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Cryptosporidium and its potential as a food-borne pathogen

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Abstract

Cryptosporidium species are intestinal protozoan parasites and are excreted in animal feces as stable oocysts. *Cryptosporidium* has now been detected in the feces of a wide range of ruminant and non-ruminant farmed animals, wild animals, domestic pets and birds and the parasite appears to be well adapted to survive and persist in feces for extended periods, ranging from several weeks to many months. Because of this persistence, these materials are important as potential vehicles of transmission within herds, farms, the water chain, the fresh food chain, and the wider environment. Appropriate handling of animal waste is necessary to control spread of this pathogen and to limit the significant risks of human infection. While water is a well-recognized vector of *Cryptosporidium*, it has only recently emerged that food may play a more significant role than previously realized in the transmission of the *Cryptosporidium* to humans. In the last 3–5 years, research efforts have been directed both at the development of suitable methods for isolation and detection of the parasite in foods and at the application of these methods to assess the prevalence and persistence of the parasite in a range of foods. Additionally, molecular subtyping methods have been used to establish the transmission routes of the parasite. This paper summarizes the general biology of *Cryptosporidium* and overviews the current research on *C. parvum* in the food chain. The risks posed by certain foods, such as salad/vegetable crops and beef, are discussed and control measures which may be useful in the farm-to-fork chain for these products are described.

Keywords: *Cryptosporidium*, food-borne pathogens

History

Cryptosporidium was first reported by Tyzzer (1910), who gave a clear description of a parasite which he observed in the gastric glands of laboratory mice. Unsure of its taxonomic status he named it *Cryptosporidium muris*. In 1912, a second, related species was identified as *Cryptosporidium parvum* (Tyzzer, 1912). After these initial observations, little attention was paid to this genus for almost 50 years, although a further species, *C. meleagridis*, was identified and linked to illness in young turkeys (Slavin, 1955). Interest in the parasite increased in 1970s, when *Cryptosporidium* was recognized as a causative agent of bovine diarrhea (Panciera *et al.*, 1971) and it was also around this time that it was first recognized as a cause of cryptosporidiosis in humans (Meisel *et al.*, 1976; Nime *et al.*, 1976). In the 1980s, cryptosporidiosis was documented in a range of animals, including cattle (Mann *et al.*, 1986; Anderson, 1988), sheep and deer (Tzipori and Campbell, 1981; Casemore, 1989). However, it was only when the United States Center for Disease Control (CDC) reported *Cryptosporidium* as the causative agent of severe protracted diarrhea in acquired immune deficiency syndrome (AIDS) patients that this parasite was recognized as an important human pathogen (MMWR, 1982).

Classification and taxonomy

The biological characteristics of the genus *Cryptosporidium* place it taxonomically within the empire

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Eukaryota, kingdom Protozoa and phylum Apicomplexa (Levine, 1986). Currently, up to nine species of *Cryptosporidium*, infecting up to 152 hosts, have been proposed. These include *C. parvum* in many different hosts (Fayer *et al.*, 2000), *C. muris* in mice and ruminants (Tyzzer, 1910), *C. felis* in cats (Iseki, 1979), *C. wrairi* in guinea-pigs (Vetterling *et al.*, 1971), *C. meleagridis* in turkeys (Slavin, 1955), *C. baileyi* in chickens (Current *et al.*, 1986), *C. serpentis* in reptiles (Levine, 1980), *C. nasorum* in fish (Hoover *et al.*, 1981) and most recently *C. andersoni* in cattle (Lindsay *et al.*, 2000). The genus also includes subspecies division, and *C. parvum* is known to comprise two distinct genotypes described as 'human' (genotype 1) and 'bovine' (genotype 2) (O'Donoghue, 1995). Despite such terminology, both genotypes are capable of causing disease in humans, while only genotype 2 is infective to cattle (Peng *et al.*, 1997). In 2002, *C. parvum* genotype 1 was renamed as a new species, *C. hominis* (Morgan-Ryan *et al.*, 2002).

Morphology

The life cycle of *Cryptosporidium* is mostly endogenous, occurring within the gastrointestinal tract of the host (Fayer, 1997), and involves the development and release of thin-walled oocysts, which remain in the host gut, developing and releasing sporozoites to autoinfect the host (Current and Reese, 1986). However, approximately 20% of *Cryptosporidium* oocysts develop into an alternative encysted stage, which has a thick, two-layered, environmentally resistant oocyst wall, and escapes from the host gut within feces, to contaminate and infect new hosts. The environmentally robust oocyst wall consists of an inner and an outer layer, with a unique suture at one end of the structure. Each oocyst contains four infective units, termed sporozoites, which can exit (excyst) through the suture, and once taken up by a host can restart the cycle of infection (O'Donoghue, 1995). *C. parvum* oocysts are ovoid in shape and range in size from 4.5 × 5.0 to 4.2 × 5.4 μm (Tyzzer, 1912; Fayer, 1997).

Cryptosporidium parvum in humans

In symptomatic immunocompetent patients, cryptosporidiosis most commonly presents as mild to perfuse diarrhea leading to rapid weight loss and dehydration, which, particularly in young patients, can necessitate parenteral fluid therapy (Arrowood and Sterling, 1987). The disease is usually self-limiting, symptoms normally lasting between 3 and 12 days. Cryptosporidiosis, however, presents a much greater challenge to immunocompromised patients, with the severity and duration of illness dictated by the extent of the individual patient's immune deficiency. Fluid loss in AIDS patients can be considerable (as high as 17 liters per day has been reported) (CDC, 1982). If sustained, such losses and related disruption of metabolism can be fatal.

Current and Garcia (1991) reviewed more than 100 studies on the prevalence of *Cryptosporidium* in people from over 40 countries. They noted that the prevalence rates in the more industrialized countries of North America and Europe were between 1 and 3%, while in underdeveloped countries rates ranged from 5% in Asia to 10% in Africa, with some increases during warmer and/or wetter months. As noted above, the rate, course and outcomes of cryptosporidiosis infection are significantly more severe in immunocompromised individuals, particularly AIDS patients (Lopez-Velez *et al.*, 1995).

Cryptosporidium in animals and birds

Cryptosporidiosis has been reported in domesticated, wild and captive mammals (O'Donoghue, 1995). Young animals, particularly calves, are reportedly more susceptible to infection (Ernst *et al.*, 1987; Naciri *et al.*, 2000; Zorana *et al.*, 2002) and *C. parvum* is regarded as the most common enteropathogen in suckling calves (Lorenzo-Lorenzo *et al.*, 1993; McDonough *et al.*, 1994; Scott *et al.*, 1994, 1995). Most animals exhibit spontaneous recovery within 1–2 weeks of infection, but significant mortality rates may occur, especially in younger animals (Fayer and Ungar, 1986; O'Donoghue, 1995). Recent studies have detected species of *Cryptosporidium* other than *parvum* in the feces of both calves and cattle, including the calf genotype of *C. muris*, which has recently been renamed as *C. andersoni* (Anderson, 1988; Lindsay *et al.*, 2000; Enemark *et al.*, 2002) .

Ovine cryptosporidiosis was first reported in 1974 in 1- to 3-week-old diarrheic lambs in Australia and since then numerous outbreaks have been recorded (Meutin *et al.*, 1974; Angus *et al.*, 1982; Villacorta, 1991). A study of artificially reared lambs reported that 10 out of every 48 lambs became naturally infected with *Cryptosporidium* and subsequently died (Tzipori *et al.*, 1981). *Cryptosporidium* is also commonly isolated from kid goats (Fayer, 1997).

Cryptosporidium infection in pigs is generally asymptomatic but infections can occur in 6- to 12-week-old pigs (Lindsay and Blagburn, 1991). Two species cause cryptosporidiosis in birds: *C. meleagridis* in turkeys and *C. baileyei* in chickens (Fayer, 1997; Morgan *et al.*, 2001).

Many animals are also asymptomatic carriers of *Cryptosporidium* species and excrete oocysts in their feces, thus contaminating the environment and posing a risk of transmission of the parasite. There are major differences among reported prevalence rates for *Cryptosporidium* in ruminant feces, perhaps due to dif-

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ferences among the ages of animals examined and among the methods used to examine the feces. Prevalence ranging from 1.1% in a random sample of adult cattle feces in California (Hoar *et al.*, 2001) to 79% in symptomatic calves in Maryland, USA have been reported (Fayer *et al.*, 2000). Similar variance has been reported in Europe, *Cryptosporidium* being reported at levels ranging from 6.38 to 62.4% in feces of apparently healthy cattle in Spain (Villacorta *et al.*, 1991) and Scotland respectively (Scott *et al.*, 1995).

Transmission to humans

Cryptosporidium parvum can be transmitted to humans via a number of routes, including person-to-person, animal-to-animal, animal-to-human, water-borne (potable, surface and recreational water) and food-borne.

