovery (1 min wash)	Robertson and Gjedre (2001b)
Vegetables				
Lettuce (Iceberg)	Detergent elution, agitation, sonication, centrifugation & IMS	IFA	25–31% recovery (5 min wash) 37–57% recovery (1 min wash)	Robertson and Gjedre (2000)
Lettuce (Green Lollo)	Detergent elution, agitation, sonication, centrifugation & IMS	IFA	43–53% recovery (1 min wash)	Robertson and Gjedre (2000)
Chinese leaves	Detergent elution, agitation, sonication, centrifugation & IMS	IFA	31–55% recovery (1 min wash)	Robertson and Gjedre (2000)
Autumn salad mix	Detergent elution, agitation, sonication, centrifugation & IMS	IFA	33–43% recovery (1 min wash)	Robertson and Gjedre (2000)
Bean sprouts	Detergent elution, agitation, sonication, centrifugation & IMS	IFA	1–5% recovery (5 min wash) 15–43% recovery (1 min wash)	Robertson and Gjedre (2000)
Cabbage & lettuce	Detergent elution, sonication, centrifugation	IFA	1% recovery	Bier (1991)
Lettuce	IMS (Dynal) IMS (ImmunoCell)	IFA	18–66% (mean = 42%) recovery 27–66% (mean = 45%) recovery	Robertson and Gjedre (2001b)
Bean sprouts	IMS (Dynal) IMS (ImmunoCell)	IFA	18–99% (mean = 30%) recovery 4–58% (mean = 22%) recovery	Robertson and Gjedre (2001b)
Shellfish				
Mussels *(Mytilus edulis)*	Homogenization of mussel tissue + IMS	IFA + PCR-RFLP (18S rRNA gene) + automated sequencing	qualitative study	Lowery *et al.* (2001a)
Blue Mussel *(Mytilus edulis)*			51.1% recovery. Detection limit 19 oocysts per 0.7 ml of mussel tissue	Graczyk *et al.* (1999)
Asian freshwater clams (*Corbicula fluminea*)	Tissue aspiration from gill and stomach	(i) Acid fast staining (AFS) (ii) IFA	(i) 94.2% recovery (ii) 94.7% recovery	Graczyk, Fayer, Cranfield, and Conn (1998)
Dairy products				
Low fat (1%) milk	sucrose flotation	Microscopic determination	82.3% recovery	Deng and Cliver (1999)
Yoghurt (98% fat-free	ethyl ether extraction + sucrose flotation	Microscopic determination	62.5% recovery	Deng and Cliver (1999)
Ice-cream (9% fat)	sucrose flotation	Microscopic determination	60.7% recovery	Deng and Cliver (1999)
Raw milk	Detergent recovery	PCR + oligonucleotide chemiluminescence hybridization	1–10 oocysts/20 ml raw milk	Laberge *et al.* (1996)

(*continued on next page*)

Table 4 (*continued*)

Product	Isolation method	Detection method	Recovery efficiency/limit of detection	Reference
Beverages				
Apple juice	Formalin-ethyl acetate sedimentation	(i). Acid-fast staining (AFS)	(i) 10^4 oocysts/100 ml	Deng and Cliver (2000)
		(ii) Direct immunofluorescence	(ii) 3×10^2 oocysts/100 ml	
	Sucrose flotation	(i) Acid-fast staining (AFS) (ii) Direct immunofluorescence (iii) PCR	(i) 10^3 oocysts/100 ml (ii) 10^2 oocysts/100 ml (iii) 3×10^1 oocysts/100 ml	Deng and Cliver (2000)
	Flotation + immunomagnetic capture	(i) Direct immunofluorescence	(i) 1×10^1 oocysts/100 ml	Deng and Cliver (2000)
		(ii) PCR	(ii) 3×10^1 oocysts/100 ml	
Water*	Filtration + sucrose gradient	IFA	ND	Tsushima *et al*. (2001)
	Filtration + IMS	IFA	23–42% recovery, mean 33.5%	Department of the Environment (1999)
	Filtration	(i) light microscopy (ii) EIA	(i) 94% sensitivity (ii) 100% sensitivity	Siddons, Chapman, and Rush (1992)
	Filtration + chelex resin + IMS	PCR (18S rRNA gene) (i) nested PCR (ii) RT-PCR	10 oocysts/ml water Detection level 5 oocysts Detection level 27 oocysts	Lowery *et al*. (2000) Monis and Saint (2001)
	Chemical flotation Percoll-sucrose gradient centrifugation	RT-PCR + southern hybridisation	Detection level 1 oocyst	Stinear *et al*. (1996)
	Filtration + centrifugation	RT-PCR + lateral flow detection	Detection level 1 oocyst/l	Kozwich *et al*. (2000)
	Filtration + IMS	(i) IFA (ii) ChemScan laser scanning	49% recovery 73% recovery	Rushton, Place, and Lightfoot (2000)
	Calcium carbonate flocculation/ centrifugation + IMS	(i) flow cytometry	82.3–86.3% recovery	Reynolds, Slade, Sykes, Jonas, and Fricker (1999)
	Filtration + IMS + freeze-thaw	(i) PCR (ii) Nested PCR	(i) 10^5 oocyts/ml sensitivity (ii) 10^2 oocysts/ml sensitivity	Kostrzynska *et al*. (1999)
	Filtration + EnviroAmp™ kit	(i) PCR (ii) Nested PCR	(i) 10^5 oocyts/ml sensitivity (ii) 10^2 oocysts/ml sensitivity	Kostrzynska *et al*. (1999)

