

Chapter 2

Epidemiology of Human Cryptosporidiosis

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Abstract *Cryptosporidium* species are protozoan parasites that infect the epithelial cells of the gastrointestinal tract of vertebrates. In humans, cryptosporidiosis is usually a self-limiting infection in immunocompetent individuals, but severe diarrhoea and dissemination to extra-intestinal sites can occur in high-risk individuals, such as the very young, the elderly and immunosuppressed individuals, particularly those with HIV infection. The oocyst, the infectious stage of *Cryptosporidium*, is immediately infectious upon excretion with the host faeces, which favours direct transmission. Oocysts have the capacity to persist in the environment and to withstand standard water treatment and some species of *Cryptosporidium*, particularly *C. parvum*, have a wide host range and can be transmitted to humans by direct contact with animals or through ingestion of water and food contaminated with oocysts. Due to the presence of multiple transmission routes, the epidemiology of cryptosporidiosis is complex. The investigation of sporadic cases and outbreaks of cryptosporidiosis has contributed to a better understanding of risk factors and infection sources. Genotyping techniques have enabled a better understanding of the epidemiology of cryptosporidiosis in different geographical, seasonal and socioeconomic context.

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2.1 Introduction

Cryptosporidia are obligate intracellular parasites of many species from all vertebrate classes. First described in laboratory mice by Tyzzer in 1912, *Cryptosporidium* was recognized as a cause of diarrheal disease in animals and then in humans during the 1970s. In the following decade, cryptosporidiosis emerged worldwide as a common cause of severe or life-threatening infection in immunocompromised patients, especially those with AIDS, and of acute, self-limiting gastroenteritis in otherwise healthy subjects, especially children (see Chap. 9).

In humans, infection is caused mainly by the zoonotic species *Cryptosporidium parvum*, which is highly prevalent in young livestock, and *Cryptosporidium hominis*, which is essentially a human parasite. With the development of improved genotyping methods, an increasing number of species and genotypes have been recognized as human pathogens, albeit with low prevalence (Putignani and Menichella 2010). The epidemiology of the infection involves both direct transmission from animals to humans or from person to person, as well as indirect transmission through ingestion of water and food contaminated with infectious oocysts (Cacciò et al. 2005; Smith et al. 2006b).

This chapter focuses on the epidemiology of human infections. The molecular epidemiology of human cryptosporidiosis is presented in Chap. 3.

2.2 Life Cycle of *Cryptosporidium*

The *Cryptosporidium* life cycle is represented in Fig. 2.1, and the major phases are briefly described here. The life cycle of the parasite is completed within a single host (i.e., a monoxenous cycle), and involves both asexual and sexual replication. For a more detailed account, the readers are referred to previously published articles (Chen et al. 2002; Smith et al. 2005; Fayer 2008).

Excystation: after ingestion of oocysts by a susceptible host, the first step towards infection is excystation, the process by which the oocyst wall opens along a suture to allow the release of four infectious sporozoites. This process has been studied under in vitro conditions and several factors that mimic the transit through the acidic stomach to the alkaline small intestine have been shown to enhance excystation. In particular, temperature and pH appear to be the most important triggers. *Cryptosporidium* species that infect the stomach of the host (like *Cryptosporidium muris*) respond more rapidly to those triggers compared to species that infect the intestine of the host, indicating the need of gastric species to rapidly excyst and release the sporozoites upon ingestion (Widmer et al. 2007). Therefore, the role of host derived triggers during the excystation process varies for different *Cryptosporidium* species (Smith et al. 2005).

Attachment and invasion: like other apicomplexans, *Cryptosporidium* possesses an apical complex formed by the apical ring, the conoid and secretory

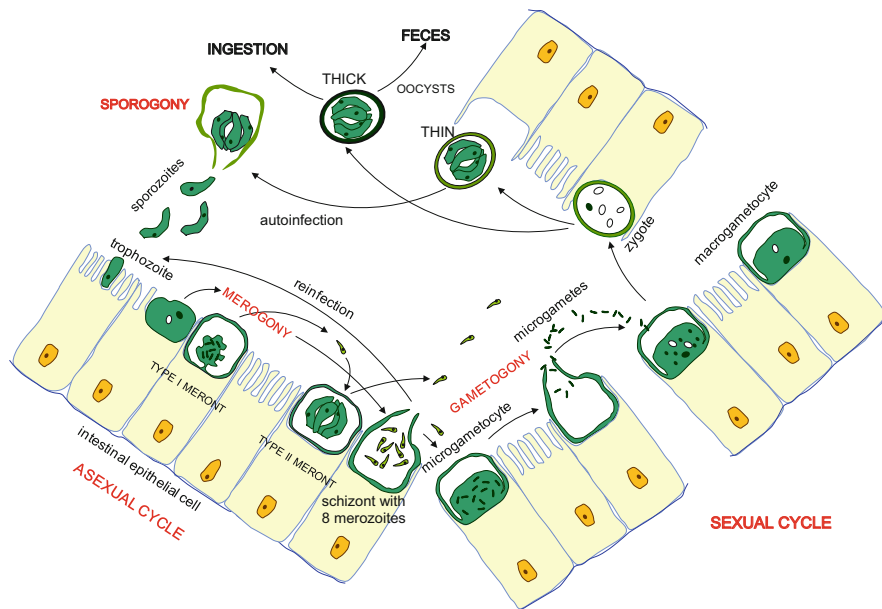


Fig. 2.1 Life cycle of parasite. The infection is acquired through the ingestion of sporulated oocysts. Motile sporozoites, from opened oocyst, attach to intestinal epithelial cells. The trophozoite undergoes an asexual replication (merogony), resulting in the production of eight merozoites (type I meronts). Merozoites, released into the intestinal lumen, infect new intestinal epithelial cells, and originate type II meronts, characterized by four merozoites. The merozoites can undergo a sexual cycle (gametogony) and develop into macrogametocytes. The microgametocyte produces numerous microgametes which are released into the intestinal lumen. A microgamete will fuse with a macrogamete and the resulting zygote undergoes sporogony. Fully sporulated thick and thin oocysts are shed into the intestinal lumen at the completion of sporogony. The infectious thick oocysts are excreted in the feces, thus completing the life cycle. An autoinfection in which excystation takes place within the same host may also be possible and is mediated by ‘thin-walled’ oocysts

organelles (a single rhoptry and micronemes). The apical complex is intimately involved in the process of attachment and invasion of host cells (Borowski et al. 2008). When the sporozoite contacts the host cell membrane, the rhoptry extends to the attachment site, while micronemes and dense granules move to the apical region. The content of secretory organelles is released, which triggers the process of recognition and attachment to the host cell, invasion, and formation of the parasitophorous vacuole in which the parasite replicates. The sporozoites within the parasitophorous vacuole are not directly in contact with the host cell, and occupy a unique intracellular but extracytoplasmic niche, typical of *Cryptosporidium* parasites (also named parasitophorous sac, Valigurová et al. 2008). A complex process at the host-parasite interface leads to the formation of the so-called feeder organelle, which is thought to be essential in salvaging nutrients and directly separates the parasite cytoplasm from the affected host cell (Umemiya

et al. 2005). As the internalization process progresses, the sporozoite differentiates into a spherical trophozoite.

Asexual reproduction: merogony is characterized by the division of the trophozoite nucleus and cytoplasm to generate meronts. In *C. parvum*, there are two types of meronts, type I and type II. Type I meronts have six or eight nuclei. Each gives rise to a merozoite, which is structurally similar to the sporozoite. Mature merozoites leave the meront and invade other host cells, where they can develop into another Type I or into a Type II meront. Type II meronts have four nuclei and generate four merozoites.

Sexual reproduction: upon infection of new host cells, merozoites from Type II meronts can initiate the sexual phase by differentiating into either a microgamont or a macrogamont. Nuclear division in the microgamont leads to the production of numerous microgametes (equivalent to sperm cells) that are released from the parasitophorous vacuole. Each macrogamont (equivalent to an ovum) may be fertilized by a microgamete. The product of fertilization, the zygote, develops into an oocyst.

Sporogony: the zygote differentiates into four sporozoites (sporogony) within the oocyst. It is thought that fully sporulated oocysts are released into the lumen of the intestine and pass out of the body with the faeces, where they are immediately infectious for other susceptible hosts. Oocysts that sporulate in the respiratory tract are found in nasal secretions and sputum (Mor et al. 2010). *Cryptosporidium* oocysts are spherical, measuring only 3–6 µm in diameter. Each contains four haploid sporozoites and possesses a thick wall.

It is believed that some of the oocysts possess a thin wall and can cause autoinfection in the same parasitized host by liberating their sporozoites in the gut lumen. The process of autoinfection is believed to occur only in species of *Cryptosporidium* and *Caryospora*. Upon their release, sporozoites undergo the developmental processes of schizogony, gametogony and sporogony in enterocytes of the same infected host. Therefore, the life cycle of *Cryptosporidium* ensures the production of very large numbers of infective oocysts, due to the recycling of merozoites to produce further type I generations of schizonts, and the endogenous re-infection from thin-walled oocysts.

2.3 *Cryptosporidium* Species Infecting Humans

The first human cases of cryptosporidiosis were reported in 1976 (Nime et al. 1976; Meisel et al. 1976). The patients were a 3-year-old child and a 39-year old individual who had severe bullous pemphigoid (a skin disease characterized by blisters) and received treatment with cyclophosphamide and prednisolone. Both patients lived on a farm with cattle and had a dog. They presented with severe watery diarrhea. Diagnosis was based on microscopic examination (including electron microscopy) of rectum and jejunal biopsy specimens. At that time, identification at the level of species was not possible, and infections were attributed to

Cryptosporidium spp. In 1978, it was shown that oocysts are shed with the feces of infected hosts (Pohlenz et al. 1978) and since then, the diagnosis of cryptosporidiosis has been based on the demonstration of oocysts in feces.

