Environmental Aspects of Cryptosporidium

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Abstract

Cryptosporidium, formerly classified to subclass Coccidia has been relocated to genus Gregarina which includes free living stages, enabling host-free multiplication and therefore may constitute an additional risk factor for human infection. The free stage of this parasitic protozoon, the oocyst, is incredibly durable under various environmental conditions, elucidating its long survival potential. The present review describes, based on published literature, the survival and behavior of Cryptosporidium oocysts in water, soil and other environmental surroundings as related to public health and infection prevention. As a zoonotic disease, cryptosporidiosis starts by infecting the intestinal tract of a large variety of mammals (~152 species) including humans. The excretion rate of oocysts (Cryptosporidium environmental stage) is high and favors the spread of this parasite to water and food sources. Such an extensive number is highly supportive for the wide spread of the parasite. Furthermore, a recent publication revealed that Cryptosporidium is not only an intracellular parasite but is also able to form a large or gigantic gamont-like stage as an additional extracellular life stage in vivo, where biofilm on gut cell surfaces may support Cryptosporidium growth and multiplication without the prerequisite for host cell invasion (inside the cell it acquires a unique epicellular location). This newly reported feature raises new question about its environmental survival. The agricultural link is obvious and its environmental impact involves soil, water and food once untreated waste water and effluents are applied for irrigation. These sources should be monitored for presence of Cryptosporidium to prevent infection and disease, and measures should be taken, such as water filtration, to prevent its spread to extended populations.

ABBREVIATIONS


INTRODUCTION

Cryptosporidium life cycle

According to traditional classification, Cryptosporidium parvum belongs to phylum Apicomplexa and order Eucoccidiorida. However, recent studies, based on genetics and physiology have now relocated the genus Cryptosporidium from Coccidia to Gregarina, which includes free living stages, enabling host-free multiplication and so may constitute an additional risk factor for human infection [1]. Cryptosporidium is a parasite infecting the intestinal tract of a large variety of mammals (~152 species) including humans [2-4]. There are at least two genotypes (based on oocyst wall protein phenotypes): genotype 1, exclusively in humans, and genotype 2, found in livestock such as sheep, cattle, goats as well as in rodents, which may also infect humans. The parasitic, epicellular development comprises asexual and sexual stages. The asexual multiplication results in merozoite formation, enabling reinfection of intestinal epithelial cells and the sexual cycle culminating in thin or thick-walled sporozoite containing oocysts (4 to 6 μm with an ovoidal or round shape). The thin-walled oocysts are a source of internal sporozoite infection of intestinal epithelium, whereas the durable thick-walled oocysts excreted in feces serve as an infection source through ingestion of contaminated drinking water or food, followed by excystation in the small intestine and finally cell invasion by excysted...
sporozoites. Sporozoite or merozoite development is termed epicellular, as it occurs between the epithelial cell cytoplasm and cells’ membrane. The life cycle is shown in Figure 1. During acute infection, oocysts are excreted in feces in high numbers (10^5 to 10^10 oocytes/gram feces). The following may exemplify the multiplication potential of this parasite: A Holstein calf (4 days of age) infected orally with 2.5 x 10^7 oocysts will start to excrete, at 6-8 days post-infection (DPI), during peak oocyst shedding period, between 2 x 10^9 and 2 x 10^10 oocysts during a 24 hr period. These oocysts have several attributes which favor their environmental distribution and persistence: environmental survival for months, stickiness that promotes adherence and infection and a relatively low infection dose (ID_{50} for the Iowa strain of C. parvum had been calculated as ~132 oocysts in healthy humans) [47]. Diarrheic feces from infected calves (the main human related infected hosts), are washed into water and soil sources, therefore contaminating human environment [5]. Infection with Cryptosporidium oocysts can take place by direct contact with infected stools (fecal-oral route), contaminated food and water, person to person (sexual contact) and aerosols. Clinical manifestations include watery diarrhea, anorexia, nausea/vomiting and abdominal pain. In immune competent persons the disease is self-limiting while in immune compromised patients it can be fatal (rapid dehydration due to 10-15 liters/day of diarrheic outings) and sometimes accompanied by infections of other organs, like pancreas and lungs. From the clinical point of view, the main problem with Cryptosporidium infection is that to date there is no effective prophylactic drug, but only clinical measures to contain gastro-intestinal symptoms (e.g. management of fluids and electrolytes, use of antimotility agents and antiparasitic drugs, nutritional support, and/or reversal of immune suppression) [49,50].