[a] References cited are a representation of numerous papers published on detection of *Cryptosporidium parvum* in water samples.

treated with "labelling reagent", fluorescein isothiocyanate (FITC)- conjugated goat anti-rabbit immunoglobulin G. The FITC is a fluorescent dye, while the goat antibody preparation will react with any rabbit antibody (i.e. "primary antibody") remaining on the filter, so that oocysts may then be detected by their fluorescence, using epifluorescence microscopy. FITC-labelled oocysts are a bright apple green, appearing luminous around the periphery and along any surface wrinkles, including the "suture" line (Ongerth & Stibbs, 1987). Direct assays, where the primary antibody is FITC-conjugated, have also been described, although more background fluorescence may be experienced (Musial, Arrowood, Sterling & Gerba, 1987). Fluorescent monoclonal antibodies are routinely used for the detection of oocysts in environmental samples and various commercial kits are now presently available (e.g. Meridian Diagnostics; Northumbria Ltd; Alexon, Inc.).

Many researchers experience poor IFA staining of *Cryptosporidium* oocysts relative to *Giardia* staining. Hoffman, Stanridge, Prieve, Cucunato, and Bernhardt (1997) found that sample age was a factor in the detection process and suggested that the problem might be that commercially available antibodies are raised against *Cryptosporidium* epitopes that are lost after a time. Since oocyst structural integrity is not maintained within the environment, it is recommended that antibodies are raised against aged oocysts and cysts, or even that antibody cocktails against fresh and aged oocysts and cysts are produced (Hoffman, Stanridge, Prieve, Cucunato, & Bernhardt, 1995).

Nonspecific fluorescence associated with other organisms is a problem with IFAs, as is background fluorescence due to debris and the filter itself (Ongarth and Stibbs, 1987; Rodgers, Flanigan, & Jakubowski, 1995). Algae may cause false positives because some stain nonspecifically with the IFA reagents and may be difficult to separate from *Cryptosporidium* oocysts by appearance. Rodgers *et al.* (1995) found that 24 of 54 tested algae species showed some degree of fluorescence with a *Cryptosporidium-Giardia* IFA assay. Often this was described as a dull fluorescence, but *Navicula minima* (a fresh water diatom with wide geographical distribution) and *Synechococcus elongatus*, a cyanobacterium, fluoresced a bright apple green. *N. minima* is also somewhat similar to cysts and oocysts in shape and size, typically 10—17 μm long to 4—7 μm wide. Both algae fluoresced when stained with labelling reagent alone (i.e. the FITC-conjugated anti-rabbit antibody preparation), which suggested a method of solving this problem. Goat serum was added to the filter with the primary antibody addition, to block subsequent non-specific binding by the FITC-labelled goat antibody. Blockage of most, but not all, non-target fluorescence was achieved, and background fluorescence was substantially reduced (Rodgers *et al.*, 1995).

Infectivity studies

It is assumed that environmental isolates vary in viability, potential infectivity in man and in virulence (Casemore *et al.*, 1997). In well-adapted strains in gnotobiotic hosts, the minimum infective dose (MID) may be as low as one oocyst (Blewett, Wright, Casemore, Booth & Jones, 1993). The infective dose was reported to be higher in normal, colostrum-fed lambs but infection was slower to develop (Casemore *et al.*, 1997).

There are currently four methods by which oocyst viability can be asesssed (see Table 5), including: (i) animal infectivity, (ii) *in vitro* excystation, (iii) the exclusion/inclusion of vital fluorogenic dyes, and (iv) reverse transcriptase-polymerase chain reaction (RT-PCR).

Animal infectivity

There are few reliable *in vitro* culture systems for *Cryptosporidium* spp. where the completion of the life-cycle and proliferation of the parasite can be achieved. However, Hajjawi, Meloni, Morgan, and Thompson (2001) have recently described the *in vitro* cultivation of the entire life cycle of the parasite using the HCT-8 cell line, which was mainly attributed success with auto-re-infection. Animal models are more common for assessing viability of the organism and for propagating sufficient numbers of oocyts for experimental work. Problems associated with the use of animals include the fact that the MID may vary for different animals and may not be truly representative of the infectivity of the parasite. The 50% infectious dose (ID_{50}) for 5-day-old Swiss-Webster mice (*Mus musculus*) was reported to be between 100 and 500 oocysts (Ernest, Blagburn, Lindsay, & Current, 1986). For suckling BALB/c mice, it was 60 to 1000 oocysts (Korich, Mead, Madore, Sinclair, & Sterling, 1990; Riggs & Perryman, 1987), for 3- to 5-day-old C57BL/6J mice, it was 600 oocysts (Aguirre, Mason & Perryman, 1994) and for 4-day-old CD-1 mice, it was 79 oocysts (Finch, Daniels, Black, Schaefer, & Belosevic, 1993).