A series of experiments performed using parasite isolates from different hosts (a calf, a lamb, a human and a deer) showed that *Cryptosporidium* could be transmitted to newborn animals (mice, rats, guinea pigs, piglets, calves and lambs), thus demonstrating a lack of host specificity and underlining a zoonotic potential (Tzipori et al. 1980). It was therefore argued that a single species, *C. parvum*, was the causative agent of cryptosporidiosis in mammals, including man.

The role of *Cryptosporidium* as a serious human pathogen was firmly demonstrated in the 1980s in individuals with HIV/AIDS, who experienced a persistent and life threatening infection, often involving parasite dissemination to the hepatobiliary and the respiratory tracts in addition to the entire gastrointestinal (GI) tract (Ma and Soave 1983; Navin and Juranek 1984). The next major event that attracted worldwide interest towards the parasite was the 1993 massive waterborne outbreak in Milwaukee, Wisconsin, that involved an estimated 403,000 person (MacKenzie et al. 1995). This event demonstrated the ability of *Cryptosporidium* to resist water treatment and be transmitted through drinking water. Following these major events, molecular methods for the detection and identification of *Cryptosporidium* species on different matrices were actively developed and their application has dramatically changed our understanding of the taxonomy and epidemiology of *Cryptosporidium*.

In the early 1990s, the application of Southern blotting (Ortega et al. 1991), Western blotting (Nina et al. 1992) and isoenzymes profiles (Ogunkolade et al. 1993) provided the first evidence of genetic heterogeneity among *C. parvum* isolated from humans and livestock. These studies demonstrated for the first time that humans were infected with two types of *Cryptosporidium* parasites, one being apparently the same as found in cattle and the other exclusively found in humans. However, due to the large amount of biological material needed, these techniques have not been used to characterize field isolates, and have been largely replaced by DNA amplification techniques (PCR). Different assays, including PCR followed by restriction length fragment polymorphism (PCR-RFLP) (Ortega et al. 1991), PCR followed by sequencing (Morgan et al. 1997), Random Amplification of Polymorphic DNA (RAPD) (Morgan et al. 1995), and length polymorphisms of simple DNA repeats (Cacciò et al. 2000), were developed to investigate genetic heterogeneity among isolates.

A number of studies, based on the analysis of single or multiple genetic markers (e.g., Peng et al. 1997; Spano et al. 1998), confirmed the presence of two genetically distinct subgroups within *C. parvum*, which were referred in the literature to as 'human' and 'cattle,' H and C, or Type 1 and Type 2. This result was subsequently confirmed in many laboratories and demonstrated the existence of two distinct transmission cycles of human cryptosporidiosis, one comprising ruminants and humans (potentially zoonotic cycle) and the other exclusively comprising humans (solely anthroponotic cycle).

In 2002, on the basis of accumulating observations of genotypic and biological differences, a new species, *C. hominis*, was finally proposed for *C. parvum* parasites exclusively infecting humans (Morgan-Ryan et al. 2002; see Chap. 1 for the taxonomy of *Cryptosporidium*).

The increasing application of genotyping techniques in epidemiological surveys has demonstrated that the large majority (>90 %) of human cases of cryptosporidiosis are due to *C. hominis* and *C. parvum* (Xiao 2010). However, it is now clear that other species have the potential to cause infection in humans, including *Cryptosporidium meleagridis* and, more occasionally, *C. canis*, *C. felis*, *C. ubiquitum* and *C. viatorum*, and other *Cryptosporidium* genotypes of unknown taxonomic status. The prevalence of these less common species and genotypes varied geographically: for example, *C. meleagridis* was found to be as prevalent as *C. parvum* in children from Peru and in Thailand (Cama et al. 2008; see Chap. 3).

2.4 Incubation Period

Based on evidence from experimental infections, it has been estimated that the incubation period is between 5 and 7 days. Data from experimental infection of healthy volunteers (Okhuysen et al. 1996; Chappel et al. 2006, 2011), and investigations of waterborne and foodborne outbreaks are the main source of information.

Experimental infections of healthy volunteers have been performed using the three main human pathogens, namely *C. parvum*, *C. hominis* and *C. meleagridis*. For *C. parvum*, volunteers were challenged with different inocula (10 to >10,000 oocysts) of three strains of animal origin (Iowa, TAMU and UCP, MD, Okhuysen et al. 1996). Results showed that 12 (86 %) of 14 volunteers who received the TAMU isolate developed diarrhoea, as did 15 (52 %) of 29 who received the Iowa isolate and ten (59 %) of 17 who received the UCP isolate (Okhuysen et al. 1996). The mean incubation period was 7.7 days (for IOWA), 7 days (for UCP) and 4 days (for TAMU).

In the study with *C. hominis*, 21 healthy adults were challenged with 10–500 oocysts of the TU502 (Xu et al. 2004) isolate, and 13 developed diarrhoea, while nine had oocysts detected in faecal samples (Chappel et al. 2006). The mean incubation period was 5.4 days (range, 2–10 days). Finally, in the case of *C. meleagridis*, the study involved five volunteers, who were challenged with 10⁵ oocysts of the isolate TU1867 (Akiyoshi et al. 2003). The incubation period was of 5.3 days (range, 4–7 days) (Chappel et al. 2011).

During the large waterborne outbreak of cryptosporidiosis in Milwaukee, the mean incubation period was estimated to range from 3 to 7 days, but it appeared to be shorter in the elderly (5–6 days) compared to either children (7 days) or adults (8 days) (Naumova et al. 2003).

In 1996, another large waterborne outbreak occurred in Japan, and was caused by contamination of the town's potable water (Yamamoto et al. 2000). An

estimated number of 9,140 individuals were affected. The median incubation period for the 14 persons for whom this calculation was possible was 6.4 days (range, 5–8 days).

In 2002, an outbreak of cryptosporidiosis occurred among visitors to a public swimming pool in Sweden and affected an estimated with about 800–1,000 individuals (Insulander et al. 2005). The median incubation period was estimated at 5 days (range, 2–13 days).

Three investigations of foodborne cryptosporidiosis have also provided data on incubation period. In 1993, an outbreak linked to consumption of contaminated apple cider occurred in central Maine (Millard et al. 1994). The median incubation period was 6 days (range, 10 h to 13 days).

In 1997, an outbreak of acute gastroenteritis occurred among members of a group attending a dinner banquet catered by a restaurant in Spokane (Anon 1998). Foodborne transmission was implicated through a contaminated ingredient in multiple menu items. The incubation period was estimated between 3 and 9 days. In 2006, acute gastroenteritis caused by *C. parvum* was reported by four members from the same company who had eaten a raw meat dish called “Yukke: Korean-style beef tartar” and raw liver at a rotisserie in Sakai City, Japan (Yoshida et al. 2007). Based on information from interviews, the median incubation period was 5.5 (range, 5–7 days).

Further data comes from other investigations. A study of an outbreak of GI illness among a class of 96 undergraduate veterinary students in New Zealand (Grinberg et al. 2011) indicated a median incubation period of 5 days (range, 0–11 days).

Variability in the estimated duration of the latent period can be attributed to inaccuracies in the estimation of the time from exposure to the onset of symptoms, or to difference in inoculum size (Chappel et al. 2006), but may also reflect alterations in the incubation period due to partial immunity derived from prior exposure to the parasite. Phenotypic differences among parasite isolates may also explain the observed range.

2.5 Asymptomatic Infection

Little information is available on asymptomatic carriage of *Cryptosporidium* in humans. In studies of experimental infection of healthy volunteers with *C. parvum*, it was noted that some individuals passed oocysts in their stools in the lack of overt symptoms (diarrhoea), indicating that asymptomatic shedding of the parasite occurs (Okhuysen et al. 1999). In contrast, asymptomatic shedding was not seen in any of the volunteers experimentally challenged with *C. hominis* oocysts (Chappel et al. 2006).

A prospective study in the US found asymptomatic cryptosporidiosis in 12 of 78 (6.4 %) immunocompetent and 11 of 50 (22 %) immunodeficient children (Pettoello-Mantovani et al. 1995). By comparison, *Cryptosporidium* was found in

4.4 % of immunocompetent and 4.8 % of immunodeficient children of a control symptomatic population. A recent study in the UK screened 230 asymptomatic children in preschool day care centres, using a highly sensitive detection protocol based on immunomagnetic separation and DNA typing (Davies et al. 2009). The observed prevalence (1.3 %) was lower than that from the study of Pettoello-Mantovani et al. (1995), that was based on the less sensitive microscopic technique. However, the detection of *C. ubiquitum* and of the skunk genotype in those asymptomatic children raises the possibility that some species/genotypes may have low pathogenicity and be more common than previously thought.

In Scandinavian countries (Denmark, Sweden, Norway and Finland) a meta-analysis of asymptomatic and symptomatic cryptosporidiosis in adults showed a lower prevalence (0.99 %) in the former compared to the latter (2.99 %). In a community-based study in Melbourne, Australia, 1,091 faecal specimens from asymptomatic individuals were screened for the presence of bacteria, viruses and parasites (Hellard et al. 2000), and *Cryptosporidium* was found in four samples (0.4 %). The role of carriers in the transmission of *Cryptosporidium* was also suggested by a large-scale case-control study of sporadic cases in the United Kingdom (Hunter et al. 2004), that identified changing diapers as an independent risk factor for infection with *C. hominis*, even if the child did not have diarrhoea.

The prevalence of asymptomatic infection in other countries is only partially known. A study of 377 Aymara school students (5–19 years of age) from villages of the northern Bolivian Altiplano (Esteban et al. 1998) found a very high prevalence of *Cryptosporidium* (31.6 %) based on microscopy. The authors argued that the mild or asymptomatic infections observed in children were due to some level of immunity that develops after continuous exposure to the parasite.