![Figure 1 Cryptosporidium parvum life cycle](http://www.dpd.cdc.gov/dpdx/html/Cryptosporidiosis.htm)
**Cryptosporidium life cycle and new scientific developments**

*Cryptosporidium* pathogenesis is now under revision considering the following points: 1. large or gigantic gamont-like stages as additional extracellular life stages that may exist in vivo; 2. biofilm on gut cell surface may support *Cryptosporidium* growth and multiplication without the prerequisite for host cell invasion (unique epicellular location) and 3. Beside host cell encapsulation, additional pathways of multiplication may exist, in order that *Cryptosporidium* will persist and cause subsequent disease symptoms. There are three different environments in the host gut: closely associated with the host mucosa in its unique epicellular location and extracellular in both the lumen and mucosal biofilms [1,6]. According to these new findings, it is critical to understand gut-biofilm interaction with *Cryptosporidium* and its aspect on this protozoan parasite growth, multiplication and survival.

**Cryptosporidium oocyst structure**

Up to date there is no available data on chemical structure of oocysts, but only the main biochemical composition e.g. carbohydrates, proteins and lipids. Among the first researcher to raise the biochemical structure of oocysts important to understand disinfection prospects were Zuckerman et al., [45] that studied indirectly the possible composition of *Cryptosporidium* oocysts by subjecting oocysts to strong chitinolytic activity of the bacterium *Serratia marcescens*. These authors showed a significant viability reduction of oocysts exposed to *Serratia marcescens* for 10 days at 36°C in comparison with oocysts in buffer solution. However, exposure of oocysts for a period of 80 days to *Pseudomonas aeruginosa* and *Enterobacter faecalis* also revealed a strong viability reduction of ~55%. According to their results, they postulated that chitin is a component of the oocyst wall. However, in light of other possible bacterial enzymatic degradative actions they could not rule out the lytic activities of proteases, glycosidases and lipases that can also induce oocyst wall damage. In light of the most recent studies on the free living stage of *Cryptosporidium*, it cannot be ruled out that those results showed bacteria-parasite facilitated excystation, at the time seen as inactivation based on oocysts Propidium iodide intake by ghost oocysts! A more recent study, applying various analytical tools (TEM- Freeze fracture; Freeze substitution, GC-MS, TLC-Thin-layer chromatography, etc.) proposed the following structure of *Cryptosporidium* oocyst wall: a ~8nm external glyocalyx, below this layer a ~4nm lipid hydrocarbon, followed by a protein layer of ~13nm and finally thick layer of structural polysaccharide of ~25-40nm. According to chemical composition of these layers, several important features of oocysts can be explained: known acid-fast staining of oocysts, strength, flexibility, impermeability and resistance to various environmental pressures, immunogenicity as also attachment possibilities by the glyocalyx which may explain the ephemeral nature of oocysts as noted in hydrological transport studies [46].

**Cryptosporidium parvum and environment (temperature, rainfall, aridity, etc.)**

*Cryptosporidium* is now recognized as one of the major causes of zoonotic human diarrheal diseases worldwide. Based on a large published data base, cryptosporidiosis is mainly a water-borne zoonosis but also food-borne, as infected animals' excreta reach water sources or food and human infection occurs through consumption or direct contact with humans (sexual) or animals (Figure 2). The best known case of massive human infection occurred in 1993 in Milwaukee, Wisconsin, USA, where drinking water originating in Lake Michigan and contaminated with *Cryptosporidium* oocysts bypassed the filtration system of one of the city's water treatment plants, causing an outbreak of watery diarrhea in more than 400,000 people [7].

