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Review Article

The Role of Cryptosporidium Parvum in Waterborne Disease Outbreaks

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Abstract

Water-related diseases, particularly waterborne diseases, remain significant sources of morbidity and mortality worldwide but especially in developing countries. waterborne pathogens represent a major health risk. Cryptosporidium is one such pathogen which is globally recognized as a major cause of diarrhea in children and adults. The objective of this paper is not only to review published studies on the impact of these emerging waterborne pathogens but also to identify the various risk factors that favor their transmission. A number of envisaged and needed actions to tackle the challenge of these pathogens in Africa have also been discussed. We have searched the web of ScienceDirect, PubMed, Scopus. ISI Web of Science, Springer Link, and Google Scholar to prevent cryptosporidiosis in humans and animals, we need to understand better how the disease is spread and transmitted, and how to interrupt its transmission cycle. This review focuses on understanding cryptosporidiosis, including its infective stage, pathogenesis, life cycle, genomics, epidemiology, previous outbreaks, source of the infection, transmission dynamics, host spectrum, risk factors and high-risk groups, the disease in animals and humans, diagnosis, treatment and control, and the prospect of an effective anti-Cryptosporidium vaccine. It also focuses on the role of the One Health approach in managing cryptosporidiosis at the animal–human–environmental interface. The summarized data in this review will help to tackle future Cryptosporidium infections in humans and animals and reduce the disease occurrence.

Key words: cryptosporidium; waterborne disease; waterborne parasite; epidemiology

Introduction

Cryptosporidiosis is an enteric disease caused by a protozoon parasite belonging to the genus Cryptosporidium. It is one of the most prevalent waterborne diseases and the leading cause of waterborne disease outbreaks worldwide (Helmy and Hafez, 2022). Cryptosporidium is a unicellular parasitic protozoan in the phylum Apicomplexa. Although considered a member of coccidia, evidence indicates that it has a closer affinity with gregarines, a large group of Apicomplexa considered particularly primitive (Robertson et al., 2020). Cryptosporidium causes up to 20% of all cases of diarrhea in children in developing countries and causes fatal complications in HIV-infected persons. Cryptosporidium is also responsible for more than 8 million foodborne illness cases worldwide annually. Cryptosporidiosis primarily affects people who are living in rural and in urban slums, where there is a high probability of disease transmission and spread (Helmy and Hafez, 2022). Cryptosporidium has now emerged as a leading cause of diarrhoeal illness worldwide, posing a significant threat to young children and immunocompromised patients. It has been reported to be a leading cause of moderate-to-severe gastrointestinal morbidity in children younger than 5 years in developing countries. A recent study into the global burden of gastrointestinal disease found that Cryptosporidium spp. accounted for in excess of 1 million deaths, almost half a million of which were in children under the age of five, and over 71 million disability-adjusted life years (DALYs) between 2005 and 2015; the highest mortality rates were observed in developing countries, particularly those in sub-Saharan Africa (O'Leary et al., 2021).The human medical importance of Cryptosporidium was highlighted in 1982, after the CDC report on Cryptosporidium-induced diarrheas in patients infected with Human Immunodeficiency Virus (HIV).

The international interest in Cryptosporidium as a public health problem began in 1993 after the largest global waterborne outbreak, when more than 400,000 inhabitants in Milwaukee, Wisconsin, USA were infected with C. hominis due to the consumption of contaminated drinking water (Thompson et al., 2008). In developing countries, children under five years old are the most affected groups with Cryptosporidium. The oocysts can survive outside the host for several months and retain infectivity, despite adverse environmental conditions such as salinity and the presence of chemicals (Helmy and Hafez, 2022). For many years, only a single species, C. parvum, was really noted as the cause of human cryptosporidiosis, with C. hominis not recognized as a separate species until 2002. Among the 30 or so Cryptosporidium species now identified, C. parvum is considered of substantial veterinary relevance to young livestock (calves and lambs), being considered as one of the most important causes of neonatal enteritis in young ruminants globally (Robertson et al., 2020). To date, there are no effective chemotherapeutics for the treatment of cryptosporidiosis. Nitazoxanide and halofuginone in humans and animals are the approved drugs against Cryptosporidium infection. However, their application does not guarantee treatment efficacy (Brainard et al., 2021). Molecular biology techniques have enabled the description of species that are highly host-specific, as well as others that are capable of infecting many hosts, Cryptosporidium parvum is considered to be the most prevalent species worldwide and a major zoonotic transmission risk, using molecular approaches to genetically characterise Cryptosporidium spp. has facilitated an improved understanding of cryptosporidiosis epidemiology (Xiao, 2010). Subtype analysis using the C. parvum 60 kDa glycoprotein locus (gp60) has revealed both human- and zoonotic-specific subtypes (Mammeri et al., 2019).