In Jeddah, Saudi Arabia, a study of asymptomatic children from nurseries found that 9 of 190 (4.7 %) were positive for *Cryptosporidium*, as compared to 20 of 63 (32 %) children with diarrhoea from paediatric clinics of the same city (Al-Braiken et al. 2003). In Makkah, Saudi Arabia, *Cryptosporidium* was detected in 4 % of 589 stool samples from asymptomatic school children aged 7–12 years from 13 primary schools (Al-Harathi 2004). Finally, in a study of 276 asymptomatic aboriginal (Orang Asli) children (141 boys and 135 girls, aged 2–15 years) living in villages in the Malaysia (Al-Mekhlafi et al. 2011), *Cryptosporidium* was detected in 20 (7.2 %) children.

An interesting study aimed at determining the contribution of asymptomatic immigrants in the spreading of the disease in the Kashmir region (India), where cryptosporidiosis is not considered to be endemic. Analysis of stool samples from 45 non-diarrheic and nine diarrheic HIV-infected individuals revealed that all were carriers of *Cryptosporidium* spp. (Masarat et al. 2012). Remarkably, epidemiological traits revealed that the asymptomatic individuals were non-Kashmiri army personals and travellers (immigrants), while local emigrant merchants represented symptomatic cases. This suggests that the non-diarrheic HIV positive population may be a potential source of endemic spread of cryptosporidiosis, and that obligatory laboratory testing in HIV positive immigrant population, like merchants and

travellers, regardless of symptoms, should be mandatory to understand patterns of transmission.

2.6 Symptomatic Infection

The symptomatic period of infection is characterized by diarrhoea, abdominal pain, nausea or vomiting, mild fever, anorexia, malaise, fatigue and weight loss (Fayer and Ungar 1986; Casemore 1990). Diarrhoea can be of sudden onset and is generally watery and voluminous; between three and six stools (but sometimes many more) may be passed each day, which are sometimes offensive and may contain mucus. Symptoms usually last up to 3 weeks, but some patients experience chronic diarrhoea of a month or longer. Oocysts may continue to be shed for a mean period of 7 days (range 1–15 days) after symptoms have ceased, although exceptionally for up to 2 months (Jokipii and Jokipii 1986) (see Chap. 9).

2.7 Risk Factors

Our knowledge of risk factors for acquiring cryptosporidiosis is mainly derived from investigations of outbreaks, albeit the majority of human infection is sporadic. Outbreak investigations have demonstrated the existence of multiple transmission routes, including contact with infected animals, person-to-person transmission in households and care settings, consumption of contaminated foods and drinks, consumption of water from private and public supplies, exposure to recreational water in swimming pools or water parks, and travel to endemic countries (Nichols et al. 2009) (Fig. 2.2). It is important to stress that risk factors are likely to differ for *C. parvum* and *C. hominis* and in different geographical settings, due to the difference in host range, transmission cycles and prevalence. This underscores the importance of identifying species or genotype in epidemiologic studies. However, molecular typing has been used only in a few case–control studies, both from developed (Hunter et al. 2004; Lake et al. 2007) and developing countries (Cama et al. 2008; Molloy et al. 2011).

In industrialised countries, case–control studies have been conducted in the US (Roy et al. 2004), the UK (Hunter et al. 2004; Lake et al. 2007), and Australia (Robertson et al. 2002). In all these studies, statistically significant risk factors, identified by multivariate analysis, included contact with persons with diarrhoea, particularly young children, and contact with cattle, especially calves. Further, travel abroad was significantly associated with an increased risk in the US and UK studies (Roy et al. 2004; Hunter et al. 2004). History of travel abroad was excluded in the Australian study to allow focus on endemic disease (Robertson et al. 2002). In addition, the US study identified swimming in fresh water as a risk factor and the Australian study found swimming in a chlorinated swimming pool as

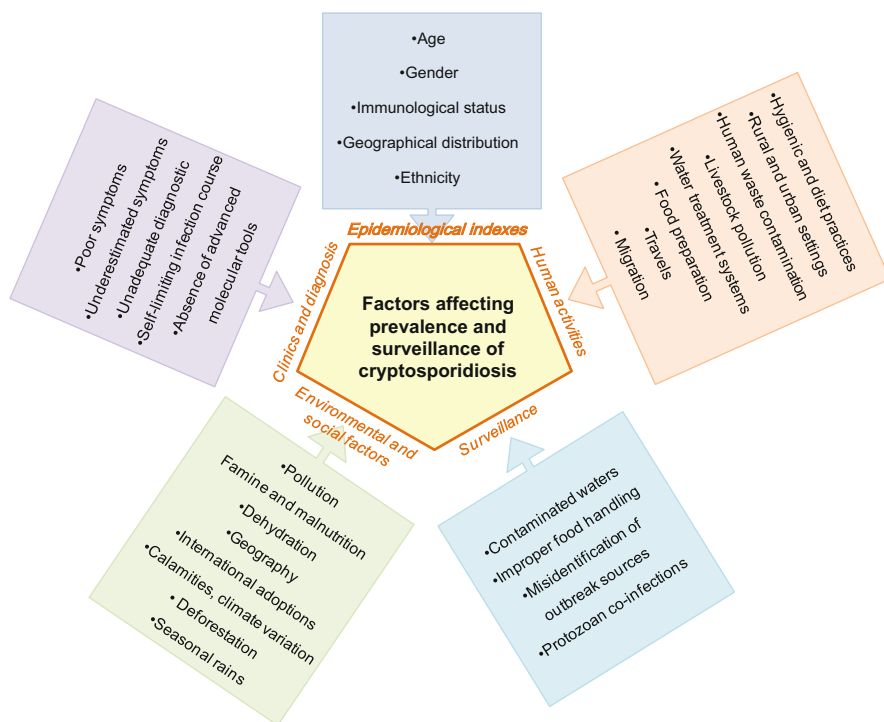


Fig. 2.2 Factors affecting prevalence and adequate surveillance of cryptosporidiosis

a risk factor. Both the UK and Australian studies identified a dose dependent risk associated with drinking unboiled water, which was also reported in a regional study in the UK (Goh et al. 2004). Negative associations with eating ice cream and raw vegetables was found in the UK and US studies.

A more recent study from the UK (Lake et al. 2007) investigated the role of wider environmental and socioeconomic factors (e.g. water supply, socioeconomic status, land use, livestock densities and healthcare accessibility) upon human cryptosporidiosis. By comparing 3,368 laboratory-confirmed cases to an equal number of controls, the authors concluded that risk factors for *C. hominis* and *C. parvum* must be considered separately. Indeed, for *C. hominis* cases the strongest risks factors were living in areas with many higher socioeconomic status individuals, living in areas with a high proportion of young children and living in urban areas. In contrast, agricultural land use surrounding the place of residence and the water supply were significant risk factors for *C. parvum* illness (Lake et al. 2007).

Despite the higher prevalence of cryptosporidiosis in developing and tropical countries, few studies have been conducted to identify risk factors in those areas. In Peru, a total of 156 cases of cryptosporidiosis were found in 109 of 553 children during a 4-year longitudinal birth cohort study (Cama et al. 2008). Investigation

into risk factors did not identify statistically significant associations between *Cryptosporidium* spp. and any of the variables considered (basic aspects of sanitation and zoonotic, foodborne, and waterborne transmission), possibly because children are exposed to these parasites through different transmission routes, which makes single exposure variables difficult to demonstrate (Cama et al. 2008) (Fig. 2.2).

In Indonesia, a study of 917 patients with acute diarrhea (715 in-patients and 202 out-patients from the Hospital of University of Airlanaga in Surabaya) found *C. parvum* oocysts in 26 (2.8 %) of the patients and in 15 (1.4 %) of 1,043 control patients. Investigation of risk factors by multiple logistic regression indicated that contact with pets (cats), rainy season, occurrence of a flood, and crowded living conditions were significant risk factors for cryptosporidiosis (Katsumata et al. 1998).

A 2-year study in the Nile River Delta in Egypt examined risk factors for cryptosporidiosis in children with diarrhea (Abdel-Messih et al. 2005). A prevalence of 17 % (241 of 1,275) was observed, and clinical findings included vomiting, persistent diarrhoea and the need for hospitalization. Children <12 months of age were 2.4 times more likely to be infected with *Cryptosporidium* ($p < 0.01$) and children 12–23 months were 1.9 ($p < 0.05$) times more likely to be infected with the organism as compared to older children. Breastfeeding had a trend towards protection against *Cryptosporidium*-associated diarrhoea ($p = 0.07$).

Finally, a study in Nigeria found that 134 of 692 children (19.4 %) were infected with *Cryptosporidium* spp. The study also provided information on the species for 49 isolates (Molloy et al. 2011). Using generalized linear mixed-effects models, risk factors were identified for all *Cryptosporidium* infections, as well as for *C. hominis* and *C. parvum* both together and separately. Malaria and absence of *Ascaris* infection were risk factors for all *Cryptosporidium* infections, whereas stunting and younger age were highlighted as risk factors for *C. hominis* infections. Stunting and malaria were identified as risk factors for *C. parvum* infection (Molloy et al. 2011).

Together, studies in developing countries identified several risk factors: age <2 years, absence of breastfeeding, contact with pets, living in overcrowded conditions, low birth weight, male gender, malnourishment and co-infections as significant risk factors for cryptosporidiosis (Putignani and Menichella 2010) (Fig. 2.2).