Zoonotic

Farm and companion animals are potentially important sources of zoonotic infections and were initially suggested as the major source of human infection (Laberge and Griffiths, 1996). There are numerous reports of humans contracting cryptosporidiosis from infected animals, such as lambs and calves (Fayer and Ungar, 1986), including student vets (Preiser *et al.*, 2003) and other persons in direct contact with animals (Casemore, 1990a; Current and Garcia, 1991). A number of cases of cryptosporidiosis have been reported within groups of children undertaking educational visits to farms during the lambing and calving seasons (Sayers *et al.*, 1996).

Water-borne

Water-borne cryptosporidiosis is associated with contamination of water with animal or human waste. There have been a number of documented outbreaks of cryptosporidiosis attributed to potable water (Smith *et al.*, 1988; Hayes *et al.*, 1989), well water (D'Antonio *et al.*, 1985; Richardson *et al.*, 1991; Dworkin *et al.*, 1996) and surface water (Kramer *et al.*, 1997). Several outbreaks have also been associated with swimming in contaminated recreational water in swimming pools, amusement park wave pools and water slides (Bayer and Wright, 1990, Fournier *et al.*, 2002; Rose *et al.*, 2002; Eurosurveillance Weekly, 2003). The ability of *Cryptosporidium* to tolerate the concentrations of chlorine (designed to eliminate bacterial pathogens) applied during water treatment processes has been a contributory factor in many of these cases.

The biggest documented outbreak of *C. parvum* occurred in April 1993, in Milwaukee USA, and was associated with a potable public water supply. The source of the outbreak was Lake Michigan water, which was shown to be contaminated with *Cryptosporidium* oocysts, and although the water was chlorinated and filtered at one of two waterworks plants before entering the main Milwaukee water supply, oocysts were not removed by the water treatment process, and it is estimated that over 403 000 residents and visitors became ill and 100 people died (MacKenzie *et al.*, 1994).

Food-borne

Recently food has been identified as a possible source of *C. parvum*, with outbreaks of cryptosporidiosis linked to dairy products, apple cider and sausages (Casemore *et al.*, 1986; Millard *et al.*, 1994) Meat products, including chicken salad, frozen tripe and raw sausages, have also been associated with cryptosporidiosis (Nichols and Thom, 1985; Casemore *et al.*, 1986). A number of food outbreaks and their suspected sources are detailed in Table 1. It is usually difficult to identify the source of infection due to the time delay in consumption of food product and onset of illness. In addition, the lack of suitable methods for the routine detection of the parasite in foods has also hampered epidemiological investigations (Sterling *et al.*, 1986; Gatti *et al.*, 1993; Orlandi *et al.*, 2002).

Methods for isolation and detection

Clinical samples

Methods for the detection of *Cryptosporidium* in clinical samples include modified acid-fast stains using auramine phenol (AP) as the fluorescent stain and carbol fuchsin as the negative stain (Casemore, 1991). Such approaches offer the advantage of allowing the simultaneous detection of a number of other parasites (e.g. *Isospora*, *Cyclospora*) in fecal smears. These 'other' parasites might not be detected by more specific methods, such as immunofluorescence or enzyme immunoassays (Fayer, 1997).

Water

The most commonly used method for the isolation of *C. parvum* from water involves filtration of large volumes of water (10–1000 litres) and recovery of captured material from the filter by a combination of centrifugation and immunomagnetic separation, with microscopic detection of the oocysts using monoclonal antibodies tagged with fluorescein isothiocyanate (FITC) (Lindquist *et al.*, 2001). Detection with FITC has the benefit of being quantitative, but lacks the ability to distinguish between viable and non-viable oocysts.

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Table 1. Summary of outbreaks of cryptosporidiosis associated with food

Suspected food	No. cases	Country	Suspected mode of transmission	Reference
Apple cider	154	USA	Unpasteurized apple cider made from apples collected from ground grazed by livestock	Millard *et al.* (1994)
Apple cider	31	USA	Unpasteurized apple cider made from apples washed and brushed with well water before pressing	Peng *et al.* (1997)
Bovine milk	8	Queensland	Drinking unpasteurized milk	Harper *et al.* (2002)
Bovine milk	50	UK	On-farm pasteurizer faulty, leading to outbreak at local farm	Gelletlie *et al.* (1997)
Milk	22	Mexico	Canadians travelled to Mexico: possible milk-borne outbreak	Elsser *et al.* (1986)
Raw goat milk	2	Australia	Consumption of unpasteurized milk	WHO (1986)
Salad	1	Mexico	Salad from street vendor consumed	Sterling *et al.* (1986)
Frozen tripe	1	UK	Oocyst contamination of tripe	Nichols and Thom (1985)
Sausage	19	Wales	Positive correlation between sausage consumption and illness	Casemore *et al.* (1986)
Chicken salad	15	USA	Food handler at social event also ran day-care centre	CDC (1996)
Green onion	54	USA	Consumption of unwashed green onions	CDC (1997)
Fruit/vegetables	148	USA	Food contamination by handler	Quiroz *et al.* (2000)

Adapted from (Millar *et al.,* 2002).

Food and environmental samples

Collation of direct evidence to implicate food in the transmission of *Cryptosporidium* has been hampered by the lack of equivalent bacteriological enrichment culture methods for the recovery of small numbers of oocysts. Methods have been described for the isolation and detection of *C. parvum* from a number of different foods, and the various approaches used and the recoveries achieved are summarized in Table 2. These methods generally employ agitation in detergents, or the more traditional bacteriological approach of homogenization to mobilize oocysts from solid matrices, followed by immunomagnetic separation (IMS) with visualization by immunofluorescent microscopy or detection of parasite genetic material by the polymerase chain reaction (PCR). Limitations in the use of these methods for isolation of *C. parvum* from food products include variations in recovery levels and difficulties due to the breakdown of the product during detachment of the oocysts from the food matrix. Such food debris can interfere with IMS recovery processes, leading to an overall reduction in oocyst recovery. Recovery of oocysts from dairy products, such as low-fat milk, yoghurt and ice-cream, have been achieved by successfully adapting methods previously employed for faecal samples, with recoveries of 62.5–82.3% reported (Deng and Cliver, 1999). Although the adaptation of these methods is very successful for liquid products, they are unsuitable for solid food products, such as vegetables and meat.

A key problem with the methods has always been the difficulty of obtaining clean extraction of the parasite from the food matrix in order to prevent food debris interfering with subsequent immunological and molecular detection methods. Recent studies have investigated the use of a novel pulsifier machine. This is a newly developed instrument that is based on a combined shock-wave generator/stirrer that drives attached microorganisms from the sample into suspension without crushing the sample (Microgen Bioproducts, UK) (Sharpe *et al.*, 2000). Methods have been developed for extraction of oocysts from beef, lettuce and environmental samples based on the use of this machine in combination with filtration, centrifugation or immunomagnetic separation, with detection by immunofluorescence microscopy using FITC-labelled antibodies (Moriarty *et al.*, 2003a; Warnes and Keevil, 2003). The methods were shown to be simple and consistent to perform. When applied to lettuce, recovery ranged from ~40% at the lowest level of 100 oocysts per gram, rising to >80% at 100 000 oocysts per gram (Warnes and Keevil, 2003). For beef surfaces, recovery ranged from 15.7–39.8% for an inoculum of 105 oocysts per cm^2 to 85–128.4% for an inoculum of 35 000 per cm^2 (Moriarty *et al.*, 2003a).

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Table 2. Summary of techniques used for isolation and detection of *Cryptosporidium* oocysts from food

Product	Isolation method	Recovery (%)	Reference
Strawberries	Detergent washing procedures and sonication followed by IMS	27–54	Robertson and Gjerde (2000)
Bean sprouts	Detergent washing procedures and sonication followed by IMS	15–43	Robertson and Gjerde (2000)
Lettuce	Detergent washing procedures and sonication followed by IMS	37–57	Robertson and Gjerde (2000)
Vegetables	Washing, centrifugation, suspension in 2.5% potassium dichromate	–	Ortega *et al.* (1997)
Bean sprouts	Detergent elution, agitation, sonication, centrifugation and IMS	1–5	Robertson and Gjerde (2000)
Salad leaves	Detergent elution, agitation, sonication, centrifugation and IMS	33–43	Bier (1991)
Salad leaves	Pulsifier, filtration, centrifugation	40 to >80	Warnes and Keevil (2003)
Apple juice	Sucrose flotation with IMS	–	Deng and Cliver (2000)
Milk (1% fat)	Sucrose flotation	82.3	Deng and Cliver (1999)
Ice-cream (9% fat)	Sucrose flotation	60.7	Deng and Cliver (1999)
Yogurt (98% fat-free)	Sucrose flotation	62.5	Deng and Cliver (1999)
Shellfish	Homogenization	–	Gomez-Bautista *et al.* (2002)[a]
Beef carcasses	Pulsifier, filtration, centrifugation	15.7–128.4	Moriarty *et al.* (2003)

Adapted from (Millar *et al.*, 2002).
[a]Detection by PCR; in all other studies detection was by immunofluorescence.
IMS, immunomagnetic separation.