Animal infectivity does not provide information on the numbers or ratio of viable to non-viable organisms present in a sample, or whether there may be attenuated organisms present. What it does assess, however, is whether there are viable organisms present in sufficient numbers to cause infection, i.e. that the sample contains infective organisms, not just those that are viable, but parasites with the ability to establish infection in the host animal. In addition, the application of animal infectivity as a means of assessing oocyst viability in environmental samples is limited by practical considerations, time constraints, economic and ethical reasons. Subsequently, the practical inconvenience of *in vivo* bioassays has led to the development of alternative measures.

Table 5. Laboratory techniques used to assess viability of cryptosporidial oocysts in foods and water

Food type	Viability assay	Reference
Shellfish (oyster, clam)	Immunofluorescent antibody technique (IFTA)	Freiro-Santos, Oteiza-Lopez, Castro-Hermida, Garcia-Martin, and Ares-Mazas (2001)
	Infectivity method using suckling murine model	
Apple juice/orange juice	Excystation using bile salts and trypsin Cell culture foci detection method	Slifko, Raghubeer, and Rose (2000)
Milk	Neonatal mouse infectivity model	Harp, Fayer, Pesch, and Jackson (1996)
Milk products low fat milk, ice-cream, yoghurt	Exclusion of fluorogenic dye propidium iodide (PI-IFA staining)	Deng and Cliver (1999)
Water	Excystation	for review article see Quintero-Betancourt Peele, and Rose (2002)
	Inclusion or exclusion of fluorogenic dyes (DAPI) Nucleic acid stains Reverse transcriptase-PCR Fluorescence *in situ* hybridization (FISH) Infectivity methods using mice models Cell culture	
General	RNA detected by Nucleic-acid-sequence-based amplification (NASBA)	Esch, Locascio, Tarlov, and Durst (2001) Esch, Baeumner, and Durst (2001) Baeumner, Humiston, Montagna, and Durst (2001) Lowery, Moore, Thompson, and Dooley (2001b)

Cell culture and *in vitro* excystation

Numerous *in vitro* intestinal cell models of development of *Cryptosporidium* spp. have been described (Chen & LaRusso, 2000; Hijjawi *et al*., 2001). *In vitro* excystation provides a quantitative method of assessing the viability and survival of oocysts after exposure to environmental and laboratory stresses. It is more economic and requires less time than animal infectivity studies, providing results in approximately four hours, compared with days or even weeks required to obtain results from animal infectivity trials. *In vitro* excystation involves the enumeration of totally excysted, partially excysted and unexcysted oocysts after exposure of oocysts to an excystation medium consisting of 0.75% sodium taurocholate, 0.25% trypsin, in calcium- and magnesium-free Hanks' Balanced Salt Solution, and incubated at 37°C (Rose, 1990). The kinetics of the particular test must be determined before it is applied, but maximum excystation generally occurs within 1–4 h (Rose, 1990; Campbell, Robertson, & Smith, 1992). In addition, a ratio of sporozoites to empty or excysted oocysts can be counted. Good correlation has been demonstrated between *in vitro* excystation and infectivity in mice (Korich *et al*., 1990).

Exclusion/inclusion of vital fluorogenic dyes

Campbell *et al*. (1992) showed that the dyes 4′, 6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI), correlated highly with *in vitro* excystation (correlation coefficient = 0.997). The assay is based on the inclusion (+) or exclusion (−) of the dyes by individual oocysts as determined by microscopy. Oocysts recorded as DAPI+/PI- could excyst, and were described as "viable at assay". PI+ oocysts had disrupted sporozoites or broken membranes and were considered dead. In addition, DAP-/PI- oocysts were also obtained and these were morphologically intact, but required an additional trigger to excyst (acidification mimicking conditions of the *in vivo* infective process). Following dilute acid pretreatment, such oocysts became DAP+/PI-. The finding that permeability of oocyst walls may vary has significance, as the degree of permeability of an individual oocyst may affect its ability to withstand environmental stresses (Campbell *et al*., 1992). For this reason, it is imperative that an optimized *in vitro* excystation assay includes a pretreatment step to trigger full excystation.

PCR and reverse transcriptase-PCR (RT-PCR)

Empty oocysts are not detected by PCR because they lack DNA, but DNA may be preserved for a week or

more in nonviable oocysts, so that PCR alone cannot ensure that only viable oocysts are detected (Fayer, Speer, & Dubey, 1997). Filkhorn, Wiedenmann, and Botzenhart (1994) used PCR to detect a 452-bp sequence of an undefinded genomic DNA region in *Cryptosporidium* spp. The authors noted that even under inappropriate conditions sporadic excystation of sporozoites or disintegration of oocysts occurs with the release of free DNA into the environment. To detect viable oocysts only, a DNA-digest prior to excystation destroys free DNA in the sample and, coupled with an excystation protocol prior to PCR, all DNA detected will be that of excysted sporozoites. Sensitivity detection levels were not determined. Wagner-Wiening and Kimmig (1995) used PCR to detect and specifically identify a 873-bp region of a 2,359-bp DNA fragment encoding a repetitive oocyst protein of *C. parvum*. An excystation protocol before DNA extraction allowed the differentiation between live and dead *C. parvum* oocysts. One hundred sporozoites (equivalent to 25 oocysts) could readily be detected using the assay, and with second round amplification as few as 10 sporozoites could be detected.