The role of host genetics in susceptibility to infection was studied in a cohort of 226 Bangladeshi children aged 2–5 years, who were prospectively followed for >3 years (Kirkpatrick et al. 2008). Ninety-six children (42.5 %) were diagnosed with *Cryptosporidium* infection. A total of 51 (22.6 %) had asymptomatic infection, whereas 58 (25.7 %) had symptomatic cryptosporidiosis, of whom 17 (29.3 %) had recurrent disease. Infected children, both asymptomatic and symptomatic, were more likely to carry the human leukocyte antigen (HLA) class II DQB1*0301 allele ($P = 0.009$), and a strong association was found between the DQB1*0301/DRB1*1101 haplotype and the development of both asymptomatic and symptomatic infection ($P = 0.009$). Infected children were also more likely to carry the

B*15 HLA class I allele. This was the first description of a genetic component of the immune response to *Cryptosporidium* infection, which included HLA class I and II alleles (Kirkpatrick et al. 2008).

2.8 Post-Infectious Sequelae

Little is known on the long-term consequences of *Cryptosporidium* infection in humans. The gut epithelium generally recovers after resolution of symptoms, but there are some indications that long-term sequelae may arise (Cacciò et al. 2009).

In immunocompetent individuals, the medium-term health effects of cryptosporidiosis is characterized by the recurrence of loss of appetite, vomiting, abdominal pain, and diarrhoea, independently if the patients have been infected by *C. parvum* or by *C. hominis* (see Chap. 9).

Interestingly, significant differences in the occurrence of extra-intestinal symptoms as sequelae of cryptosporidiosis following infections with *C. hominis* and *C. parvum* have been reported in immunocompetent persons (Hunter et al. 2004). Indeed, eye pain and recurrent headache are associated with *C. hominis* infection but not with *C. parvum* infection, whereas other symptoms, such as fatigue and joint pains, are present after infections with both species, but are significantly more common after *C. hominis* infection (Hunter et al. 2004). The relatively small number of case patients who reported joint pains (13 control subjects versus 36 case patients) means that the establishment of firm conclusions about the nature and distribution of joint symptoms will require further investigations (Hunter et al. 2004).

The potential association of *Cryptosporidium* with the inflammatory bowel syndrome (IBS), a common GI disorder characterized by abdominal pain and alterations in bowel habits, is still uncertain. Indeed, a study of intestinal mucosal biopsies and serology from patients with IBS did not support a major role for the parasite in its pathogenesis (Chen et al. 2001). However, experimental *C. parvum* infection in a rat model resulted in jejunal hypersensitivity to distension, which was also associated with activated mast cell accumulation at 50 days post-infection (Khaldi et al. 2009). These findings are consistent with the observations that IBS patients have a marked increase in mast cell numbers and higher tryptase concentrations in jejunal fluid. Thus, further studies are needed to understand the role that *Cryptosporidium* infection may have in the establishment of IBS.

A seronegative reactive arthritis secondary to cryptosporidiosis has been reported in adults (Hay et al. 1987; Özgül et al. 1999; Collins and Highton 2004) and children (Shepherd et al. 1989; Cron and Sherry 1995), including one report of Reiter's syndrome (arthritis, conjunctivitis and urethritis) (Cron and Sherry 1995).

2.9 Burden of Disease

2.9.1 Overall Prevalence of Infection

In the developed countries, diarrhoea is the most common reason for missing work, while in the developing world, it is a leading cause of death. Internationally, the mortality rate is 5–10 million deaths each year (Nemes 2009). In this scenario, *Cryptosporidium* is a major cause of diarrheal disease, globally (Shirley et al. 2012). Unlike many common causes of infectious enteritis, control and treatment of this infection are still problematic. Indeed, control is focused mainly on prevention and no widely effective vaccine or drug-based intervention strategies are available (see Chap. 11). Furthermore, control strategies are particularly deficient for infections of severely immunocompromised individuals, the elderly, children or malnourished people, especially in developing countries. Cryptosporidiosis also presents a significant burden on immunocompetent individuals, and can have permanent effects on physical and mental development of children infected at an early age (Jex et al. 2011). Therefore, a tight monitoring of global infection is nowadays even more essential than in the past, because of the need to include control strategies for different categories of individuals, also in the absence of symptomatic evidence.

Moreover, *Cryptosporidium* is considered an emergent pathogen, often underappreciated for limitations or absence of appropriate diagnostics tools (e.g., microscopy often fails to detect low parasite load) and/or for only indirect association to severe medical growth faltering, malnutrition, and diarrheal mortality.

Recently, significant advances in molecular typing and subtyping analyses have yielded new insights into the epidemiology of cryptosporidiosis; however, in developing countries, point-of-care sites remain crucial for control of enteritis, especially in large areas such as the Indian subcontinent, where they are still lacking in many territories, while highly active antiretroviral therapy (HAART) is not equally distributed. In industrialized countries, outbreaks due to food-borne and water-borne transmission routes, growing transplantation surgery in routine medical practice and related long-term steroid treatments given in combination, are strongly contributing to the increase in disease incidence (Putignani and Menichella 2010; Shirley et al. 2012).

2.9.2 Seasonality

The incidence of cryptosporidiosis exhibits strong seasonality, with low endemic levels followed by pronounced seasonal outbursts (McLauchlin et al. 2000). In a recent meta-analysis on the seasonality of cryptosporidiosis, which was based on 61 published studies, increases in temperature and precipitation were associated with an increase in the incidence of cryptosporidiosis (Jagai et al. 2009).

Precipitation was found to be a strong seasonal driver for cryptosporidiosis in moist tropical climates. On the other hand, in temperate climates, the incidence of cryptosporidiosis peaked with an increase in temperature. The study further shows that while climatic conditions typically define a pathogen habitat area, meteorological factors affect timing and intensity of seasonal outbreaks (Jagai et al. 2009).

Clearly, the seasonal patterns tend to vary with location. For example, in India, the incidence of cryptosporidial diarrhea among children residing in the more temperate northern part of India correlated positively with temperature and negatively with humidity, but this correlation is not observed for children residing in the more tropical southern region. In another study from North-Eastern India, the highest prevalence of cryptosporidiosis was observed during the rainy months, and symptomatic as well as asymptomatic cryptosporidiosis in children was found to increase with increasing rainfall in Kolkata (Desai et al. 2012). In Kuwait, peak incidence occurred during the months of March and April, with no cases during the hottest months of July and August (Daoud et al. 1990).

Due to difference in transmission routes, it is likely that seasonal patterns may vary for different *Cryptosporidium* species. Indeed, genotyping studies conducted in the United Kingdom and New Zealand have found that human cases due to *C. parvum* peak in the late spring whereas those caused by *C. hominis* peak in the fall (McLauchlin et al. 2000; Learmonth et al. 2004). This was interpreted as increased exposure to animal oocysts following the calving and lambing season for *C. parvum*, and to increased travel, exposure to water and attendance to day care centres for *C. hominis*. The dramatic decline in human cases due to *C. parvum* observed in the United Kingdom after the large food-and-mouth outbreak of 2001, was initially explained by a reduced exposure of people to animals and, therefore, reduced zoonotic transmission (Smerdon et al. 2003). However, as the decline has continued, intervention measures, such as the introduction of water regulations and major structural changes in public water supply, are now believed to have played a major role (Sopwith et al. 2005).

2.9.3 Geographic Distribution

Cryptosporidiosis has a worldwide distribution, but the prevalence of infection is assumed to be higher in developing countries (Putignani and Menichella 2010). It should be noted, however, that in many developed and developing countries, surveillance systems for routine detection of cryptosporidiosis are not in place, and few studies have been conducted to estimate how prevalence can vary over time (Nichols 2008).

The distribution of the major *Cryptosporidium* species infecting humans varies geographically. Previous studies have shown that *C. parvum* and *C. hominis* are responsible for >90 % of human cases of cryptosporidiosis in most areas (Xiao and Ryan 2008). In the United Kingdom, in other European countries and in New

Zealand, *C. parvum* is responsible for slightly more infections than *C. hominis* (Xiao 2010). In the Middle East, *C. parvum* is the dominant species in humans. In contrast, *C. hominis* is responsible for more infections than *C. parvum* in the United States, Australia, China and Japan, as well as in most developing countries. Notably, the prevalence of *C. meleagridis* can be as high as that of *C. parvum* in certain areas of the world (Cama et al. 2008). Major differences in transmission routes may account for the observed differences in the distribution of *Cryptosporidium* species (Xiao 2010). The distribution of *C. parvum* and *C. hominis* can also vary within a single country; for example, *C. parvum* is more common than *C. hominis* in rural states in the United States, Ireland and New Zealand (Feltus et al. 2006; Zintl et al. 2009; Snel et al. 2009). Further geographic differences can be observed in the distribution of *C. parvum* and *C. hominis* subtypes (see Chap. 3).

2.9.4 Infection in Children

Cryptosporidiosis occurs more frequently in infants and children than in adults, both in developed and developing countries (Snelling et al. 2007; Xiao 2010; Putignani and Menichella 2010). This is likely to reflect both exposure and immunity. In the United States, endemic parasitic infections are more frequent than commonly perceived. Cryptosporidiosis occurs mainly in children aged 1–9 years, with the onset of infection peaking in the summer in association with communal swimming venues and recreational water use (Barry et al. 2013).

Needless to say, however, the impact of cryptosporidiosis is much higher in the poorest regions of the world. Globally, one in ten child deaths result from diarrhoeal disease during the first 5 years of life, and most occur in sub-Saharan Africa and south Asia (Liu et al. 2012). The role of various pathogens have been very recently studied in 9,439 children with moderate-to-severe diarrhoea and 13,129 control children without diarrhoea (Kotloff et al. 2013). Remarkably, *Cryptosporidium* was identified as a significant pathogen regardless of HIV prevalence and site of collection, and was the second most common pathogen in infants, and was associated with an increased risk of death in toddlers aged 12–23 months (Kotloff et al. 2013).