Molecular methods

A variety of molecular techniques have been developed for the detection and characterization of *Cryptosporidium*. In general these techniques are very sensitive, and rapidly provide information on the viability and possible source of the oocysts. A number of PCR methods have been reported for *C. parvum* and are generally based on the amplification of the thrombospondin-related adhesive protein (TRAP-C2), *Cryptosporidium* outer wall protein (COWP) or 18s rRNA genetic regions (Cai *et al.*, 1992; Peng *et al.*, 1997; Spano *et al.*, 1997; Lowery *et al.*, 2000). A novel multiplex PCR has been reported recently for the detection of *C. parvum*, types H and C, *C. canis*, and *C. felis* in fecal and soil samples (Lindergard *et al.*, 2003).

Subtyping methods used to characterize *Cryptosporidium* isolates include random amplified polymorphic DNA analysis (RAPD) (Morgan *et al.*, 1995), restriction fragment length polymorphism-PCR (RAPD-PCR) (Bonnin *et al.*, 1996), nucleic acid based sequence analysis (NASBA) (Cook, 2003), and the use of gp40/15 as a subtyping marker gene and multilocus microsatellite-fingerprinting assays (Cacciù *et al.*, 2000, 2001; Enemark *et al.*, 2002), all of which allow detailed characterization of specific isolates and provide valuable epidemiological information.

Oocyst infectivity and assessment of viability

The viability of *C. parvum* can vary depending on the length of time they have been in the environment and the conditions to which they have been exposed. The assessment of oocyst infectivity and viability is important in assessing persistence and survival in the environment and the impact of treatment processes, and in quantifying the risk that *Cryptosporidium* may pose to public health. In particular, it is now recognized that in performing quantitative risk assessments for the parasite the exposure assessments should include data on the viability of oocysts and not just prevalence data. Several methods of assessing viability/infectivity have been reported and are summarized below.

Animal infectivity

Animal models are regarded as the gold standard for assessing the ability of oocysts to cause infection, the mouse being the most commonly used model for *C. parvum* infection. However, there are problems associated with the use of animal models. For example, the minimum infective dose may vary among different species of animals and may not be a true representation of the infectivity of the parasite to humans (Ernest *et al.*,

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1986; Finch *et al.*, 1993). Equally, this assay can only be performed in specialized facilities and is unsuitable for use in routine laboratories

Cell lines

At least 20 cell lines have been shown to support *C. parvum* infection, including cell lines of human ileocecal adenocarcinoma (HCT-8) (Upton *et al.*, 1995), bovine kidney (MDBK) (Gold *et al.*, 2001), canine kidney (MDCK) and human colonic adenocarcinoma Caco-2 (Yu *et al.*, 2000). There is currently no consensus as to which cell line is most appropriate for studying the *in vivo* development of *C. parvum*. A comparison of 11 cell lines reported that HCT-8 cells produced approximately twice as many intracellular life cycle stages compared with MDBK, MDCK or Caco-2 cells (Upton *et al.*, 1994). However, other investigators have reported that there were no differences among the amounts of infection in Caco-2, HCT-8 and HT29 cell lines (Maillot *et al.*, 1997). In a recent study, a good correlation (R^2 = 0.85, n = 25) was observed between infection in HCT-8 cell lines and mouse infectivity, demonstrating that cell lines may present a suitable alternative to the gold standard mouse infectivity, while avoiding ethical and interspecies problems related to the use of mouse models (Rochelle *et al.*, 2002). However, specialized facilities are needed to perform this assay.

Exclusion/inclusion of vital fluorogenic dyes

The most commonly used method for assessing viability is the inclusion or exclusion of the dyes 4′,6-diamidino-2-phenylindole and propidium iodide by oocysts, as determined by epifluorescence microscopy and examination of individual oocyst contents using differential interface contrast. This method is very fast (<3 h) and has the benefit of providing information on the relative percentages of viable and non-viable oocysts. It is also a method that is suited to use in routine laboratories while the use of animal models and cell line assays is limited to specialist facilities and staff. However, although it is a good indicator of viability, it has been reported that this assay can overestimate oocyst viability, particularly in relation to some treatments, e.g. low-level ozonation (Bukhari *et al.*, 2000), which would lead to overestimation of risk, particularly when this method is used for risk assessment.

Nucleic acid-based assays

Because of the difficulties in performing the assays or with the accuracy of results obtained with the above assays, approaches based on detection of specific nucleic acids are now being assessed and recognized as alternative approaches to determining oocyst viability.

The combination of a DNA digest and a viability assay followed by a PCR has been reported (Filkorn *et al.*, 1994). A DNA digest was employed to destroy free DNA in the sample and the oocysts were then subjected to an *in vitro* excystation protocol (releasing sporozoites from viable oocysts). The nucleic acids released at that stage were then detected by a PCR reaction. Dead sporozoites within intact oocyst walls were not detected

mRNA is rapidly degraded in the cell and thus provides a potential molecular target for viability assessment. The development of a viability assay for *C. parvum* oocysts based on immunomagnetic separation and reverse transcription PCR (IMS-RT-PCR) has been described (Hallier-Soulier and Guillot, 2003). The procedure is based on the hybridization and detection of hsp70 mRNA, which is induced by heat shock and thus produced only in viable oocysts.

The isothermal amplification method NASBA, which amplifies RNA, has been reported for the detection of viable oocysts of *C. parvum* in environmental samples (Baeumner *et al.*, 2001). The target molecule was a 121-nucleotide sequence from the *C. parvum* heat-shock protein hsp70 mRNA. Amplified RNA was hybridized with specific DNA probes and quantified with an electrochemiluminescence detection system. Although a relatively recent method, NASBA has the potential for adoption as a diagnostic tool for environmental pathogens.

Prevalence and persistence in the food chain

Environment

Animal and human wastes (manures, slurries, sewage, etc.) may contain pathogenic microorganisms, and without suitable treatment (composting, digestion, etc.) there is potential for the pathogens present to contaminate the food chain. This can occur as a result of run-off into adjacent surface waters or from direct application of the untreated wastes to crops, or the pathogens can be spread directly to man or farm and domestic animals using the land. Understanding the survival of potential human and animal pathogens in these wastes before and after their application to land is critical in delivering safe agricultural products to the marketplace. These concerns are particularly pertinent when considering *C. parvum* oocysts, which can reportedly remain viable for about 18 months in a cool, damp or wet environment (Fayer *et al.*, 2000).

Some recent research has concentrated on the survival of *Cryptosporidium* in soil environments. An experiment designed to assess the effects of drying and temperature on *Cryptosporidium* oocysts placed on semipermeable membranes on pastures showed that oocysts were sus-

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ceptible to drying (Svoboda *et al.*, 1997). Estimated viability declined to undetectable levels after 2–4 weeks in summer, whilst in winter the combined effects of drying and freezing temperatures appeared to kill oocysts rapidly after only a few days. This study also found that up to 90% of oocysts applied to the soil in excreta could be recovered in the soil. Viable oocysts could then be leached from the soil matrix for extended periods of at least 3 months. In an experiment on the survival of *Cryptosporidium* oocysts in stacked manure heaps, the parasite was shown to decline rapidly, 4 weeks at 20°C being sufficient for the total kill of all oocysts (Svoboda *et al.*, 1997). Research conducted by Warnes and Keevil (2003) monitored the survival of *C. parvum* in slurry (cattle, pig), different soil types (clay, chalky, sandy) and in dirty water over a period of about 162 days at pH 4, 7 and 9 and at 4, 10 and 20°C. The results showed that, in general, the parasite survived best at 4°C at an acid or neutral pH (4 and 7), and under these conditions it was often still viable at 162 days. In contrast, Barrick *et al.* (2003) conducted a study on factors associated with the likelihood of *Cryptosporidium* in soil from dairy farms, and reported a decreased risk of contamination in soil with low pH. Kato *et al.* (2002) conducted a study on the effect of freezing (−10°C) on the survival of *C. parvum* in soil, which indicated that inactivation rates for the parasite were greater in water than in soil and greater in dry soil than in moist and wet soils. Overall, the results indicated that 99% of oocysts were non-viable after 50 days at this temperature.

Overall, the results suggest that animal wastes should be either stored for sufficient periods of time or actively treated to remove pathogenic microorganisms before application to land, particularly land that is to be used for growing crops or for grazing by livestock.