Due to its environmental robustness and distribution, adequate mechanical and chemical treatments are required for oocyst removal from water prior to human consumption. The main process for oocyst removal is filtration through a large variety of matrices that strain these particles and thus obviate their further movement into clean water outlets. In this context, Parker and Smith [8] reported that shaking a mixture of *Cryptosporidium parvum* oocysts with sand particles can induce oocyst destruction, especially after addition of chlorine. Agitation of oocysts with sand for 5 minutes and subsequent chlorination for additional 5 minutes resulted in 68.02% inactivation. This phenomenon can be applied to large water filtration facilities, saving expensive processes used today to inactivate this parasitic protozoon.

The main season of oocysts outbreaks occurs during spring time when newborn calves are present and exposed to infections from infected cows or low hygienic conditions. People working in dairy farms are the first to be infected, followed by watershed contamination that may carry oocysts to long distance into drinking water sources.

Naumova et al., [9] proposed an analytical and conceptual framework for assessment of disease seasonality. Among six enterically transmitted reportable diseases (EDs) in Massachusetts, the authors quantified the timing and intensity of seasonal peaks of cryptosporidiosis incidence and examined the synchronization in timing of these peaks with respect to ambient temperature for a 10-year period. Relative to its incidence at recorded peak temperature (27-30°C), *Cryptosporidium* exhibited a significant delay of ~40 days in its incidence. The long delay was explained by the following attributes: different transmission routes, person-to-person infection amplification, pathogen environmental survival, incubation time, different disease manifestations and combination thereof. Hu et al., [10] also pointed at temperature as a significant factor involved in *Cryptosporidium* transmission in Australia through data analysis with two models: time series Poisson regression and seasonal auto-regression integrated moving average (SARIMA). Nevertheless, it should be remembered that during warm summer months people drink more and practice more outdoor activities, increasing the risk of infection.

In a more predictive study, Casman et al., [11] analyzed qualitatively the impact of climate changes on cryptosporidiosis. Heuristically they showed that increased temperature, variations in river flow and elevated water pollution could increase cryptosporidiosis in the USA. However, in a developed country like US with an advanced public health management and high standard of living, the negative effects of climate can be overcome, even in cases of climatic catastrophes.
In contrast, in a developing country like Indonesia, Katsumata et al., [12] applied a multiple logistic regression model to find out the significant risk factors for *Cryptosporidium* infection. Their results revealed that direct contact with animals (in this particular case, cats) increased precipitations, floods and socio-economical aspects (e.g. crowded living conditions) are indeed the significant risk factors that public health management has to deal with to prevent future outbreaks. An additional social aspect regarding cryptosporidiosis, not covered by Casman et al., [11] report, is the age factor, as children display a higher susceptibility to infections, especially in developing countries (with highest susceptibility among children < 2 years old) [12,13].

It is clear now that rainy seasons are a major factor in the spread of *Cryptosporidium parvum* and its high prevalence in the surrounding. However, rain has another impact on pathogen pollution, namely its resultant dilution factor [12,14,15]. If the main source of *Cryptosporidium parvum* is calf manure (with elevated oocysts output), rain can counteract the risk of oocyst spread through manure distribution by diluting its content. A direct consequence of such an event is additional contaminated water sources with this specific pathogen but at lower numbers at each site. Therefore, in order to contract an infection, a person has to be exposed to larger volumes of water. Proof of this perception was reported by Noordeen et al., [16] in Sri Lanka. The authors screened three agro climatic zones in rural communities to determine presence of various *Cryptosporidium* species among goats. Oocysts were detected in goats in all agro climatic zones, with highest prevalence in the dry zone (33.6%), followed by intermediate zone (24.7 %) and wet zone (21.7%) (p< 0.001). It is surprising that the drier zone had higher prevalence, since water is an important parameter in infection and its extent. Their logical explanation was that in dry regions, goats encounter nutritional stress under extensive management system without supplementary feed, and in consequence - lower immunity to parasitic infection. They also pointed to an additional environmental condition, e.g. crowding in sheds with low hygienic standards favoring a continuous fecal-oral transmission in those animals. A third possibility for the higher infection rate in the dry zone is detainment of higher concentrations of the parasite in excreta without the rain dilution factor, increasing the chance of infection based on infection dose.