Children in developing countries are uniquely vulnerable to persistent infection because of the independent and synergistic effects of immune naiveté, malnutrition, and HIV infection (Mor and Tzipori 2008). In these areas, cryptosporidiosis is most prevalent during early childhood, with as many as 45 % of children experiencing the disease before the age of 2 years (Valentiner-Branth et al. 2003). *Cryptosporidium* also plays a causal role in childhood malnutrition and has been linked to impaired physical fitness in late childhood. Studies in Sub-Saharan Africa (reviewed by Mor and Tzipori 2008) have documented significantly higher cryptosporidiosis prevalence among malnourished children. It is difficult to ascertain the direction of this association, i.e., if malnutrition predisposes to infection or if infection actually impairs nutrient absorption and therefore causes weight loss

and growth stunting. Similar findings have been reported from longitudinal studies in Peru (Checkley et al. 1998) and Brazil (Bushen et al. 2007).

The prevalence and predictors of *Cryptosporidium* infection, and its effect on nutritional status, have recently been explored among 276 children (aged 2–15 years) in aboriginal villages in the Malaysian state of Selangor (Al-Mekhlafi et al. 2011). Faecal smears were examined by microscopy while socio-economic data were collected using a standardized questionnaire. Nutritional status was assessed by anthropometric measurements. *Cryptosporidium* infection was detected in 7.2 % of the children, and was found to be significantly associated with low birth weight (≤ 2.5 kg), being part of a large household, and prolonged breast feeding (> 2 years).

The impact of *Cryptosporidium* on children has been demonstrated also in Arab countries, such as Egypt, Jordan, Kuwait, Libya, Palestine, Saudi Arabia and Tunisia. Prevalence rates of 1–43 % (mean 8.7 %) in diarrheic immunocompetent children and of 1–82 % (mean 41 %) in immunocompromised children and adults were reported (Ghenghesh et al. 2012). Higher infection rates were found in children living in rural and semi-urban areas than in those residing in urban areas. *Cryptosporidium*-associated diarrhoea occurred mainly in children aged 1 year or less and was inversely correlated with age (Ghenghesh et al. 2012).

Access to quality drinking water in poor regions of the world is thought to be important in limiting gastro-intestinal infections. A study was conducted to determine whether or not bottled drinking water, intended such as “protected” water supply, could prevent or delay cryptosporidiosis among children in an endemic semi-urban community in Southern India (Sarkar et al. 2013). A total of 176 children were enrolled and received either bottled ($n = 90$) or municipal ($n = 86$) drinking water. Weekly surveillance visits were conducted until children reached their second birthday. Stools were collected every month and during diarrheal episodes, and tested for the presence of *Cryptosporidium* spp. by PCR. Cryptosporidiosis, mostly in an asymptomatic form, was observed in 118 of 176 (67 %) children during the follow-up period at a rate of 0.59 episodes/child-year. Diarrhea associated with *Cryptosporidium* spp. tended to be longer in duration and more severe. Stunting at 6 months and a higher disease burden were associated with a higher risk of cryptosporidiosis, but interestingly drinking bottled water was not associated with a reduced risk of cryptosporidiosis. The lack of association between drinking bottled water and cryptosporidiosis suggests possible spread from asymptotically infected individuals involving multiple transmission pathways.

2.9.5 Infection in Immunocompromised Individuals

Cryptosporidiosis is a leading cause of severe diarrhoea and extraintestinal infection in immunocompromised individuals (see Chap. 9). Besides HIV-infected patients, individuals at high risk include those with X-linked hyperimmunoglobulin

M syndrome (XHIM), CD40 ligand or gamma-interferon deficiency, children with leukemia, and organ transplant recipients (Cacciò et al. 2009).

The importance of cryptosporidiosis in HIV-infected people is well established and the subject has been extensively reviewed from the point of view of the epidemiology and clinical features (Hunter and Nichols 2002; Del Chierico et al. 2011), as well as from the perspective of treatment and control (Abubakar et al. 2007). In short, the risk of faecal carriage, the severity of illness and the development of unusual complications of cryptosporidiosis are related to the CD4 cell count (Pozio et al. 1997). Indeed, patients with CD4 counts of less than 50 are at greatest risk for both the severity of the disease and prolonged carriage. In severely immunocompromised persons, the parasite can also colonize extra-intestinal sites, particularly the gall bladder, biliary tract, pancreas, and lungs (Hunter and Nichols 2002).

The introduction of the highly active antiretroviral therapy (HAART) has had a remarkable impact on many opportunistic viral, bacterial and parasitic infections, resulting in a marked reduction in their occurrence and clinical course, at least in developed countries (Pozio and Gomez Morales 2005). The HAART therapy is based on a combination of nucleoside and non-nucleoside reverse transcriptase inhibitors and HIV protease inhibitors, and results in immune restoration, characterized by an increase in memory and naïve CD4⁺C T cells and the recovery of CD4⁺C lymphocyte reactivity against opportunistic pathogens. Cryptosporidiosis, however, remains a major problem for patients failing HAART, for most individuals living with AIDS in developing countries without access to HAART, and for severely malnourished children.

An extended study on intestinal parasitic infections, including cryptosporidiosis, was carried out in Congo by enrolling hospitalized AIDS patients (Wumba et al. 2010). Stool samples were collected from 175 patients older than 15 years. Parasites were detected by microscopy, immunofluorescence antibody tests and PCR (for diagnosis of microsporidia). At baseline, 19 patients (10.8 %) were under HAART and 156 (89.2 %) were eligible for it. Hospitalization was essentially due to intestinal infection associated with diarrhoea (49.7 % of the patients). A parasite was found in 47 of 175 (26.9 %) patients, and 27 out of 175 (15.4 %) were infected with at least one opportunistic parasite. The overall prevalence rate for *Cryptosporidium* sp. was 9.7 %, and increased to 12.6 % when only patients with diarrhoea were considered. A number of other protozoan and helminths were observed, but no significant relationship was established between any individual parasite and diarrhoea. These results underline the importance of opportunistic infections in symptomatic AIDS patients regardless of diarrhoea at the time of the hospitalisation, and showed that routine microscopic examination for *Cryptosporidium* spp. should be considered due to the absence of clinical markers.

In HIV patients, hepatic parenchymal and biliary tract diseases are common. A recent study focused on clinical aspects of AIDS-related cholangiopathy (De Angelis et al. 2009). Although the etiology is unclear, several opportunistic infections (including *Cryptosporidium* and cytomegalovirus) are suspected to cause it. The most common finding after endoscopic retrograde cholangiopancreatography is

diffuse sclerosing cholangitis in combination with papillary stenosis. Clinically, the most common manifestations are right upper quadrant pain and fever accompanied by an elevated serum alkaline phosphatase level. In vitro experiments have shown that concurrent active HIV replication and *C. parvum* infection synergistically increase cholangiocyte apoptosis and thus jointly contribute to AIDS-related cholangiopathies (De Angelis et al. 2009).

Primary immunodeficiencies are rare inherited disorders of the innate, cellular, and/or humoral immune system. A particular susceptibility to infection with *Cryptosporidium* is observed in children with X-linked hyper-immunoglobulin M syndrome (XHIM), resulting from CD40 ligand deficiency (CD40L), hyper-IgM syndrome type 3 caused by CD40 deficiency, primary CD4 lymphopenia, severe combined immunodeficiency syndrome, and gamma interferon deficiency (Cacciò et al. 2009). These patients are unable to clear the infection and extraintestinal infection, particularly of the bile tract, may result in chronic liver inflammation or even lead to liver cirrhosis. Colonization of the biliary system may also predispose to the development of sclerosing cholangitis (SC) and cholangiocarcinoma.

In a study of the association between XHIM and tumors of the pancreas, liver, and biliary tree, 14 of 20 boys (70 %) were found to be infected with *Cryptosporidium* (Hayward et al. 1997). In these patients, cholangiopathy and/or cirrhosis preceded the development of the tumors, suggesting that infection or inflammation of bile ducts caused by *Cryptosporidium* may play an important role in the development of malignancy. Another study in Poland found chronic cryptosporidiosis in three out of five patients with XHIM and in a single patient with primary CD4 lymphopenia, and reported SC in these patients (Wolska-Kusnierz et al. 2007). Further support for the association between *Cryptosporidium* infection and SC was found in a study in the United Kingdom that enrolled 35 children with clinical evidence of liver disease and found 12 of 27 children (44 %) infected with the parasite, among whom nine had SC (Rodrigues et al. 2004).

In a recent analysis of 126 patients with XHIM syndrome reported to the European Society for Immunodeficiency registry, approximately one-sixth developed liver disease, and in more than 50 % of cases this was associated with *Cryptosporidium* species infection (Toniati et al. 2002). These figures may even be an underestimate, because more-sensitive molecular techniques reveal that a number of patients are colonized by *Cryptosporidium* without evidence of its presence on conventional microbiology screening (Rodrigues et al. 2004; Wolska-Kusnierz et al. 2007). Thus, unrecognized cases of cryptosporidiosis in children with primary immunodeficiencies may lead to serious consequences, with development of sclerosing cholangitis, liver cirrhosis, and cholangiocarcinoma.

Data on cryptosporidiosis in solid-organ transplant recipients are limited, but those available illustrate the need of a high index of suspicion in any transplant patient who presents with severe diarrhea (Cacciò et al. 2009; Krause et al. 2012). Intestinal infections with *Cryptosporidium* species have been reported in renal transplant patients. Some cases resulted in either mild disease or asymptomatic carriage, but severe cryptosporidiosis can occur in these patients, including biliary involvement treated with reduction of immunosuppression and a short course of

antiparasitic agents (Abdo et al. 2003; Hong et al. 2007). In the recent study of Krause et al. (2012), *Cryptosporidium* was detected as the cause of gastroenteritis in six children (four kidney recipients, one liver and kidney recipient, and one heart transplant recipient). All patients were hospitalized due to prolonged diarrhoea, fever, abdominal pain and weight loss, and most presented deterioration of kidney functions and abnormal values of liver enzymes.