The presence of mRNA has been correlated with the viability of an organism, (Mahbubani *et al.*, 1991). Heat shock proteins (hsps) are known to be synthesized with a high level of efficiency and the transcripts are present in large numbers in stressed organisms (Lindquist, 1986). Stinear, Matusan, Hines, and Sandery (1996) developed a (RT)-PCR coupled with IMS that can detect the presence of a single oocyst spiked into concentrated environmental water samples. The test is based on the detection of *Hsp70* mRNA, produced only from viable oocysts, and then isolated by hybridization to oligo$(dT)_{25}$-coated beads.

Unlike PCR used to determine bacterial foodborne pathogens in foodstuffs, PCR determination of cryptosporidial oocysts in foodstuffs, is further complicated by the inability to subculture the parasite from the foodstuff and then perform the PCR reaction, following DNA extraction. The relative non-homogeneity of the chemical composition of foodstuffs and the range of PCR inhibitors in foodstuffs (Wilson, 1997) makes PCR detection directly from foodstuffs problematical.

Control of *C. parvum* within the food processing environment

Prevention of transmission of viable oocysts from contaminated foodstuffs is an important public health control strategy in minimizing foodborne human cryptosporidiosis. The prevention and control of oocysts in foodstuffs may be achieved through an integrated HACCP approach, tailored specifically for the elimination of viable oocysts in food processing (Fig. 1). However, a reliance on absolute control measures, i.e.

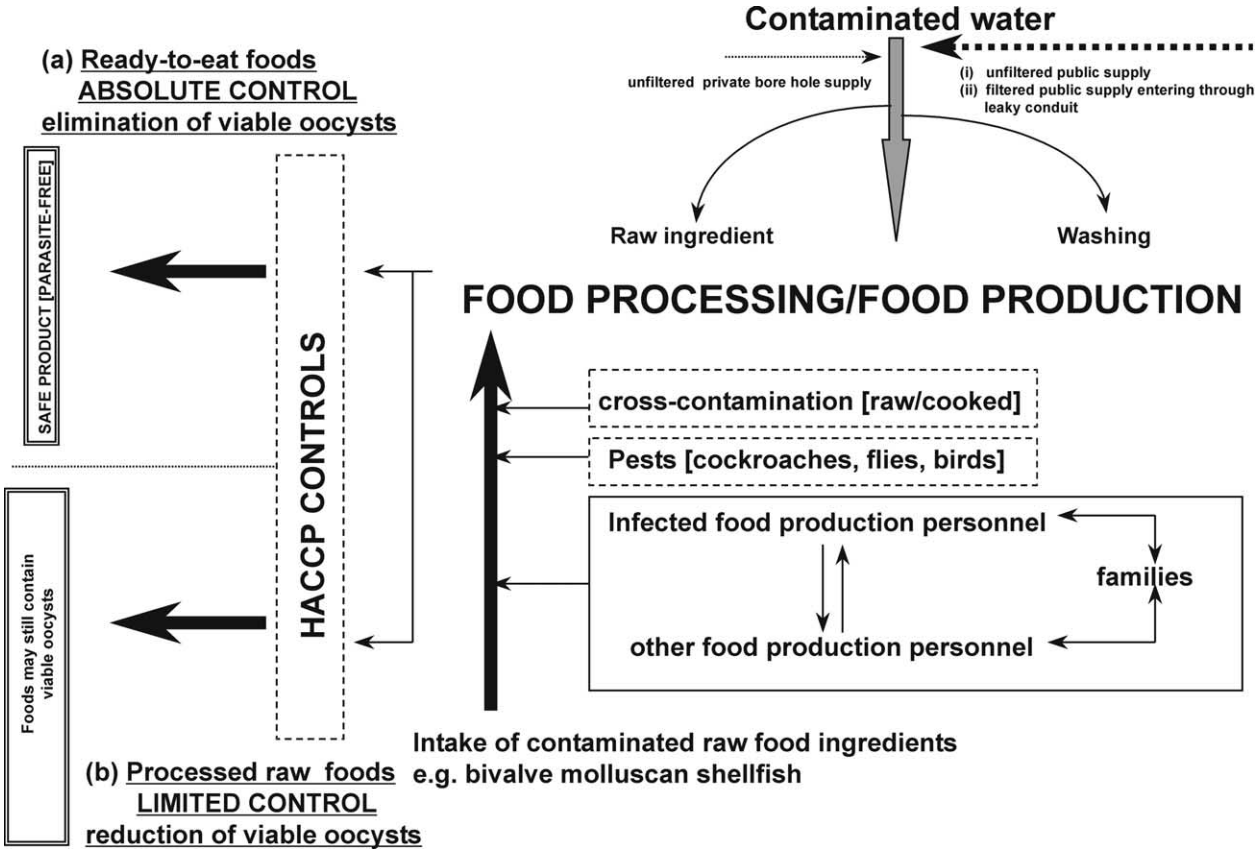

Fig. 1. Structured overview of the entry and control of *Cryptosporidium* in the food industry.

elimination of cryptosporidial ocysts, requires empirically obtained survival data from the control process being examined. Table 6 indicates the range of control processes and survivability of oocysts, which have already been used to control oocysts in foodstuffs.