Food

C. parvum cannot replicate in food, but oocysts can potentially survive in contaminated foods and infect people, either directly as a result of eating raw or undercooked foods or indirectly as a result of cross-contamination. Foods of animal origin, including raw milk/dairy products and raw meats and fresh fruit and vegetables grown in a contaminated environment, are most likely to pose a risk. A study in Costa Rica found oocysts on cilantro leaves and roots, lettuce, radishes, tomatoes, cucumbers and carrots (Monge and Chinchilla, 1996). A study in Peru found that, in addition to some of the above foods, cabbage, basil, parsley, celery, leeks, green onions and ground green chilli were also contaminated (Ortega *et al.*, 1997). Studies conducted in Norway recovered *Cryptosporidium* from 19 of 475 fruit and vegetable samples tested, of which five were in lettuce and 14 in mung bean sprouts (Robertson and Gjerde, 2001b). A further study by these authors recovered *Cryptosporidium* from 8% (14/171) of seed sprout samples (alfalfa sprouts, mung beans, radish) examined (Robertson *et al.*, 2002).

Cryptosporidium has been reported at high concentrations (80–10 000 oocysts/0.5 kg) in zucchini (Armon *et al.*, 2002)

At a beef abattoir in the Republic of Ireland, Moriarty *et al.* (2003b) conducted a study on the prevalence of *Cryptosporidium* oocysts in the feces of cattle immediately after slaughter (n = 288) and on their corresponding carcasses following evisceration (n = 288). *Cryptosporidium* species were isolated from 21/288 (7.3%) fecal samples at a level of 25–37 500 per gram. The isolates were speciated and genotyped, using restriction fragment length polymorphism-PCR, as *C. andersoni* (54.5%) or *C. parvum* genotype 2 (45.5%). The parasite was not detected on carcass meat. The prevalence of *Cryptosporidium* oocysts in water used to wash the beef carcasses was also determined at an abattoir with a bore-hole supply (n = 46) and a further abattoir with a river water supply (n = 49) (McEvoy *et al.*, 2003). Both supplies were chlorinated and the river water supply was additionally treated by slow sand filtration. Each water sample (50 litres) was collected at the point of application to the carcass. *Cryptosporidium* species were not isolated from bore-hole water but were detected in 12 of 49 river water samples at a level of 0.08–9.0 oocysts per litre. This highlights the need for assessment and optimization of the efficiency of treatment systems for water that is used in food production. It also emphasizes that the quality of the raw water source directly influences the level of treatment required, and consequently drinking water and water destined for use in food production should preferentially be from a protected water source. The reason *Cryptosporidium* was not detected on the beef carcasses, although clearly present in the abattoir environment, may have been related to a number of issues, including the fact that the number of oocysts was low in the majority of samples of feces (12/21 had counts <100 oocysts per gram) and water (5/12 positive had counts <0.4 per litre), which may have precluded transmission onto the carcass. Studies conducted on bacteria suggest that the transmission rate from feces to carcass may be as low as 1:10 0000 organisms (Cassin *et al.*, 1998). The negative results may also have been partly attributable to high variability in the number of oocysts that the method would have recovered, with as few as 15.37% of oocysts recovered from a sample with very low numbers of the parasite present (Moriarty *et al.*, 2003). Alternatively, it may reflect low adhesion of oocysts to beef tissue, but further research would be necessary to establish this.

In a study conducted by Lowery *et al.* (2001) in Northern Ireland, *C. parvum* genotype 1 (human origin) oocysts were detected in the marine filter-feeding mussel *Mytilus edulis*, collected from the shores of Belfast

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Lough. Gomez-Couso *et al.* (2003) reported the recovery of *C. parvum* in 69 of 203 (34%) shellfish samples from Spain and the parasite was also reported in six of 133 (13%) Portuguese oysters grown in The Netherlands (Schets *et al.*, 2003). Similarly, studies in the USA have reported the presence of *Cryptosporidium* in shellfish in Atlantic coastal waters and in the Chesapeake bay (Fayer *et al.*, 2002, 2003).

Detection of *Cryptosporidium* (particularly genotype 1) in shellfish destined for human consumption confirms the organism as a potential hazard in seafood.

Control measures

In order to control the transmission of *C. parvum* through the food chain, a farm-to-fork approach must be taken, with efforts focused at various points along the chain. On-farm controls should include the management of animals on the farm to reduce animal-to-animal transmission of infection and the contamination of the farm environment. Of equal importance is the correct storage and/or treatment of animal wastes to limit the transmission of the parasite into the farm environment, where, as outlined above, it may survive for considerable periods. Active treatment processes currently used include composting, heat-drying and anaerobic digestion. The addition of lime (quicklime or slaked lime to raise the pH to 12 for at least 2 h) has been reported to result in significant reduction of bacterial pathogens (Bujoczek *et al.*, 2001). However, with conflicting reports on the effect of pH on *Cryptosporidium*, further research would be needed to establish the benefits of this treatment.

During food processing different intervention measures that are commonly employed may be or may not be successful in reducing or eliminating the parasite. The effects of a number of typical control measures are outlined below. At the domestic level, as with all foodborne pathogens, the risk of cryptosporidiosis can be reduced by adequate cooking of raw foods and the use of good hygiene practices in the home to avoid the risk of cross-contamination.

Disinfectants

Cryptosporidium has been shown to be resistant to the action of many common antimicrobial agents and chlorine-based disinfectants, including hospital disinfectants such as bleach (Campbell *et al.*, 1982). Because comparatively high concentrations of disinfectants (e.g. 80 mg/litre of free chlorine or monochloramine) with long exposure times (e.g. 90 min) are necessary to achieve inactivation of 90% of oocyst populations, it is suggested (Campbell *et al.*, 1982) that conventional disinfection practices will have little impact on *Cryptosporidium*.

Water activity

A limited number of studies have investigated the effect of drying on *Cryptosporidium* in foods. Studies have shown that drying of oocysts suspended in water on glass surfaces at ambient temperature resulted in 97% loss of viability after 2 h and total death after 4 h (Robertson *et al.*, 1992). Deng and Cliver (1999) demonstrated that the viability of oocysts on the surface of stainless steel was significantly affected by desiccation, only 5% of the oocysts remaining viable after 4 h of air-drying at room temperature. Slifko *et al.* (1997) studied the survival of *Cryptosporidium* oocysts in two model products with water activity values of 0.85 (pancake syrup) and 0.95 (9.0% sodium chloride and 2% dextrose, to simulate bread dough) at temperatures of 7 and 28°C. Infectivity was evaluated with the cell culture foci detection method. Excystation and staining procedures were also used as conservative measures of oocyst viability. Under a_w conditions of 0.85, more than 99.9% of the oocysts appeared to be non-infectious after storage for 24 h at 28°C or 1 week at 7°C. Oocyst infectivity was reduced by >99.99% after storage at a_w 0.95 for 1 week at 28°C or 2 weeks at 7°C.

pH

Cryptosporidium oocysts are susceptible to low pH values, as was shown in several studies on the survival of oocysts in beverages. Oocysts do not survive at the low pH values in beer, cola and cider (Friedman *et al.*, 1997). Jenkins *et al.* (1998) studied the effect on oocyst viability of ammonia concentrations between 0.007 and 0.148 M. Although a significant decrease in oocyst viability was shown, a small fraction of oocysts remained viable. Exposure to pH values corresponding to the applied ammonia concentrations showed minimal effects of alkaline pH values.

Freezing

Studies have shown that snap-freezing to −22°C, rendered all oocysts non-viable (as determined by an inclusion/exclusion vital dye assay), although oocysts that were slowly frozen, stored at −20°C for 14 days, and slowly thawed, were still infective to mice (Robertson *et al.*, 1992).

A study by McEvoy *et al.* (2003) investigated the survival of *C. parvum* inoculated onto lean and fat beef trimmings that were boxed, blast-frozen (to −20°C within 60 h), stored (−20°C, 21 days) and tempered (48 h at −3°C) under commercial conditions prior to burger production. After the freeze/tempering process, 9.46% of the inoculum was still viable on lean trimmings, and 7.17% of the inoculum was still viable on fat trimmings. The results

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showed that freezing/tempering does not inactivate all oocysts present on meat. However, current commercial processing can lead to the inactivation of very significant proportions of a contaminating oocyst population. Such processing, if carefully and rigorously applied within HACCP and related schemes, can provide useful and highly desirable reductions in the numbers of viable oocysts present on contaminated trimmings and derived products. It is, however, important to add the caveat that the viability results in this study were obtained using a dye exclusion assay which may have overestimated viability (Bukhari *et al.*, 2000), and it would be useful to do further research to assess infectivity as well as viability following freezing and thawing.

Heat

C. parvum is susceptible to heat treatments, such as those employed to pasteurize milk and dairy products (Harp *et al.*, 1996). Oocysts (in water) were rendered non-infectious to mice and chickens by heating at 55°C for 30 s, 60°C for 15 s or 70°C for 5 s (Fujino *et al.*, 2002).