About ten cases of cryptosporidiosis has also been reported in liver transplant recipients. In a study from Belgium on 461 children following liver transplantation, three (0.65 %) developed diffuse cholangitis associated with intestinal *Cryptosporidium* species carriage (Campos et al. 2000). All three recipients required reoperation on the bile duct anastomosis, but biliary cirrhosis developed in one patient, requiring retransplantation. In a retrospective study from Pittsburgh, four (0.34 %) pediatric cases of cryptosporidiosis were identified among 1,160 nonrenal, abdominal organ transplant recipients (Gerber et al. 2000). Three of these four cases occurred in patients receiving liver transplants, and one occurred following a small bowel transplantation. All four patients spontaneously resolved their infections. Manz and Steuerwald (2007) reported a case of cryptosporidiosis in an adult patient treated with interferon and ribavirin for recurrent hepatitis C after liver transplantation. The patient did not have HIV infection or immunoglobulin deficiency and recovered after the treatment was stopped and the dosage of immunosuppressant was lowered. A case of multiple infections with distinct *Cryptosporidium* species has been described for a transplanted ileum (Pozio et al. 2004). In Iran, among 44 liver transplant children in Shiraz Nemazee hospital, *C. parvum* and *C. meleagridis* were detected in 11.36 % of the children (Agholi et al. 2013).

The study by Sulżyc-Bielicka et al. (2012) evaluated the prevalence of *Cryptosporidium* spp. in 87 patients with diagnosed colorectal cancer, by using a ProSpecT *Cryptosporidium* Microplate Assay. *Cryptosporidium* sp. was found in 12.6 % of the patients, with a prevalence comparable to patients with immune deficiency; however, no specific correlation was found between *Cryptosporidium* spp. infection and gender, age, neoplasm differentiation grade, or neoplastic tumour localisation.

2.9.6 Infection in Travellers

In 2011, approximately 980 million people travelled internationally. More than 522 million people from developed countries travelled overseas; an estimated 50–100 million people travelled to developing countries (Ross et al. 2013). Approximately 8 % of travellers to the developing world require medical care during or after travel and more than a quarter of those who seek medical assistance present with GI symptoms. Traveller diarrhoea (TD) occurs in 20–60 % of European or North American travellers in inter-tropical areas (Cavallo and Garrabé 2007). Bacteria, viruses and parasites all may contribute to TD, but their relative importance is still uncertain; however, protozoan infections with *Giardia* and *Cryptosporidium* are frequently identified as the cause of GI complaints in returning travellers

(Freedman et al. 2006; Thielman and Guerrant 1998; Okhuysen 2001). Travelling represents an important risk factor for acquiring infection also with spore-forming protozoa such as *Cyclospora*, Microsporidia, and *Isospora* (Goodgame 2003). Several studies have shown that a large proportion of travellers and immigrants from tropical and subtropical countries are affected by GI disorders and harbour intestinal pathogens in the absence of evident GI problems (Saiman et al. 2001; Freedman et al. 2006; Ansart et al. 2005; Caruana et al. 2006; Whitty et al. 2000; Fotedar et al. 2007).

One of the first studies of travellers returning from developing countries was performed in Germany in 1997 (Jelinek et al. 1997). To estimate the prevalence of *C. parvum* and *Cyclospora cayetanensis*, 978 stool samples were taken from 795 patients (469 suffering from diarrhoea) returning from developing countries. Of the 795 patients, infection with *C. cayetanensis* was detected in five subjects (1.1 %), while 13 patients (2.8 %) were infected with *C. parvum*. All patients with either *C. parvum* or *C. cayetanensis* infection suffered from watery diarrhoea, suggesting that in cases of persistent watery diarrhoea these pathogens should be always considered in the differential diagnosis (Jelinek et al. 1997).

In the Netherlands, a new diagnostic strategy was recently implemented for the routine diagnosis of intestinal parasites in returning travellers and in immigrants (ten Hove et al. 2009). Over a period of 13 months, unpreserved stool samples, patient characteristics and clinical data were collected from those attending a travel clinic. Stool samples were analysed on a daily basis by microscopic examination and antigen detection, and compared with a weekly performed multiplex real-time PCR analysis for *Entamoeba histolytica*, *Giardia*, *Cryptosporidium* and *Strongyloides stercoralis*. Microscopy and antigen detection screening of 2,591 stool samples showed *E. histolytica*, *Giardia*, *Cryptosporidium* and *S. stercoralis* in 0.3 %, 4.7 %, 0.5 %, and 0.1 % of the cases. These detection rates were lower than those obtained with real-time PCR. PCR positivity was 0.5 %, 6.0 %, 1.3 %, and 0.8 % (ten Hove et al. 2009), showing that high-throughput molecular screening could provide more accurate estimates of the prevalence of these pathogens.

It has been hypothesized that travellers may be exposed to parasite species/genotypes that do not circulate in their home countries, and that symptomatic infection with these parasites occurs because of the lack of or insufficient cross-protection resulting from previous exposures. In agreement with this hypothesis, Tanriverdi et al. (2008) used microsatellite typing to show that the *C. hominis* isolates infecting patients that had travelled to the UK from Pakistan less than 2 weeks prior to the isolation of the parasites were significantly different from the large cluster of autochthonous cases from the UK. Furthermore, a novel *Cryptosporidium* species was recently identified among travellers with gastro-intestinal symptoms returning to the UK from the Indian subcontinent (Elwin et al. 2012). Based on morphological and molecular data, this parasite was designated a new species, *Cryptosporidium viatorum*. The name was chosen to underscore its link to foreign travel (Elwin et al. 2012). The epidemiology of *C. viatorum* cases was found to be different from that of individuals infected with *C. parvum* and *C. hominis*: most *C. viatorum* cases occurred in the first months of the year, vomiting

was reported less often, but the duration of symptoms was longer. The cases of *C. viatorum* were all travellers to the Indian subcontinent, whereas cases of *C. hominis* and *C. parvum* were more likely associated to travel elsewhere (Elwin et al. 2012).

Another study on *Cryptosporidium* infections contracted whilst travelling abroad used a PCR-coupled single-strand conformation polymorphism analysis, followed by targeted sequencing of the gp60 gene (Jex and Gasser 2008). The study investigated *C. hominis* and *C. parvum* isolates ($n = 115$) from UK citizens inferred to have been infected while travelling abroad (to 25 countries) or in the UK. Isolates were classified to the genotype and sub-genotype levels, leading to the identification of five *C. hominis* and four *C. parvum* gp60 genotypes. A particular *C. hominis* subgenotype (IbA10G2R2) was found in the majority (71 %) of the isolates, collected from individuals who have travelled to 14 different countries, while other subgenotypes appeared to be quite rare (Jex and Gasser 2008).

2.10 Transmission Routes

There are multiple transmission routes through which humans can acquire *Cryptosporidium* infections. The main routes of transmissions are shared by the two major human pathogens, *C. hominis* (Fig. 2.3) and *C. parvum* (Fig. 2.4); however animal pollution and environmental contamination particularly affect zoonotic transmission of *C. parvum* (Fig. 2.4).

2.10.1 Person-to-Person Transmission

Cryptosporidium is easily transmitted among children and staff members in nurseries (Hannah and Riordan 1988), day care centres (Heijbel et al. 1987), and schools (Lee and Greig 2010).

Nosocomial infection is also well documented, and both direct (Baxby et al. 1983; Koch et al. 1985) and indirect (Martino et al. 1988; Navarrete et al. 1991) person-to-person transmission (via contaminated hands) has been incriminated as the likely route. Nosocomial infection can cause secondary cases among roommates (Bruce et al. 2000) and family members (Pandak et al. 2006), further supporting the highly infectious capacity of the parasite.

Direct transmission among HIV-positive men who have sex with men occurs more frequently than in HIV-positive drug users (Pedersen et al. 1996), and a case-control study in Australia (Hellard et al. 2003) has shown that men having more than one sexual partner are more likely to have *Cryptosporidium* diarrhoea, therefore indicating that sexual contacts represent a risk factor for faecal-oral parasite transmission.

In developing countries, the high prevalence of *C. hominis* and of anthroponotic subtypes of *C. parvum*, particularly in children, has also been