Manufacturers should place special emphasis on developing suitable and efficient HACCP strategies for the critical control of oocysts depending on the type of unit operation employed and foodstuffs being processed. Given the relative recent emergence of *C. parvum* as a foodborne pathogen, many manufacturers may not, as yet, have given careful technical consideration to this parasite, through the introduction of modified and more stringent HACCP plans for its control. Lateral transfer of established HACCP control plans for other enteric bacterial pathogens, e.g. *Campylobacter jejuni*, should be discouraged, as the survival kinetics for this parasite differ significantly from viral, bacterial and fungal pathogens.

The primary role of water treatment is to prevent transmission of infectious diseases. Physical removal of oocysts through coagulation, sedimentation, and filtration is the primary barrier against waterborne cryptosporidiosis (Rose *et al*., 1997). Deficiencies in any one of these processes has been shown to be directly correlated to the rate of *Cryptosporidium* oocysts reported in water (Leland, McAnulty, Keene, & Stevens, 1993). Properly operated conventional treatment (coagulation, sedimentation, filtration) can remove 99% or more of oocysts. One of the critical times when oocysts can breach the filtration barrier is following backwash (Rose *et al*., 1997). For this reason, optimization of the backwash procedure, including addition of coagulant, or filtering the waste can minimize the passage of oocysts.

For control of the waterborne protozoan, a combination of filtration and disinfection is required. Chlorination alone has not been successful for eliminating waterborne *Cryptosporidium* oocysts. As much as 80 mg/l of free chlorine or monochloramine required 90 min to produce 90% oocyst inactivation (Korich *et al*., 1990). Peeters, Mazas, Masschelein, Dematurana, and Debacker (1989) reported that 0.43 mg/l of chlorine dioxide (ClO_2) reduced infectivity within 15 min, although some oocysts remained viable. Korich *et al*. (1990) reported approximately 90% inactivation of oocysts exposed to 1.3 mg/l of chlorine dioxide for 60 min. Ozone has shown the most promise as an effective disinfectant against oocysts. An initial concentration of 1.11 mg/l ozone for 6 min was shown by Peeters *et al*. (1989) to inactivate viable oocysts at a concentration of 10^4 oocysts/ml. Viability was assessed on the basis of infectivity in neonatal mice. Korich *et al*. (1990) reported that exposure to 1 mg/l ozone inactivated between 90 and 99% of oocysts (2.8×10^5/ml) in water at 25°C. An inactivation level of 99–99.9% was achieved when the exposure time was increased to 10 min.

More recently, ultra violet (UV) treatment of water has become an attractive and popular means of disinfection of water supplies containing viable *C. parvum* oocyts. Health concerns associated with other disinfection processes, including treatment with ozone, has resulted in a renewed interest in the potential of employing UV irradiation, as a means of inactivating oocysts (see Table 6).

Management of *Cryptosporidium* in food processing

Cryptosporidium spp. present several potential hazards within the food processing sector. These hazards may be subdivided into (i) those where the parasite is introduced to the foodstuff through contaminated raw ingredients, e.g. unwashed lettuce destined for ''ready-to-eat'' (RTE) salads; (ii) where the parasite is introduced during food processing due to addition of contaminated water as an important ingredient of the foodstuff, e.g. in soft drinks production; (iii) where the parasite is introduced during food processing, as a contaminant of cleaning of equipment with non-potable water or contaminated potable water; (iv) introduction of the parasite through pest infestations, e.g. cockroaches, house flies, mice and rats; and (v) introduction of the parasite to processed foodstuffs from infected food handlers. The associated risk from each of these potential routes of entry of oocyst into the foodstuff should be controlled through an integrated HACCP approach for the reduction/elimination of viable oocyts in the final food product (Fig. 1). Where manufacturers are producing RTE foodstuffs requiring no further processing, (domestic cooking) then the critical control points in such circumstances are required to be absolute, i.e. complete elimination of the hazard from the RTE foodstuff. Manufacturers should also take into account the globalization of food production, including the sourcing of raw materials from several different countries. This may open new mechanisms for the transmission of this parasite, therefore food processors must be diligent in sourcing ingredients with stringent HACCP-controlled specifications and a commensurate degree of product sampling/testing, to verify the efficacy of such controls. Although the industry should strive to obtain this objective even when processing raw foodstuffs (e.g. raw meats) the critical control points in such circumstances are in practice less stringent, as these foods will receive sufficient cooking to render viable oocysts non-infective. However, contaminated raw produce may pose an important cross-infection hazard with the potential indirect transmission through contaminated utensils and work surfaces.