Beef carcasses are often washed with water before chilling to removal visible dirt. Depending on the temperature of the water used, this may also have an anti-microbial effect. A study by Duffy *et al.* (2003) investigated the survival of *C. parvum* oocysts on lean beef (knuckle) following thermal treatments at 60, 75 and 82°C with a view to establishing the effect of a hot-water carcass washing procedure on the survival of oocysts. The results indicate that at 60°C oocyst viability dropped gradually from 83.5% after 15 s to 64.2% after 1 min. After 15 s at 75°C there was a large loss in viability (53.7%), which continued to decrease, reaching 11.2% after treatment for 1 min. The final treatment of 82°C proved highly effective in inactivating oocysts, with a survival of only 17.9% after 15 s, dropping to zero survival following treatment for 1 min. Cell lines were also used in this study to assess the infectivity of the oocysts recovered from the beef surfaces. This study observed that agreement between a high viability (>50%) and infection of the cell lines did not always occur. After heat treatment at 60°C for 45 s, 71.6% of recovered oocysts were determined to be viable by vital staining, but 0% of recovered oocysts were able to establish infection in HCT-8 cell monolayers. This is in agreement with studies that state that the vital dye assay consistently overestimates the viability of an oocyst suspension following treatment. Care must therefore be taken when interpreting vital dye assay results.

The results from this study indicate that adequate cooking times will inactivate *C. parvum*. The parasite is less resistant than most food-poisoning bacteria, so industrial processes such as pasteurization and cooking that are in place to inactivate bacteria are sufficient to inactivate this parasite.

Hydrostatic pressure

Slifko *et al.* (2000) studied the effects of high hydrostatic pressure (HHP) on *C. parvum* oocysts. By exposing foods to pressure for a short time, foods can achieve the benefits of pasteurization without the undesirable effects of heat (changes in texture, flavor and color). Oocysts were suspended in apple and orange juice and HHP-treated at 5.5×10^8 Pa (80 000 p.s.i.) for 0, 30, 45, 60, 90 and 120 s and viability was assessed by excystation using bile salts trypsin while the cell culture foci detection (FDM-MPN) method was used to assess infectivity. Results indicated that HHP inactivated *C. parvum* oocysts by at least 3.4 log 10 after 30 s of treatment. No infectivity was detected in samples exposed to ≥60 s of HHP and >99.995% inactivation was observed.

Conclusion

Cryptosporidium parvum is frequently present in animal wastes and can persist in these matrices for considerable periods of time, posing a risk of transmission to humans via direct contact from handling animals or fecal material, or the handling or consumption of contaminated produce. There is a need to develop practical and economical treatments to reduce/eliminate pathogens from animal manures/slurries, especially when destined for use in fruit and vegetable production. The sensitivity of the oocysts to high temperatures, freezing, drying and pH variation may also be exploited in the treatment of stored slurries and other animal wastes (e.g. application of lime, raising the ammonia concentration or adding acids), but this has to be in conjunction with knowledge and awareness of the effect of the treatments on other pathogens (particularly bacteria) present in the waste. These data should be incorporated into risk analysis models and used to develop recommendations for the storage, treatment and land application of animal wastes.

Evidence is now growing to support the theory that *Cryptosporidium* can enter and persist in the food chain in a similar fashion to well-recognized bacterial food-borne pathogens (*Salmonella*, *Campylobacter* and *E. coli* O157:H7, etc.). A number of studies have now reported the parasite as a natural contaminant in foods, including salad/vegetable crops and shellfish, and in the beef abattoir, as catalogued above. However, much of this information has been generated from snapshot studies in narrow geographical areas. Further research and surveillance on the pathogen conducted in a cohesive manner as part of national surveillance studies are now necessary to build up a clear picture of the presence, numbers and transmission routes for *C. parvum* in the food chain. This can be achieved using the newly developed protocols for the examination of food samples. Further research will be needed to refine and improve

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the recovery rates (consistency and percentage recovery) achievable. This is critical because without the classical bacterial enrichment step the parasite may be present in food at numbers which are at or below the threshold of detection limits for the methods, but still at levels sufficient to cause illness. Research and surveillance studies should incorporate an assessment of both the viability/infectivity of the oocysts and the numbers present, as this is essential information in generating an exposure assessment of the parasite that can be used in developing quantitative risk assessment models for this parasite and for use as part of the risk analysis approach to control this emergent pathogen in the food chain.

References

Angus KW, Tzipori S and Gray EW (1982). Intestinal lesions in specific pathogen free lambs associated with a *Cryptosporidium* from calves with diarrhea. *Veterinary Pathology* **19**: 67–78.

Armon R, Gold D, Brodsky M and Oron G (2002). Surface and subsurface irrigation with effluents of different qualities and presence of *Cryptosporidium* oocysts in soil and crops. *Water Science and Technology* **46**: 115–120.

Arrowood MJ and Sterling CR (1987). Isolation of *Cryptosporidium* oocysts and sporozoites using discontinuous sucrose and isopycnic gradients. *Journal of Parasitology* **73**: 314–319.

Baeumner AJ, Humiston MC, Montagna RA and Durst RA (2001). Detection of viable oocysts of *Cryptosporidium parvum* following nucleic acid sequence based amplification. *Analytical Chemistry* **73**: 1176–1180.

Barrick RS, Mohammed HQ, White ME and Bryant RB (2003). Factors associated with the likelihood of *Giardia* and *Cryptosporidium* spp. in soil from dairy farms. *Journal of Dairy Science* **86**: 784–791.

Bayer MR and Wright AE (1990). *Cryptosporidium* and water. *Letters in Applied Microbiology* **11**: 272–277..

Bier JW (1991). Isolation of parasites on fruits and vegetables. *Southeast Asian Journal of Tropical Medicine in Public Health* **22** (Suppl.): 144–145.

Bonnin A, Fourmaux MN, Dubremetz JF, Nelson RG, Gobet P, Harly G, Buisson M, Puygauthier-Toubas D, Gabriel-Pospisil F, Naciri M and Camerlynck P (1996). Genotyping human and bovine isolates of *Cryptosporidium parvum* by polymerase chain reaction-restriction fragment length polymorphism analysis of a repetitive DNA sequence. *Microbiology Letters* **137**: 207–211.

Bujoczek G, Reiners RS and Olaszkiewicz JA (2001). Abiotic factors affecting inactivation of pathogens in sludge. *Water Science and Technology* **44**: 10, 79–84.

Bukhari Z, Marshall MM, Korich DG, Fricker CR, Smith HV, Rosen J and Clancy JL (2000). Comparison of *Cryptosporidium parvum* viability and infectivity assays following ozone treatment of oocysts. *Applied and Environmental Microbiology* **66**: 2972–2980.

Cacciù S, Homan H, Camilli R, Traldi G, Kortbeek T and Pozio E (2000). A microsatellite marker reveals population heterogeneity within human and animal genotypes of *Cryptosporidium parvum*. *Parasitology* **120**: 237–244.

Cacciù S, Spano F and Pozio E (2001). Large sequence variation at two microsatellite loci among zoonotic (genotype C) isolates of *Cryptosporidium parvum*. *International Journal for Parasitology* **31**: 1082–1086.

Cai J, Collins MD, McDonald V and Thompson DE (1992). PCR cloning and nucleotide sequence determination of the 18S rRNA genes and internal transcriber spacer 1 of the protozoan parasites *Cryptosporidium parvum* and *Cryptosporidium muris*. *Biochimica et Biophysica Acta* **1131**: 317–320.

Campbell I, Tzipori S, Hutchinson G and Angus KW (1982). Effects of disinfectants on survival of *Cryptosporidium* oocysts. *Veterinary Record* **111**: 414–415.

Casemore DP (1989). Sheep as a source of human cryptosporidiosis. *Journal of Infection* **19**: 101–104.

Casemore DP (1991). Laboratory methods for diagnosing cryptosporidiosis. *Clinical Pathology* **44**: 445–451.

Casemore DP, Jessop EG, Douce D and Jackson FB (1986). *Cryptosporidium* plus *Campylobacter*: an outbreak in a semi-rural population. *Journal of Hygiene (London)* **96**: 95–105.

Cassin MH, Lammerding AM, Todd EC, Ross W and McColl RS (1998). Quantitative risk assessment for *Escherichia coli* in ground beef hamburgers. *International Journal of Food Microbiology* **41**: 21–44.

CDC (Center for Disease Control) (1982). Cryptosporidiosis: assessment of males with acquired immune deficiency syndrome (AIDS). *Morbidity and Mortality Weekly Report* **31**: 589–592.

CDC (Center for Disease Control and Prevention) (1996). Foodborne outbreak of diarrheal illness associated with *Cryptosporidium parvum* Minnesota 1995. *Morbidity and Mortality Weekly Report* **45**: 783–784.