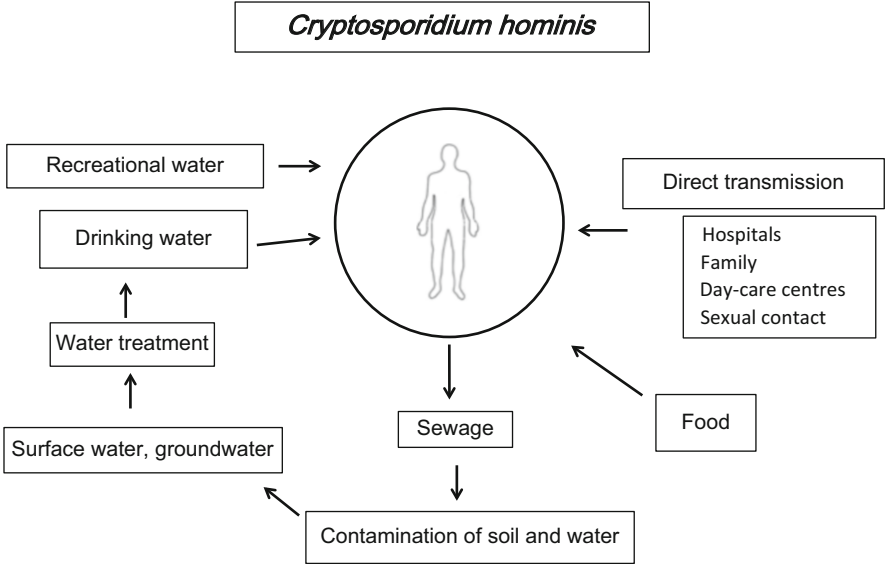


Fig. 2.3 Main transmission routes of *Cryptosporidium hominis*

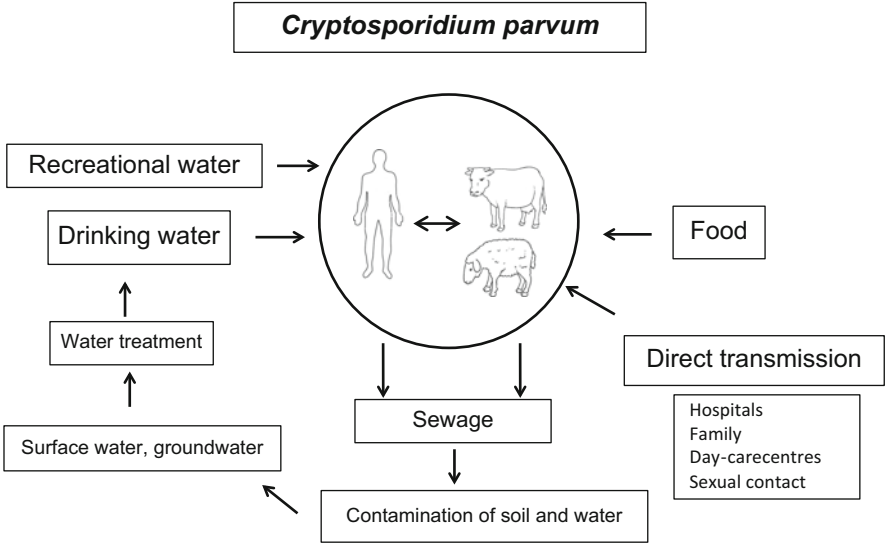


Fig. 2.4 Main transmission routes of *Cryptosporidium parvum*

taken as an indication of the importance of person-to-person transmission (Xiao 2009) (Figs. 2.3 and 2.4).

2.10.2 Zoonotic Transmission

Cryptosporidium parvum is the most important zoonotic agent of cryptosporidiosis, with a large range and abundance of animal reservoirs, mainly in young farmed animals (see Chap. 4). Therefore, individuals who come in contact with those animals, either for occupational or recreational reasons, may be at risk. Outbreaks of cryptosporidiosis have been reported among veterinarians and veterinary students (Pohjola et al. 1986; Preiser et al. 2003; Gait et al. 2008), other people exposed to agricultural animals (Stantic-Pavlinic et al. 2003) and children visiting farms (Shield et al. 1990; Stefanogiannis et al. 2001; Hoek et al. 2008). Contact with farmed animals was identified as a significant risk factor for sporadic cases of human cryptosporidiosis in the UK (Hunter et al. 2004; Goh et al. 2004).

Due to the high prevalence of *C. parvum* infection and the high numbers of oocysts shed in faeces (up to $>5 \times 10^6$ oocysts per gram), calves are considered to pose the most significant threat to environmental contamination and transmission to humans (Current et al. 1983). Initially, it was believed that human infection with *C. parvum* were all of zoonotic origin and calves have been implicated as the main source of infectious oocysts, but further studies based on highly polymorphic markers have shown that certain *C. parvum* subtypes are found in humans but not in animals, and are likely to be transmitted through an anthroponotic cycle (Mallon et al. 2003). Thus, a significant fraction of human *C. parvum* infections may not originate from livestock reservoirs (Grinberg et al. 2008). Nevertheless, calves are frequently infected with a *C. parvum* subtype that is commonly found in humans in the same geographic areas (Xiao 2009) and epidemiologic studies have supported the occurrence of zoonotic transmission (Hunter et al. 2007).

Epidemiologic investigations have demonstrated the role of sheep in human cryptosporidiosis more than 20 years ago (Casemore 1989), and this has been further supported by molecular studies, particularly in the UK, where five human outbreaks have been linked to contacts with lambs (Chalmers and Giles 2010). Recently, a case of zoonotic transmission of a rare *C. parvum* subtype from infected lambs to a children has been reported in Italy (Cacciò et al. 2013).

On the contrary, little is known about the role of goats in zoonotic cryptosporidiosis. Goats can be infected with *C. xiaoi*, which is a non-zoonotic species, but also with *C. parvum* (Rieux et al. 2013).

The zoonotic potential of canine and feline cryptosporidiosis has been a major concern to both veterinarians and physicians, but the actual risk of transmission from pets to human appears to be minimal, at least in develop countries. To date, there have been only 26 *C. canis* and 97 *C. felis* cases reported in people, and the majority was from immunocompromised individuals (Lucio-Forster et al. 2010). Epidemiological investigations in the UK and USA (Goh et al. 2004; Glaser et al. 1998) have failed to establish a significant relationship between owning a dog and infection with *Cryptosporidium*, whereas owning a dog or a cat was found to be a risk factor for cryptosporidiosis in Guinea Bissau and Indonesia (Miron et al. 1991; Katsumata et al. 1998). There is only one report of possible transmission

of *C. canis* between a dog and two siblings living in a household in Peru (Xiao 2009). It is likely that only immunocompromised individuals are at risk of acquiring cryptosporidiosis from pets.

Rabbits are the natural host of *Cryptosporidium cuniculus* (Robinson et al. 2010), a species that is now known to infect humans (Chalmers et al. 2009). First identified as a human pathogen during a waterborne outbreak, *C. cuniculus* appears to be the third most commonly identified species in patients with diarrhea in the UK, after *C. parvum* and *C. hominis* (Chalmers et al. 2011). The existence of identical *C. cuniculus* subtypes in humans and pets or wild rabbits suggest zoonotic potential (Zhang et al. 2012).

Zoonotic transmission of *C. meleagridis*, an avian parasite that can infect humans, has been recently reported in Sweden (Silverlås et al. 2012). Interestingly, results of molecular characterization suggest laying hens or broiler chickens as the source of infectious oocysts.

Fish, reptiles and amphibians appear not to pose a risk for human cryptosporidiosis (see Chap. 5).

2.10.3 Waterborne Cryptosporidiosis

Waterborne infectious diseases are a globally emerging public health issue. Various community outbreaks due to contamination of water have highlighted the importance of intestinal protozoa in public health. Among these important pathogens are *Giardia duodenalis*, *E. histolytica*, *C. cayetanensis*, *Isospora belli*, Microsporidia and, of greater relevance, *Cryptosporidium* (Karanis et al. 2007).

The ubiquitous presence of *Cryptosporidium* spp. in the aquatic environment is explained by the large number of hosts, the extremely high number of oocysts shed by these hosts, and the remarkable stability of oocysts (Smith et al. 2006b). Thus, water represents a very important vehicle of infection for the population, and waterborne cryptosporidiosis is a serious public health concern, particularly for populations at risk of severe infection (pregnant women, children, HIV-positive and transplanted patients) (Chap. 12).

Indeed, out of the 71 *Cryptosporidium*-linked outbreaks described in the last decade, 40 (56.3 %) appear to be correlated to waterborne transmission. Geographically, the outbreaks seem to be concentrated in the USA, Canada, Australia and Europe, especially in the UK and Ireland, and affect both adults and children (Putignani and Menichella 2010; Chalmers 2012). Surveillance data has revealed the presence of *Cryptosporidium* spp. in the entire water treatment system (see Chap. 12), which represents an unacceptable health risk, particularly for at risk populations (pregnant women, children, HIV-positive and transplanted patients). Such evidence suggests that focus ought to be placed on prevention of human and animal waste contamination especially in authorized recreational waters. Remarkably, cryptosporidiosis is the most frequently reported gastrointestinal illness in

outbreaks associated with treated (disinfected) recreational water venues in USA (Yoder and Beach 2007; Chap. 12).

Following several large outbreaks linked to drinking water in UK and USA, most notably the 1993 Milwaukee outbreak that involved an estimated 403,000 cases of cryptosporidiosis (MacKenzie et al. 1995), emphasis on monitoring and intervention of water supplies, and greater awareness and investigation of water as a transmission vehicle, has led to a decrease in the number of outbreaks due to drinking water (Chap. 12).

Remarkably, however, cryptosporidiosis remains the most frequently reported GI illness in outbreaks associated with treated (disinfected) recreational water venues in the UK and the USA (Hlavsa et al. 2011; Smith et al. 2006a; Yoder and Beach 2007; Yoder et al. 2010). Outbreaks have occurred in swimming pools, paddling or wading pools, water parks and fountains, and were caused by *C. hominis* and *C. parvum* (see Chap. 12). During the summer of 2007, Utah experienced a statewide outbreak of GI illness caused by *Cryptosporidium* (CDC, MMWR Report 2012). Of 1,506 interviewed patients with laboratory-confirmed cryptosporidiosis, 1,209 (80 %) reported swimming in at least one of approximately 450 recreational water venues during their potential 14-day incubation period. Because swimmers were the primary source of *Cryptosporidium* contamination, healthy swimming campaigns are needed to increase awareness and practice of healthy swimming behaviours, especially not swimming while ill with diarrhoea. The healthy swimming campaign, as part of a multipronged prevention effort, might have helped prevent recreational water-associated outbreaks of cryptosporidiosis in Utah (CDC, MMWR Report 2012). Local and state health departments can use cryptosporidiosis surveillance data to better understand the epidemiologic characteristics and the disease burden of cryptosporidiosis in the USA, to design prevention strategies that reduce disease spread and to establish research priorities. The role of water in the transmission of *Cryptosporidium* in developing countries is less known. Clearly, the potential for transmission is enhanced by the absence of sanitary and parasitological drinking water monitoring, and the burden of the infection is surely underestimated due to the scarcity of appropriate surveillance programs and the relative inadequacy of laboratory diagnosis (Mak 2004). A Quantitative Microbiological Risk Assessment (QMRA) conducted in Africa (Hunter et al. 2009) demonstrated that interruptions in water supplies that forced people to revert to drinking raw water caused a greater risk of infection, particularly in young children. Thus, poor reliability of drinking water supplies has an impact on the achievement of health improvement targets (Hunter et al. 2009).