Presently, there is much debate with regard to the management of *Cryptosporidium*-positive food handlers within the food processing sector. Correct management

Table 6. Treatment methods which may be used to disinfect food and drinking water containing *Cryptosporidium parvum*

Treatment/storage method	Test milieu	Treatment details	Effect on oocyst viability	Reference
Pressure				
High hydrostatic pressure (HHP)	Apple juice	5.5×10^8 Pa applied for	(i) 30 s (i) 99.959% (3.4 log) inactivation	Slifko *et al.* (2000)
			(ii) 120 s (ii) >99.983% (>3.8 log) inactivation	
	Orange juice	5.5×10^8 Pa applied for	(i) 30 s (i) >99.993% (>4.1 log) inactivation (ii) 120 s (ii) >99.995%(>4.3 log) inactivation	Slifko *et al.* (2000)
Dessication	Stainless steel surface	(i) 10 min, (ii) 1 h, (iii) 2 h, (iv) 4 h	(i) 81% viability (similar to oocyst control suspension), (ii) 69% viability, (iii) 45% viability (iv) 5% viability	Deng and Cliver (1999)
Extreme temperatures				
Extreme high temperatures	Milk	High temperature short time pasteurization (i) 5 s, 71.7°C (ii) 10 s, 71.7°C (iii) 15 s, 71.7°C	 (i) 100% deactivation (ii) 100% deactivation (iii) 100% deactivation	Harp *et al.* (1996)
	Apple cider	Flash pasteurization (i) 5 s, 70°C (ii) 5 s, 71.7°C Pasteurization (i) 10 s, 71.7°C (ii) 20 s, 71.7°C	 (i) 3.0 log (99.9%) reduction (ii) 4.8 log (99.998%) reduction (i) minimum 4.9 log (99.999%) reduction (ii) minimum 4.9 log (99.999%) reduction	Deng and Cliver (2001) Deng and Cliver (2001)
	Water	High temperature short time pasteurization (i) 5 s, 71.7°C (ii) 10 s, 71.7°C (iii) 15 s, 71.7°C	 (i) 100% deactivation (ii) 100% deactivation (iii) 100% deactivation	Harp *et al.* (1996)
Freezing temperatures	Ice-cream	−20°C	$t=0$, (18–40% viability remaining) $t=24$ h (0–8% viability remaining) $t=48$ h (0–5% viability remaining)	Deng and Cliver (1999)
	Water	(i) −10°C (ii) −15°C (iii) −20°C (iv) −70°C	(i) At 8, 24 and 168 h viable oocysts detected (ii) At 8 and 24 h viable oocysts detected, 100% deactivation at 168 h (iii) At 1, 3, 5 and 8 h viable oocysts detected, 100% deactivation at 24 and 168 h (iv) 100% deactivation at 1,8 and 24 h	Fayer and Nerad (1996)

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Table 6 (*continued*)

Treatment/storage method	Test milieu	Treatment details	Effect on oocyst viability	Reference
Prolonged storage	Yogurt	Incubated at 37°C for 48 h then held at 4°C		
		$t = 0$ (pH 6.3)	83% viability remaining	Deng and Cliver (1999)
		$t = 4$ h (pH 6.0)	81% viability remaining	
		t-24 h (pH 4.8)	70% viability remaining	
		$t = 48$ h (pH 4.8)	61% viability remaining	
		$t = 96$ h (pH 4.8)	62% viability remaining	
		$t = 240$ h (pH 4.8)	61% viability remaining	
Ultra violet light				
	Water	Medium pressure, 60 mJ/cm^2	100% deactivation	Belosevic *et al*. (2001)
	Water	Low pressure, 120 mJ/cm^2	100% deactivation	Drescher, Greene and Gadgil (2001)
	Water	250–275 nm	approx. 2 log inactivation	Linden, Shin, and Sobsey (2001)
Filtration	Water	(i) membrane filtration (ii) diatomaceous earth filtration 1gpm/sq foot (2.5 m/h)	(i) 6 log removal of oocysts (ii) 6.25 log reduction	States *et al*. (2000)
High energy irradiation	Water	Dose of 10 kGy ^{60}Co	2 $\log_{10}$ inactivation of *C. parvum* oocysts	Thompson and Blatchley (2000)
Electric field photo oxidation	Tap water	Photocatalysis generating biocidal hydroxyl radicals via two treatment types:- (i) thermal film reactor, (ii) sol-gel reactor	Maximum inactivation of oocysts was with thermal film reactor. These initial studies show potential for the disinfection of water	Curtis, Walker, Dowling and Christenson (in press)
Disinfectants				
Sodium hypochlorite	Water	1.31, 2.63 and 5.25% sodium hypochlorite examined for 10, 30. 60 and 120 min	Survival of oocyts in all permutations tested	Fayer (1995)
	Stainless steel surface	5% bleach [final NaOCl concentration 0.26%] 30 min contact time	Viability not significantly affected	Deng and Cliver (1999)
Chlorine	Water	80 mg chlorine/l	approx. 90% inactivation after 90 min	
Monochloramine	Water	80 mg monochloramine/l	approx. 90% inactivation after 90 min	Korich *et al*. (1990)
Chlorine dioxide	Water	(i) 1.3 mg ClO_2/l	(i) 90% inactivation after 1 h	Korich *et al*. (1990)
		(ii) pH 8; 21°C; 1000 mg min/l	(ii) 99% inactivation	Chauret, Radziminski, Lepuil, Creason, and Andrews (2001)
		(iii) pH 8; 20°C; 50 mg min/l	(iii) 1 log inactivation	Arora, LeChevallier, and Battigelli (2001)
		(iv) pH 8; 20°C; 100 mg min/l	(iv) 2 log inactivation	Arora, LeChevallier, and Battigelli (2001)
Ozone	Water	1 mg O_3/l; pH7; 25°C; 5 min	>1.0 log (>90%) inactivation in 1 min	Korich *et al*. (1990)