CDC (Center for Disease Control and Prevention) (1997). Foodborne outbreak of cryptosporidiosis Spokane Washington, 1997. *Morbidity and Mortality Weekly Report* **73**: 353–355.

Cook N (2003). The use of NASBA for the detection of microbial pathogens in food and environmental samples. *Journal of Microbiological Methods* **53**: 165–174.

Current WL, Upton SJ and Hanes TB (1986). The life cycle of *Cryptosporidium baileyi* n. sp. (Apicomplexa: Cryptosporidiidae) infecting chickens. *Journal of Protozoology* **33**: 289.

Current WL and Garcia LS (1991). Cryptosporidiosis. *Clinical Microbiology Reviews* **4**: 325–358.

Current WL and Reese NC (1986). A comparison of endogenous development of three isolates of *Cryptosporidium* in suckling mice. *Journal of Protozoology* **33**: 98–108.

D·Antonio RG, Winn RE, Taylor JP, Gustafson TL, Current WL, Rhodes MM, Gray GW and Zadac RA (1985). A waterborne outbreak of cryptosporidiosis in normal hosts. *Annals of Internal Medicine* **103**: 886–888.

Deng MQ and Cliver DO (1999). *Cryptosporidium parvum* studies with dairy products. *International Journal of Food Microbiology* **46**: 113–121.

Deng MQ and Cliver DO (2000). Comparative detection of *Cryptosporidium parvum* oocysts from apple juice. *International Journal of Food Microbiology* **54**: 155–162.

Duffy G, McEvoy JM, Moriarty EM and Sheridan JJ (2003). A study of *Cryptosporidium parvum* in beef. Teagasc Project Report RMIS 4723. Dublin: Teagasc, National Food Centre.

Dworkin MS, Goldman DP, Wells TG, Kobayashi JM and Herwaldt BL (1996). Cryptosporidiosis in Washington State: an outbreak associated with well water. *Journal of Infectious Diseases* **174**: 1372–1376.

Elsser KA, Moricz M and Proctor EM (1986). *Cryptosporidium* infections: a laboratory survey. *Canadian Medical Association Journal* **135**: 211–213.

Enemark HL, Ahrens P, Lowery CJ, Thamsborg SM, Enemark JMD, Billie-Hansen V and Lind P (2002). *Cryptosporidium andersoni* from a Danish cattle herd: identification and

https://doi.org/10.1079/AHRR200357 Published online by Cambridge University Press

preliminary characterization. *Veterinary Parasitology* **107**: 37–49.

Enemark HL, Juel CD and Cacciù S (2003). Effects of environmental conditions on *Cryptosporidium* oocysts viability. In: Duffy G (editor). *Conference proceedings, Cryptosporidium parvum in Food and Water held at Grand Hotel Malahide, Dublin January 29th 20003*. Dublin: Teagasc, National Food Centre, pp. 36–47.

Ernest JA, Blagburn BL, Lindsay DS and Current WL (1986). Infection dynamics of *Cryptosporidium parvum* (Apicomplexa: Cryptosporidiidae) in neonatal mice (*Mus musculus*). *Journal of Parasitology* **72**: 796–798.

Ernst JV, Steward TB and Witlock DR (1987). Quantitative determinations of coccidia oocysts in beef calves from coastal plain area of Georgia (USA). *Veterinary Parasitology* **23**: 1–10.

Eurosurveillance Weekly (2003). *Cryptosporidium parvum* outbreak in swimming pool in Alcudia, Majorca, Spain. Eurosurveillance Weekly **7** (33). www.eurosurveillance.org/ew/2003.

Fayer R (editor) (1997). *Cryptosporidium* and cryptosporidiosis. NewYork: CRC Press.

Fayer R and Ungar LP (1986). *Cryptosporidium* spp. and cryptosporidiosis. *Microbiological Reviews* **50**: 458–483.

Fayer R, Trout JM, Graczyk TK and Lewis EJ (2000). Prevalence of *Cryptosporidium*, *Giardia* and *Eimeria* infections in post-weaned and adult cattle on three Maryland farms. *Veterinary Parasitology* **93**: 103–112.

Fayer R, Morgan U and Upton SJ (2000). Epidemiology of *Cryptosporidium*: transmission, detection and identification. *International Journal of Parasitology* **30**: 305–1322.

Fayer R, Trout JM, Lewis EJ, Xiao L, Lal A, Jenkins MC and Graczyk TK (2002). Temporal variability of *Cryptosporidium* in the Chesapeake Bay. *Parasitology Research* **88**: 998–1003.

Fayer R, Trout JM, Lewis EJ, Santin M, Zhou L, Lal AA and Xiao L (2003).Contamination of Atlantic coast commercial shellfish with *Cryptosporidium*. *Parasitology Research* **89**: 141–145.

Filkorn R, Wiedenmann A, Botzenhart K (1994). Selective detection of viable *Cryptosporidium* oocysts by PCR. *Zentralblatt für Hygiene und Umweltmedizin* **195**: 489–494.

Finch GR, Daniels CW, Black EK, Schaefer FW III and Belosevic M (1993). Dose response of *Cryptosporidium parvum* in outbred neonatal CD-1 mice. *Applied and Environmental Microbiology* **59**: 3661–3665.

Fournier S, Dubrou S, Liguory O, Gaussin F, Santillana-Hayat M, Sarfati C, Molian J M and Derouin F (2002). Detection of microsporidia, *Cryptosporidium* and *Giardia* in swimming pools: a one year prospective study. *FEMS Microbiology Letters* **33**: 209–213.

Friedman DE, Patten KA, Ose JB and Barney MC (1997). The potential for *Cryptosporidium parvum* oocyst survival in beverages associated with contaminated tap water. *Journal of Food Safety* **17**: 125–132.

Fujino T, Matsui T, Kobay Ash F, Haruki K, Yoshino Y, Kajima J and Tsui M (2002). The effect of heating against *Cryptosporidium* oocysts. *Journal of Veterinary Medical Science* **64**: 199–200.

Gatti S, Cevini C, Bruno A, Bernuzzi AM and Scaglia M (1993). Cryptosporidiosis in tourists returning from Egypt and the island of Mauritius. *Clinical Infectious Diseases* **16**: 344–345.

Gelletlie R, Stuart J, Soltanpoor N, Armstrong R and Nichols G (1997). Cryptosporidiosis associated with school milk. *Lancet* **350**: 1005–1006.

Gold D, Stein B and Tzipori S (2001). The utilization of sodium taurocholate in excystation of *Cryptosporidium parvum* and infection of tissue culture. *Journal of Parasitology* **87**: 997–1000.

Gomez-Bautista M, Ortega-Mora LM, Tabares E, Lopez-Rodas V and Costas E (2000). Detection of infectious *Cryptosporidium parvum* oocysts in mussels (*Mytilus galloprovincialis*) and cockles (*Cerastoderma edule*). *Applied and Environmental Microbiology* **66**: 1866–70.

Gùmez-Couso H, Freire-Santos F, Martinez-Urtaza J, Garcia-Martin O and Ares-Mazas ME (2003). Contamination of bivalve molluscs by *Cryptosporidium* oocysts: the need for new quality control standards. *International Journal of Food Microbiology* **87**: 97–105.

Hallier-Soulier S and Guillot E (2003). An immunomagnetic separation-reverse transcription polymerase chain reaction (IMS-RT-PCR) test for sensitive and rapid detection of viable waterborne *Cryptosporidium parvum*. *Environmental Microbiology* **5**: 592–598.

Harp JA, Fayer R, Pesch BA and Jackson GJ (1996). Effect of pasteurization on infectivity of *Cryptosporidium parvum* oocysts in water and milk. *Applied and Environmental Microbiology* **62**: 2866–2868.

Harper CM, Cowell NA, Adams BC, Langley AJ and Wohlsem TD (2002). Outbreak of *Cryptosporidium* linked to drinking unpasteurised milk. *Communicable Diseases Intelligence* **26**: 449–450.

Hayes EB, Matte TD, O'Brien TR, McKinley TW, Logsdon GS, Rose JB, Ungar, BLP, Word DM, Pinsky PF, Cummings ML, Wilson MA, Long EG, Hurwitz ES and Juranek DD (1989). Large community outbreak of cryptosporidiosis due to contamination of a filtered water supply. *New England Journal of Medicine* **320**: 1372–1376.

Hoar BR, Atwill ER, Elmi C and Farver TB (2001). An examination of risk factors associated with beef cattle shedding pathogens of potential zoonotic. *Epidemiology and Infection* **127**: 147–155..

Hoover DM, Hoerr FJ, Carlton WW, Hinsman EJ and Ferguson HW (1981). Enteric cryptosporidiosis in a naso tang, Naso lituratus. *Journal of Fish Diseases* **4**: 25.