In a more recent publication, Nichols et al., [17] reported on a case-crossover study comparing rainfall and outbreaks of different pathogens, among them *Cryptosporidium*, during an extended time span (from 1910 to 1999). Based on weather data, they found a significant positive correlation between excessive cumulative rainfall in the previous 7 days and pathogen outbreaks (p=0.001). Interestingly, low rainfall (< 20 mm) for three weeks prior to outbreaks was also highly correlated (p=0.002). Based on these results, these authors suggested that any climate change should examine both scenarios.

*Cryptosporidium in soil*

From the moment that oocysts are excreted in feces, they are expected to eventually reach soil environment and move along soil column to ultimately reach groundwater. Jenkins et al., [18] studied the survival of *Cryptosporidium parvum* oocysts in three soil types (silty clay loam, silt loam, and loamy sand) at different temperatures (4, 20, and 30°C) and water potentials (-0.033, -0.5 and -1.5 MPa). According to their experimental results, parasite survival in soil was not affected by the experimental water potential, but was affected to a certain extent by soil
results of this study revealed that Cryptosporidium parvum while using effluents for soil irrigation. Khashiboun et al., [22] determined the geographical factors that contribute to low temperature, including freeze–thaw cycles, can also occur as already pointed out by Robertson and Gjerde [19] in Norway, for infection through water and soil contamination. However, (the main parasite carriers) are penned, hence reduced chance related to disease prevalence, as during winter time, animals implicated animal-human infection path as the main factor (winter season) no such association was found [20]. The authors to maximum river flow. However, between December and March and November cryptosporidiosis rates were positively related were used as environmental factors. In general, between April and November cryptosporidiosis rates were positively related to maximum river flow. However, between December and March (winter season) no such association was found [20]. The authors implicated animal-human infection path as the main factor related to disease prevalence, as during winter time, animals (the main parasite carriers) are penned, hence reduced chance for infection through water and soil contamination. However, as already pointed out by Robertson and Gjerde [19] in Norway, low temperature, including freeze–thaw cycles, can also occur in England and Wales and should be considered Graczyk et al., [21] determined the geographical factors that contribute to watershed contamination with Cryptosporidium parvum in cattle farms in the flood plain area in Lancaster County, Pennsylvania, U.S.A. namely, large areas of ranches, stream crossings by herds and unlimited access and closeness to infected calves and manure that can provide a continuous supply of oocysts to water sources. As already mentioned, the waterborne characteristic of Cryptosporidium parvum should be taken into consideration while using effluents for soil irrigation. Khashiboun et al., [22] studied the fate of Cryptosporidium parvum oocysts in history- and non-history soils irrigated with effluents. Interestingly, they found that history soil (formerly irrigated with effluents) enhances oocyst migration and infiltration into soils, concluding that effluent irrigation requires excessive treatment in order to remove soluble organic matter and oocysts to prevent soil and groundwater contamination. Another aspect of water contamination with Cryptosporidium parvum oocysts is sewage discharge into seas or oceans. Besides their survival capability in sediments of these environments, oocysts of Cryptosporidium parvum can be taken up by a large variety of bi-valves living on the sea bottom, therefore jeopardizing the shellfish food industry. It was found that sewage-borne oocysts reaching sea water can survive for up to 4 weeks at a salinity of 30 ppt at 20ºC [23-25]. Therefore, shellfish can ingest and harbor infectious Cryptosporidium parvum oocysts for extended periods of time and can serve as mechanical vectors of this pathogen [26-29]. As already mentioned above, it can not be ruled out that this parasite can persist and multiply in shellfish similarly to the vertebrate gastrointestinal tract. From the gastronomic public health point of view, this fact is important as shellfish are mostly consumed uncooked. Finally, Gomez-Couso et al., [29] showed an interesting phenomenon upon which oocysts subjected to increased temperatures (as a result of solar water disinfection- SODIS method) oocyst up to 53.8% when exposed to a temperature of 46ºC for 12 hours. Environmental excystation of oocysts and release of unprotected sporozoites in the environment increases the inactivation rate of this pathogen, rendering this process an effective disinfection method.