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Table 6 (*continued*)

Treatment/storage method	Test milieu	Treatment details	Effect on oocyst viability	Reference
Combination treatment	Water	ozone + monochloramine	Synergy observed at all temperatures (1–20°C) Inactivation rate: (i) 20°C–5 times faster, (ii) 1°C–22 times faster Faster than no pretreatment with ozone	Driedger, Rennecker, and Marinas (2001)
Gaseous disinfection				
Ammonia	Water	Concentration: (i) 0.007M NH_3 (ii) 0.148M NH_3 Contact time (i) 10 min (ii) 24 h	Theoretical NH_3 concentration of 3.9M required to inactivate 99.999% freshly purified oocysts in 24 h.	Jenkins, Bowman, and Ghiorse (1998)
	Water	8×10^6 oocysts exposed to gaseous substance	0/10 BALB/c neonatal mice infected	Fayer, Graczyk, Cranfield, and Trout (1996)
Ethylene oxide	Water	8×10^6 oocysts exposed to gaseous substance	0/12 BALB/c neonatal mice infected	Fayer *et al.* (1996)
Carbon monoxide	Water	8×10^6 oocysts exposed to gaseous substance	12/12 BALB/c neonatal mice infected	Fayer *et al.* (1996)
Formaldehyde	Phosphate-buffered saline	8×10^6 oocysts exposed to gaseous substance	6/10 BALB/c neonatal mice infected	Fayer *et al.* (1996)
Methyl bromide	Water	8×10^6 oocysts exposed to gaseous substance	0/12 BALB/c neonatal mice infected	Fayer *et al.* (1996)

of such individuals must stem from evidence-based studies in the scientific/medical literature. The mean duration of the illness has previously been reported as 12.2 days. However, the range in duration has been reported as 2–26 days (Jokipii & Jokipii, 1986). Oocyst excretion times have varied widely from 6.9 days (range 1–15 days) after the cessation of symptoms to two months and greater in a small proportion of patients. Thus it is impossible to predict the carrier status of individuals based on cessation of symptoms. In addition, microbiological screening for carrier status in post-infected individuals is problematic as symptomatic patients may have intermittently negative stool specimens (Jokipii & Jokipii, 1986). Other studies have shown that asymptomatic carrier status in the population may be 0.4% (Hellard, Sinclair, Hogg, & Fairley, 2000) to 6.4% in immunocompetent children (Pettoello-Mantovani *et al*., 1995). However, food processors should also note that this carriage rate may increase dramatically in developing and underdeveloped countries, where raw ingredients may be sourced. Thus processors of RTE foodstuffs are potentially vulnerable to potential cross-infection of their products from symptomatic and asymptomatic carriers of viable oocyts, particularly when these individuals are involved with the handling of such products, as well as the low infective dose require to initiate new cycles of infection in customers consuming contaminated product via this mechanism. Although previous studies have shown the treatment of positive carriers with spiramycin (100 mg/kg daily for 14 days) may significantly reduce the duration of shedding of potentially infectious oocysts (Pettoello-Mantovani *et al*., 1995), such a control strategy is not feasible for food production handlers. Therefore, it is important that simple control measures are implemented within food processing to identify risks associated from these sources, including informative education programmes to report symptoms of food-poisoning to line managers prior to the commencement of production. Additionally, food manufacturers may wish to proactively screen stool specimens from personnel working in high-risk areas of the plant through either a random sampling plan or through a structured plan, e.g. on return from foreign holidays or from endemic areas.

Cryptosporidium—a potential bioterrorist agent

Cryptosporidium is also one of the few foodborne parasites that can be potentially used in bioterrorist attacks. Several factors have made *Cryptosporidium* a candidate for bioterrorist employment. Firstly, human-pathogenic *Cryptosporidium* oocysts are easily available and can be produced or isolated from infected animals or humans in large numbers. Secondly, *Cryptosporidium* is much more infectious to humans than most bacterial agents, with an ID_{50} in human volunteers as low as 9 oocysts when *C. parvum* bovine genotype isolates are used as inocula (Okhuysen *et al*., 1999), and probably even lower with the *C. parvum* human genotype. Thirdly, *Cryptosporidium* oocysts are highly resistant to most environmental factors that are otherwise detrimental to many other microorganisms, such as high salinity, low temperature and most commercial disinfectants (see Table 6). Fourthly, the severe clinical symptoms associated with human cryptosporidiosis (voluminous watery diarrhea for several days in immunocompetent persons and persistent and potentially deadly diarrhea in immunocompromized persons) and the lack of effective prophylactic and therapeutic treatment can generate the panic and fears desired by terrorists. Finally, *Cryptosporidium* has the demonstrated potential of causing large numbers of human cases via a single introduction/contamination event of water or food, and secondary transmission is frequently associated with primary infections (MacKenzie *et al*., 1994; Quiroz *et al*., 2000). Additionally, the organism is difficult to detect in the laboratory in comparison with other bacterial foodborne pathogens, such as *Campylobacter* or *Salmonella* and most primary diagnostic food and clinical laboratories would not normally have the capability in terms of necessary consumable reagents or equipment (IMS, PCR, sequencing facilities, etc.) in order to reliably detect this agent from potentially contaminated foodstuffs or water.