Iseki M (1979). *Cryptosporidium* felis sp. n. (Protozoa: Eimeriorina) from the domestic cat. *Japanese Journal of Parasitology* **28**: 285.

Jenkins MB, Bowman DD and Ghiorse WC (1998). Inactivation of *Cryptosporidium* oocysts by ammonia. *Applied and Environmental Microbiology* **64**: 784–788..

Kato S, Jenkins MB, Fogarty EA and Bowman DD (2002). Effects of freeze-thaw events on the viability of *Cryptosporidium parvum* oocysts in soil. *Journal of Parasitology* **88**: 718–722.

Kramer MH, Sorhage FE, Goldstein ST, Dalley E, Wahlquist SP and Herwaldt BL (1997). First reported outbreak in the United States of cryptosporidiosis associated with a recreational lake. *Clinical Infectious Diseases* **26**: 27–33.

Laberge I and Griffiths MW (1996). Prevalence, detection and control of *Cryptosporidium parvum* in food. *International Journal of Food Microbiology* **31**: 1–26.

Levine ND (1980). Some corrections of coccidian (Apicomplexa: Protozoa) nomenclature. *Journal of Parasitology* **66**: 830.

Levine ND (1986). The taxonomy of *Sarcocystis* (Protozoa, Apicomplexa) species. *Journal of Parasitology* **72**: 372–382.

Lindergard G, Nydam DV, Wade SE, Schaaf SL and Mohammed HO (2003). A novel multiplex polymerase chain reaction approach for detection of four human infective *Cryptosporidium* isolates: *Cryptosporidium parvum*, types H and C, *Cryptosporidium* canis, and *Cryptosporidium* felis in fecal and soil samples. *Journal of Veterinary Diagnostic Investigation* **15**: 262–277.

Lindquist HD, Bennett JW, Ware M, Stetler RE, Gauci M and Schaefer FW (2001). Testing methods for detection of *Cryptosporidium* spp. in water samples. *Southeast Asian Journal of Tropical Medicine and Public Health* **32** (Suppl 2): 190–194.

Lindsay DS and Blagburn BL (1991). *Cryptosporidium parvum* infection of swine. *Compendium* **13**: 891–895.

Lindsay DS, Upton SJ, Owens DS, Morgan UM, Mead JR and Blagburn BL (2000). *Cryptosporidium* andersoni n. sp. (Apicomplexa: Cryptosporiidae) from cattle, Bos taurus. *Journal of Eukaryotic Microbiology* **47**: 91–95.

Lopez-Velez R, Tarazona R, Garcia-Camacho A, Gomez-Mampaso E, Guerrero A, Moreira V and Villanueva R (1995). Intestinal and extraintestinal cryptosporidiosis in AIDS patients. *European Journal of Clinical Microbiological and Infectious Diseases* **14**: 677–681.

Lorenzo-Lorenzo MJ, Ares-Mazas E and Villacorta-Martinez de Maturana I (1993). Detection of oocysts and IgG antibodies to *Cryptosporidium parvum* in asymptomatic adult cattle. *Veterinary Parasitology* **47**: 9–15.

Lowery CJ, Moore JE, Millar BC, Burke DP, McCorry KAJ, Crothers E and Dooley JSG (2000). Detection and speciation of *Cryptosporidium* spp. in environmental water samples by immunomagnetic separation, PCR and endonuclease restriction. *Journal of Medical Microbiology* **49**: 779–785.

Lowery CJ, Nugent P, Moore JE, Millar BC, Xiru X and Dooley JSJ (2001). PCR-IMS detection and molecular typing of *Cryptosporidium parvum* recovered from a recreational river source and an associated mussel (*Mytilus edulis*) bed in Northern Ireland. *Epidemiology and Infection* **127**: 545–553.

MacKenzie WR, Hoxie NJ, Proctor ME, Gradus MS, Blair KA, Peterson DE, Kazmierczak DE, Addiss DG, Fox KR, Rose JB and Davis JP (1994). A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *New England Journal of Medicine* **331**: 161–167.

Maillot C, Favennec L, Francois A, Ducrotte P and Brasseur P (1997). Sexual and asexual development of *Cryptosporidium parvum* in five oocyst or sporozoite-infected human enterocytic cell lines. *Journal of Eukaryotic Microbiology* **44**: 582–585.

Mann ED, Nayar GPS and Koschik C (1986). Infection with *Cryptosporidium* spp. in humans and cattle in Manitoba. *Canadian Journal of Veterinary Research* **50**: 174–178.

McDonough SP, Stull CL and Osburn BI (1994). Enteric pathogens in intensively reared veal calves. *American Journal of Veterinary Research* **55**: 1516–1520.

McEvoy JM, Moriarty EM, Duffy G and Sheridan JJ (2003). The role of beef in the transmission of *Cryptosporidium parvum*. In: Duffy G (editor). *Conference proceedings, Cryptosporidium parvum in Food and Water, Grand Hotel Malahide, Dublin January 29th 2003*. Dublin: Teagasc, National Food Centre, pp. 25–26.

Meisel JL, Perera DR, Meligro C and Rubin CE (1976). Overwhelming watery diarrhea associated with a *Cryptosporidium* in an immunosuppressed patient. *Gastroenterology* **70**: 1156–1160.

Meutin DJ, Van Kruiningen HJ and Kein DH (1974). Cryptosporidiosis in a calf. *Journal of the American Veterinary Medicine Association* **165**: 914–914.

Millar BC, Finn M, Xiao L, Lowery CJ, Dooley JS and Moore JE (2002). *Cryptosporidium* in foodstuffs an emerging aetiological route of human foodborne illnesses. *Trends in Food Science and Technology* **13**: 168–187.

Millard PS, Gensheimer KF, Addiss DG, Sosin DM, Beckett GA, Houck-Janoski A and Hudson A (1994). An outbreak of cryptosporidiosis from freshly pressed apple cider. *Journal of the American Medical Association* **272**: 1592–1596.

Monge R and Chinchilla M (1996). Presence of *Cryptosporidium* oocysts in fresh vegetables. *Journal of Food Protection* **46**: 202–203.

Moriarty EM, McEvoy JM, Duffy G, Sheridan JJ, McDowell DA and Blair IS (2003a). Development of a novel method for isolating and detecting *Cryptosporidium parvum* from lean and fat beef carcass surfaces. *Food Microbiology*, in press.

Moriarty EM, McEvoy JM, Duffy G, Lowery CJ, Thompson HP, Finn M, Sheridan JJ, Blair IS and McDowell DA (2003b). The prevalence of *Cryptosporidium* on beef tissue and in animal faeces at slaughter. *Veterinary Record*, in press.

Morgan-Ryan UM, Fall A, Ward LA, Hijjawi N, Sulaiman I, Fayer R, Thompson RC, Olson M, Lal A and Xiao L (2002). *Cryptosporidium hominis* n. sp. (Apicomplexa: Cryptosporidiidae) from *Homo sapiens*. *Journal of Eukaryotic Microbiology* **49**: 433–40.

MMWR (1982). Cryptosporidiosis: assessment of chemotherapy of males with acquired immune deficiency syndrome (AIDS). *Morbidity and Mortality Weekly Report* **31**: 589–592

Morgan D, Allaby M, Crook S, Casemore D, Healing TD, Soltanpoor N, Hill S and Hooper W (1995). Waterborne cryptosporidiosis associated with a borehole supply. *Communicable Disease Report CDR Review* **5** (7): R93-R97.

Morgan UM, Monis P, Xiao L, Limor J, Sulaiman IM, Raidal S, O·Donoghue P, Gasser R, Murray A, Fayer R, Blagburn BL, Lal AA and Thompson RCA (2001). Molecular and phylogenetic characterisation of *Cryptosporidium* from birds. *International Journal of Parasitology* **31**: 289–296**.**

Naciri M, Lefay MP and Mancassola R (2000). Role of *Cryptosporidium parvum* as a pathogen in neonatal diarrhea complex in suckling and dairy calves in France. *Veterinary Parasitology* **85**: 245–257.

Nichols G and Thom BT (1985). Food poisoning caused by *Cryptosporidium*: a load of tripe. *Communicable Disease Report* **85**: 3.

Nime FA, Burek JD, Page DL, Holscher MA and Yardley JH (1976). Acute enterocolitis in a human being infected with the protozoan *Cryptosporidium*. *Gastroenterology* **70**: 592–598.

O·Donoghue PJ (1995). *Cryptosporidium* and cryptosporidiosis in man and animals. *International Journal for Parasitology* **25**: 139–195.

Orlandi PA, Chu DMT, Bier JW and Jackson GJ (2002). Parasites and the food supply. *Food Technology* **56**: 72–81.