**Cryptosporidium in water**

Excretion of large oocyst numbers by infected humans/animals is an important factor in water contamination (surface and groundwater). From the epidemiological point of view, oocysts (the infectious developmental stage) which reach water sources can be carried to long distances and disseminated to many people. Regularly, the main source of oocysts is infected cattle grazing in a riparian zone [30]. To further test this assumption, a Canadian study checked areas of restricted cattle access (RCA) versus unrestricted cattle access (URCA) in a riparian zone with an intermittent stream running through a small pasture [30]. As expected, the mean percent load reduction for Cryptosporidium for “all stream flows” was 321%for the RCA and 60% for the URCA. Riparian zone constraining a buffer vetiver zone was found to enhance deposition of sediment-adsorbed oocysts but not of non-adsorbed oocysts upslope of the buffer [31]. These preliminary results suggest that vegetation in a riparian zone may play a significant role in oocyst transport from a contamination site.

The second obstacle to oocyst journey from excretion to infection is the soil matrix structure and chemistry. When soil is involved, it is expected that soil particles as a matrix should serve as a potential barrier to oocyst movement and spread into groundwater sources. Harter et al., [32] characterized oocyst transport behavior in saturated, sandy sediments under strictly controlled conditions (laboratory columns). These authors found that oocysts, as colloids, are subject to velocity enhancement and travel 10-30% faster than a conservative tracer in medium and coarse sand columns. An important observation was that a significant portion of the initial deposition (retained filtered oocysts) is reversible “leading to significant asymmetry and tailing in the oocyst concentration breakthrough curve” being subject to time-dependent detachment!

These findings were also substantiated by others using different soil types: “an organic-rich (43-46% by mass) volcanic ash-derived soil from the island of Hawaii, and a red, iron (22-29% by mass), aluminum (29-45% by mass), and clay-rich (68-76% by mass) volcanic soil from the island of Oahu [33]. Oocysts and microspheres advecting through the red volcanic soil were almost completely immobilized (98% and 99% respectively) in 10-cm flow through columns. These authors’ conclusion was that volcanic ash soil could serve as a reservoir for subsequent groundwater contamination by colloids of the size of Cryptosporidium oocysts or smaller. Under extreme cases such as heavy precipitation, other factors may play an important role in oocysts transport and viability for watershed management,
The above experimental examples reveal the potential movement of oocysts in soil following contaminated discharge from humans or animals. However, an important factor is oocyst viability and infectivity as an infection source of contaminated water sources. To test these parameters, Nasser et al. [43] determined the effect of biotic and abiotic components of soil (saturated and dry loamy) on the viability and infectivity of Cryptosporidium parvum. Using immunofluorescence assay (IFA) and PCR (for viability) and tissue culture growth (for infectivity estimation), these authors showed that Pseudomonas aeruginosa played a role on digestion of the outer layer of the oocysts resulting in viability loss. Oocyst viability at 30°C in distilled water and in saturated soil was unchanged while infectivity dropped by one log. In dry loamy soil at 32°C (for 10 days of incubation) oocyst infectivity dropped by 3 log orders without any change in viability.

Previous die-off studies revealed that at low temperatures there is an agreement between viability and infectivity of Cryptosporidium parvum, with extended viability/infectivity. Under semi-arid conditions, desiccation and increased temperatures enhanced infectivity loss of oocysts. These results can be used for future management of wastewater reuse in warm environments to reduce health risks of effluents irrigation.

**DISCUSSION AND CONCLUSION**

Cryptosporidium is a sophisticated protozoan parasite that exhibits various characteristics which support its extended survival in environment:

1. An environmental extensively durable stage in form of thick-walled oocysts, which beside their sturdiness are also sticky, allowing additional spread.
2. Due to their elasticity, they can pass through filtration systems (especially in faulty ones, containing channeling) and reach drinking water.
3. Can survive very well in soil environment at largely varying temperatures, especially in moist climates. However, they can be mechanically inactivated by shaking with soil/sand particles combined with chlorine.
4. Irrigation of vegetables with untreated effluents is a major source of Cryptosporidium infection, especially with "fairy" vegetables such as zucchini.
5. The new relocation of Cryptosporidium as Gregarinia, which includes free living stages, enables host-free multiplication, thereby constituting an additional potential risk factor for human and animal infection. This risk is enhanced by the potential exocytosis of oocysts, with release and deposition of sporozoites unto biofilms encountered both in the intestine and in nature, enabling further development of its life cycle and additional enhancement of parasite numbers in a variety of surroundings.

**REFERENCES**

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