Not all C*ryptosporidium* parasites have the same potential as bioterrorist agents. Five *Cryptosporidium* parasites, including the *C. parvum* human and bovine genotypes, *C. meleagridis, C. felis, and C. canis*, are the most common causes of human cryptosporidiosis (Xiao *et al*., 2001). Others such as *C. muris, C. andersoni*, a cervine genotype and a pig genotype, have been found in a few human cases. Thus far, only the human and bovine genotypes of *C. parvum* have been identified as the cause of foodborne and waterborne outbreaks, indicating that they are probably more infectious to humans than other *Cryptosporidium* parasites. Oocysts of these two *Cryptosporidium* parasites are also more easily to obtain in large quantities, thus would be more likely to be used as bioterrorist agents than other less available *Cryptosporidium* taxa.

Within human and bovine genotypes of *C. parvum*, there is different virulence potential which may influence their employment as bioterrorist agents. Among nearly 50 subgenotypes of the *C. parvum* human genotype identified so far, only several subgenotypes have wide geographic distributions, and one such subgenotype has been found to be responsible for seven foodborne and waterborne outbreaks in North America and Europe, indicating that certain subgenotypes of the *C. parvum* human genotype are more infectious than other subgenotypes. Likewise, among the 30 subgenotypes of the *C. parvum* bovine genotype identified so far, only one or two have wide geographic distributions and one of these

subgenotypes was responsible for two waterborne outbreaks in the US (Xiao *et al.*, unpublished observations). The wide geographic distribution of these *Cryptosporidium* parasites is probably indicative of their biological fitness. The use of a common *Cryptosporidium* subgenotype would make it more difficult to identify a bioterrorist event and to track the source of the attack.

There are several possible ways for terrorists to use *Cryptosporidium* as an attack agent. Intentional contamination of processed food, fruits and beverages with purified *Cryptosporidium* oocysts is probably one of the easiest modes of attack terrorists could use. Previously, such a scheme was used by a religious sect in a bioterrorist attack of a community in Oregon. In this attack, salad bars in 10 restaurants were intentionally contaminated with *Salmonella enterica Typhimurium* over a one month period, which resulted in gastroenteritis in 751 persons. A subsequent criminal investigation revealed that members of a religious commune had deliberately contaminated the salad bars. An *S. enterica Typhimurium* strain found in a laboratory at the commune was indistinguishable from the outbreak strain (Torok *et al.*, 1997). This terrorist attack highlights the challenge of investigating outbreaks caused by intentional contamination and demonstrates the vulnerability of some foods to bioterrorists. It is conceivable that similar attacks can easily be made with *Cryptosporidium* oocysts. As *Cryptosporidium* oocysts can survive in contaminated milk and fruit juice for extended periods of time (see Table 1), coupled with reports of outbreaks of cryptosporidiosis through consumption of these foodstuffs (Table 3), the use of these products and other agricultural produce as attack vehicles is possible.

The use of shellfish, as the point of pathogen introduction to the human foodchain, is a potentially more subtle mode of bioterrorist attack. Human-pathogenic *Cryptosporidium* oocysts are frequently found in oysters and other marine or freshwater shellfish and maintain viability and infectivity for extended periods of time (see Tables 2, 4 and 5). As shellfish are efficient filter-feeders, it is possible to contaminate large numbers by intentional introduction of *Cryptosporidium* oocysts into aquaculture water. This is especially a hazard to humans due to the fact that oysters are frequently consumed uncooked. Even though there is no documented cases of human cryptosporidiosis as a result of consumption of raw shellfish, the high frequency and intensity of *Cryptosporidium* contamination in shellfish emphasize the need for protection of shellfish-rearing water against potential bioterrorist attack.

Future trends and relevance to the food industry

Cryptosporidium has recently emerged as an important gastrointestinal pathogen in humans, where the majority of attention to date has focused on water as the major vehicle of transmission of infective oocysts to humans. Currently, research work is progressing with an examination of risk assessment models to the food and water industries. One such project, funded by the EU Commission aims to establish the risk that *C. parvum* poses for the food and water industry (Boenke, 2001). The objectives are (i) to develop routine procedures for the isolation and detection of oocysts from test samples (food and water), (ii) to determine the survival and infectivity of *C. parvum* in food and water, (iii) to develop procedures for control of *C. parvum* in the food and water industries, and (iv) to develop a risk assessment model for *C. parvum* for the food and water industries.

With the development of improved laboratory detection systems for both isolation, identification and viability testing, coupled with food-related outbreaks, more attention is being placed on the potential transmission of this agent through foodstuffs. Thus, food testing laboratories will experience an increased demand for having such assays in place to routinely monitor for this organism and more importantly, its viability. As the majority of modern detection systems are based on a variety of molecular platforms, including PCR, RT-PCR, NASBA, LightCycler, this may prove a diagnostic challenge for a number of food industry laboratories, which predominantly rely on conventional detection systems.

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