The importance of stability of the treatment process and the importance of watershed protection has been stressed by a comprehensive QMRA performed on a source water survey from 66 waterworks in 33 major cities across China (Xiao et al. 2012). The annual diarrhoea morbidity caused by *Cryptosporidium* in drinking water was estimated to be 2,701 cases per 10^5 immunocompromised persons and 148 cases per 10^5 immunocompetent persons, giving an overall rate of 149 cases per 100,000 population. The burden of cryptosporidiosis associated with drinking water treated with the conventional process was higher than the reference risk level

suggested by the WHO, but lower than that suggested by the United States Environmental Protection Agency (Xiao et al. 2012).

A survey of *Giardia* and *Cryptosporidium* conducted in 206 samples of surface waters used as drinking water sources by public water systems in four densely urbanized regions of Sao Paulo State, Brazil and a QMRA, showed 102 samples positive for *Giardia* and 19 for *Cryptosporidium*, with maximum concentrations of 97.0 cysts/L and 6.0 oocysts/L, respectively. The probability of *Giardia* infection was close to the rates of acute diarrheic disease for adults (1–3 %) but lower for children (2–7 %). The daily consumption of drinking water was an important contributing factor for these differences (Sato et al. 2013).

2.10.4 Foodborne Cryptosporidiosis

Contamination of different types of food with *Cryptosporidium* oocysts has been demonstrated in studies from different regions of the world. Those studies have mainly focused on fruits and vegetables, because these foods are prone to contamination and are often consumed raw or after minimal thermal treatment, therefore increasing the possibility of transmission (Robertson and Chalmers 2013). Due to the highly variable, and usually low, rates of recovery of oocysts from food matrices, improved methods have been recently developed and validated in the UK (Cook et al. 2006).

Studies in Costa Rica and Peru (Monge and Arias 1996; Ortega et al. 1997) have shown contamination of numerous raw vegetables, including basil, cabbage, celery, cilantro, green onions, leeks, lettuce, parsley, and yerba buena. A more recent study in Costa Rica (Calvo et al. 2004) investigated the presence of *Cryptosporidium* spp., *Cyclospora* spp., and Microsporidia on lettuce, parsley, cilantro, strawberries and blackberries collected from five local markets. Fifty different samples of each product, 25 taken in the dry season and 25 in the rainy season, were evaluated. All products were found contaminated with *Cryptosporidium* spp., *Cyclospora* spp., and/or Microsporidia. *Cryptosporidium* was not detected in strawberries, microsporidia were absent on blackberries and *Cyclospora* was only isolated from lettuce during the dry season. These results show the importance of introducing good agricultural practices, especially due to the resistance of *Cryptosporidium* and *Cyclospora* to disinfecting agents (Putignani and Menichella 2010).

Food contamination with *Cryptosporidium* oocysts, however, is not limited to developing countries. In Norway, a search for parasites in fruits and vegetables was undertaken in the period from 1999 to 2001 (Robertson and Gjerde 2001). Of the 475 samples, 29 were found to be positive for *Cryptosporidium* oocysts and *Giardia* cysts, of which 19 only for *Cryptosporidium* (lettuce and mung bean sprouts). Mung bean sprouts were significantly more likely to be contaminated with *Cryptosporidium* oocysts or *Giardia* cysts than the other fruits and vegetables, even if concentrations were generally low (approximately 3 (oo)cysts per 100 g product). There was no association between imported produce and detection of parasites.

Cryptosporidium oocysts and *Giardia* cysts were also detected in water samples concerned with field irrigation and production of bean sprouts (Robertson and Gjerde 2001).

In Poland, 163 samples comprising Peking cabbages, leeks, white cabbages, red cabbages, lettuces, spring onions, celery, cauliflowers, broccoli, spinach, Brussels sprouts, raspberries, strawberries, all from local markets, were tested (Rzezutka et al. 2010). *Cryptosporidium* oocysts were detected in one leek sample, one celery sample, four cabbage samples, including all cabbage types tested. Of note, *Cryptosporidium*-positive samples came from districts with the highest number of cattle herds. In Spain, 19 fresh produce samples from local markets were tested, and oocysts were found in 33 % of Chinese cabbage, 75 % of Lollo rosso lettuce, and 78 % of Romaine lettuce (Amorós et al. 2010).

These studies demonstrated that contamination of fruits and vegetables occurs also in developed countries.

A few outbreaks have been linked to the consumption of contaminated vegetables. In 2008, a *C. parvum* outbreak in Sweden was linked to chanterelle sauce with fresh parsley added after the preparation of the sauce (Insulander et al. 2008), while in Finland a salad mixture was the suspected vehicle for a *C. parvum* outbreak (Ponka et al. 2009).

Contamination of dairy products and fruit juices has also been linked to outbreaks of cryptosporidiosis. The consumption of unpasteurized cow milk has been suggested as the cause of outbreaks in the UK and Australia (Gelletlie et al. 1997; Harper et al. 2002). Outbreaks associated with consumption of fruit juice is becoming an emergent public health problem since the early 1990s, when the first outbreak associated with apple cider was described (Millard et al. 1994). However, in the period from September to November 2003, 12 local residents in Northern Ohio were diagnosed with cryptosporidiosis for having drunk ozonated apple cider (Blackburn et al. 2006). In response to epidemiologic investigations of outbreaks in which juice is implicated, the USA Food and Drug Administration has implemented process control measures to regulate the production of fruit juice, according with the Hazard Analysis Critical Control Point (HACCP) plan. However juice operations that are exempt from processing requirements or do not comply with the regulation, continue to be implicated in outbreaks of illness. The CDC receives reports of food-associated outbreaks of illness (FoodNet, <http://www.cdc.gov/FoodNet/>) and its *Foodborne Outbreak Reporting System* has reviewed, from 1995 through 2005, ten implicating apple juice or cider, eight linked to orange juice, and three involving other types of fruit juice-associated outbreaks (Putignani and Menichella 2010). Among the 13 outbreaks of known aetiology, two were caused by *Cryptosporidium* and one by Shiga toxin-producing *E. coli* O111 and *Cryptosporidium* (Vojdani et al. 2008). The incidence of foodborne disease outbreaks caused by contaminated low pH fruit juices is increasing (Lynch et al. 2006). The association of *Cryptosporidium* with fruit juice is a raising safety concern in food industries. In 1998, CDC implemented enhanced surveillance for foodborne-diseases outbreaks by increasing communication with state, local, and territorial health departments and revising the outbreak report form. Since 2001,

reports are submitted through a web internet application called electronic Foodborne Outbreak Reporting System (Putignani and Menichella 2010).

Outbreak investigations have also put into focus the role of food handlers as a source of food contamination and subsequent transmission of cryptosporidiosis (Robertson and Chalmers 2013). In 1998, a large outbreak of gastroenteritis occurred in >100 persons at a University campus in Washington DC. A case-control study of 88 case patients and 67 control subjects showed that eating in one of two cafeterias was associated with diarrheal illness. Further epidemiologic and molecular evidence indicate that an ill foodhandler was the likely outbreak source, and *C. hominis* as identified as the etiological cause of gastroenteritis (Quiroz et al. 2000).

In 2005 an outbreak of diarrhoea, affecting a group of 99 company employees, was described near Copenhagen (Ethelberg et al. 2009). All people were ill and 13 tested positive for *C. hominis*. Disease was associated with eating from the canteen salad bar on one, possibly two, specific weekdays. Three separate salad bar ingredients were found to be likely sources: peeled whole carrots served in a bowl of water, grated carrots, and red peppers. The likely source of infection was an infected food-handler, who may have contaminated food served at the buffet (Ethelberg et al. 2009).

Recently, the role of the food handlers has been investigated in Venezuela, where cryptosporidiosis is an important public health problem (Freites-Martinez et al. 2009). Despite a basic investigation approach, 14 out of 119 fecal samples from food workers were found positive for *Cryptosporidium* spp. in association with other protozoa, the most frequently detected being *Endolimax nana*, *Blastocystis hominis*, *Entamoeba coli*, *Giardia*, and *E. histolytica*/*Entamoeba dispar*.

Finally, because of their capacity to filter large volumes of water potentially contaminated with oocysts from human and animal faecal waste, and because they are usually consumed raw, molluscs have been postulated as a route of transmission (Robertson 2007). Indeed many species, including mussels, oysters and clams, have been found to harbour *Cryptosporidium* oocysts in their digestive tract. However, there are no evidence of infections or outbreaks linked to consumption of molluscs.

2.11 Conclusions

Cryptosporidium is an important cause of diarrhoea, worldwide. In developed countries, large waterborne outbreaks continue to occur, emphasizing the need for better regulation and for improvements of drinking water treatment processes. Also, the increasing number of outbreaks linked to the use of recreational water (swimming pools, water parks) indicate the need for better control measures and guidelines. Immunocompromised individuals are particularly susceptible to *Cryptosporidium*, and may develop severe infections and extra-intestinal dissemination, yet an effective therapy to eradicate the parasite is not available. In developing

countries, the parasite is endemic and significantly associated with moderate-to-severe diarrhoea in infants, a finding that highlights the need to develop resources to diagnose, treat, and prevent cryptosporidiosis in resource-poor settings. Under this situation, routine diagnosis and effective reporting of *Cryptosporidium* to local and national surveillance organizations remain of key importance in understanding the epidemiology of this important, but often underestimated, pathogen.

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