Ortega YR, Roxas CR, Gilman RH, Miller NJ, Cabrera L, Taquiri C and Sterling CR (1997). Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from vegetables collected in markets of an endemic region in Peru. *American Journal of Tropical Medicine and Hygiene* **57**: 683–686.

Panciera RJ, Thomassen RW and Garner FM (1971). Cryptosporidial infection in a calf. *Veterinary Parasitology* **8**: 479–484.

Peng MM, Xiao L, Freeman AR, Arrowood MJ, Escalante AA, Weltman AC, Ong CSL, MacKenzie WR, Lal AA and Beard CB (1997). Genetic polymorphism among *Cryptosporidium parvum* isolates: evidence of two distinct human transmission cycles. *Emerging Infectious Diseases* **3**: 567–573.

Preiser G, Preiser L and Madeo L (2003). An outbreak of cryptosporidiosis among veterinary science students who work with calves. *Journal of American College Health* **51**: 213–215.

Quiroz ES, Bern C, MacArthur JR, Xiao L, Fletcher M, Arrowood MJ, Shay DK, Levy ME, Glass RI and Lal A (2000). An outbreak of cryptosporidiosis linked to a foodhandler. *Journal of Infectious Disease* **181**: 695–700.

Richardson AJ, Frankenberg RA, Buck AC, Selkon JB, Colbourne JS, Parsons JW and Mayon-White RT (1991). An outbreak of waterborne cryptosporidiosis in Swindon and Oxfordshire. *Epidemiology and Infection* **107**: 485–495.

Robertson LJ and Gjerde B (2000). Isolation and enumeration of *Giardia* cysts, *Cryptosporidium* oocysts, and *Ascaris* eggs from fruit and vegetables. *Journal of Food Protection* **63**: 775–778.

Robertson LJ and Gjerde B (2001a). Factors affecting recovery efficiency in isolation of *Cryptosporidium* oocysts and *Giardia* cysts from vegetables for standard method development. *Journal of Food Protection* **64**: 1799–1805.

Robertson LJ and Gjerde BK (2001b). Occurrence of parasites on fruits and vegetables in Norway. *Journal of Food Protection* **64**: 1793–1798.

Robertson LJ, Campbell AT and Smith HV (1992). Survival of *Cryptosporidium parvum* oocysts under various environmental pressures. *Applied and Environmental Microbiology* **58**: 3494–3500.

Robertson LJ, Johannessen GS, Gjerde BK and Loncarevic S (2002). Microbiological analysis of seed sprouts in Norway. *International Journal of Food Microbiology* **75**: 119–126.

Rochelle PA, Marshall MM, Mead JR, Johnson AM, Korich DG, Rosen JS and de Leon R (2002). Comparison of *in vitro* cell culture and a mouse assay for measuring infectivity of *Cryptosporidium parvum*. *Applied and Environmental Microbiology* **68**:3809–3817.

Rose JB, Huffman DE and Gennaccaro A (2002). Risk and control of waterborne cryptosporidiosis. *FEMS Microbiology Letters* **26**: 113–123.

Sayers GM, Dillion MC, Connolly E, Thornton L, Hyland E, Loughman E, O·Mahony MA and Butler KM (1996). Cryptosporidiosis in children who visited an open farm. *Communicable Disease Report* **6**: 140–144.

Schets FM, van den Berg HHJL, Verschoor F, Engels GB, Lodder WJ, van Pelt-Heerschap HML, van der Giessen JWB, de Roda Husman AM and van der Poel WHM (2003). Detection of *Cryptosporidium* and *Giardia* in Portuguese oysters (*Crassostrea gigas*) grown in the Oosterschelde, the Netherlands. In: Duffy G (editor). *Conference proceedings, Cryptosporidium parvum in Food and Water held at Grand Hotel Malahide, Dublin January 29th 2003*. Dublin: Teagasc, National Food Centre, p. 21

Scott CA, Smith HV and Gibbs HA (1994). Excretion of *Cryptosporidium parvum* oocysts by a herd of beef suckler cows. *Veterinary Record* **134**: 172.

Scott CA, Smith HV, Mtambo MMA and Gibbs HA (1995). An epidemiological study of *Cryptosporidium parvum* in two herds of adult beef cattle. *Veterinary Parasitology* **57**: 277–288.

Sharpe AN, Hearn EM and Kovacs-Nolan J (2000). Comparison of membrane filtration rates and hydrophobic grid membrane filter coliform and *Escherichia coli* counts in food suspensions using paddle-type and pulsifier sample preparation procedures. *Journal of Food Protection* **63**: 126–130.

Slifko TR, Friedman D, Rose JB and Jakubowski W (1997). An in vitro method for detecting infectious *Cryptosporidium* oocysts with cell culture. *Applied and Environmental Microbiology* **63**: 3669–3675.

Slifko TR, Coulliette A, Huffman DE and Rose JB (2000). Impact of purification procedures on the viability and infectivity of *Cryptosporidium parvum* oocysts *Water Science and Technology* **41**: 23–29.

Slavin D (1955). *Cryptosporidium meleagridis* (sp. nov.). *Journal of Comparative Pathology* **65**: 262.

Smith HV, Girdwood RW, Patterson WJ, Hardie R, Green LA, Benton C, Tulloch W, Sharp JC and Forbes GI (1988). Waterborne outbreak of cryptosporidiosis. *Lancet* **2**: 8626–8627.

Spano F, Putigani L, McLauchlin J, Casemore DP and Crisanti A (1997). PCR-RFLP analysis of the *Cryptosporidium* oocysts wall protein (COWP) gene discriminates between *C. wrairi* and *C. parvum* isolates of human and animal origin. *Microbiology Letters* **150**: 209–217.

Sterling CR, Seegar K and Sinclair NA (1986). *Cryptosporidium* as a causative agent of traveller's diarrhea. *Journal of Infectious Diseases* **153**: 380–381.

Stinear T, Matusan A, Hines K and Sandery M (1996). Detection of a single viable *Cryptosporidium parvum* oocyst in environmental water concentrates by reverse transcription-PCR. *Applied and Environmental Microbiology* **62**: 3385–3390.

Svoboda IF, Read IA, Kemp JS, Wright SE, Coop RL, Mawdsley JL, Bargett RD, Merry RJ, Pain BF, Theodorou MK, Bukhari Z and Smith HV (1997). *Cryptosporidium* on cattle farms. In: *Cryptosporidium in water: the challenge to policy makers and water managers. Symposium of the Chartered Institute of Water and Environmental Management, Glasgow*, pp. 3–20.

Tyzzer EE (1910). An extracellular coccidium, *Cryptosporidium muris* (Gen. et. sp. nov.) of the gastric glands of the common mouse. *Journal of Medical Research* **23**: 487.

Tyzzer EE (1912). *Cryptosporidium parvum* (sp. nov.), a coccidium found in the small intestine of the common mouse. *Archiv für Protistenkunde* **26**: 394–412.

Tzipori S, Angus KW, Campbell I and Clerihew LW (1981). Diarrhea due to *Cryptosporidium* infection in artificially reared lambs. *Journal of Clinical Microbiology* **14**: 100–105.

Upton SJ, Tilley M and Brillhart DB (1994). Comparative development of *Cryptosporidium parvum* (Apicomplexa) in 11 continuous host cell lines. *FEMS Microbiology Letters* **118**: 233–236.

Upton SJ, Tilley M and Brillhart DB (1995). Effects of select medium supplements on in vitro development of *Cryptosporidium parvum* in HCT-8 cells. *Journal of Clinical Microbiology* **33**: 371–375.

Vetterling JM, Jervis HR, Merrill TG and Sprinz H (1971). *Cryptosporidium wrairi* sp. n. from the guinea pig *Cavia porcellus*, with an emendation of the genus. *Journal of Protozoology* **18**: 243–247.

Villacorta I, Ares-Mazas E and Lorenzo MJ (1991). *Cryptosporidium parvum* in cattle, sheep and pigs in Galicia (N.W. Spain). *Veterinary Parasitology* **38**: 249–252.

Warnes SW and Keevil CW (2003). Survival of *Cryptosporidium parvum* in faecal wastes and salad crops. In: Duffy G (editor). *Conference proceedings, Cryptosporidium parvum in Food and Water, held at Grand Hotel Malahide, Dublin January 29th 2003*. Dublin: Teagasc, National Food Centre, p.15.

WHO (1986). Cryptosporidiosis surveillance. *Weekly Epidemiology Record* **59**: 72–73. http://www.who.int/ctd/dracun/whodocs2.htm

Yu JR, Choi SD and Kim YW (2000). In vitro infection of *Cryptosporidium parvum* to four different cell lines. *Korean Journal of Parasitology* **38**: 59–64.

Zorana M, Sofija KR and Kulisic Z (2002). *Cryptosporidium* infection in weaners, bull calves and postparturient cows in the Belgrade area. *Acta Veterinaria* **52**: 37–42.

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