2. Life Cycle and Developmental Stages of Cryptosporidium

Cryptosporidium belongs to the Coccidia class of the phylum Apicomplexa. Cryptosporidium have some features which differentiate them from all other Coccidia , including (1) intracellular and extra-cytoplasmic localization, (2) forming of a “feeder” organ, (3) presence of morphological (thin- or thick-walled) oocysts as well as functional (auto vs. new-infection) types of oocysts, (4) small size of oocysts, (5) missing some morphological characteristics such as sporocysts or micropyles, and (6) the resistance of Cryptosporidium to all the available anti-coccidial drugs (Smith and Corcoran, 2004). cryptosporidium has a complex monoxenous life cycle, which is divided into two phases: the asexual phase (sporogony and schizogony/ merogony) and the sexual (gamogony) phase. They proliferate and differentiate during the invasion of the free-living stages of Cryptosporidium within the parasitophorous vacuole under the brush border of the host cell located outside the cellular cytoplasm (Leitch and He, 2011). Cryptosporidium parasites can then attach to the cell surface and move along it for a short time using gliding mobility before they start to enter the cell. Cryptosporidium does not completely invade the cells actively, but they provoke the cells to embrace them with a host-cell-derived membrane. Additionally, at the parasite–cell interaction phase, the Cryptosporidium creates an actin-rich disk, a feeder organelle responsible for nutrition intake, as well as a channel into the cytoplasm of the host cell (Lendner and Daugschies, 2014). After Cryptosporidium internalization in the host cells, the sporozoite divides inside the parasitophorous vacuole to approximately 4 µm × 4 µm in diameter as a spherical trophozoite with an excentric cell nucleus. After three asexual divisions (merogony/schizogony), the trophozoite is divided into 5 µm × 5 µm large type-1 meront, which contains eight merozoites. The merozoites and the sporozoites are similar in shape and size; however, the nucleus of the merozoites is located more centrally to the cell compared to the sporozoites. Upon leaving the parasitophorous vacuole, the merozoites begin their asexual development cycle in the epithelial cells and develop Type-I meronts again, then the trophozoite. Otherwise, the merozoites initiate the sexual development cycle through differentiation to type-II meronts. Inside the meront, four merozoites develop by asexual division and after infection of further enterocytes, they are divided into micro- and macro-gametes (gamogony). The immature micro-gamontes are spherical, 5 µm × 4.5 µm in diameter, contain up to 16 peripherally located compact cell nuclei, and are precursors of the developing micro-gametes (Figure 1) (Mohamed, 2014). They also have stubbed front ends and cell nuclei with no flagella. The mature micro-gametes leave their host cell and fertilize the macrogametes. Macrogametes are spherical, 5 µm × 5 µm in diameter and contain granulated cytoplasm and eccentrically positioned wall-forming bodies. Tandel et al. have suggested the direct development of gametes from type I meronts (Tandel et al., 2019). The zygote grows by syngamy and then goes through sporogony-a meiosis-like process. The oocysts (thin- or thick-walled) with 4 haploid sporozoites (sporulated oocysts) develop inside the parasitophorous vacuole (Figure 1). Thin-walled oocysts (about 20%) excystate in the host intestinal tract, leading to endogenous autoinfection, and the thick-walled oocysts (about 80%) are extremely resistant to several disinfectants, are excreted with the feces to the environment and can survive outside the host for a long time (Helmy and Hafez, 2022). The thick-walled oocysts represent the exogenous stage of the Cryptosporidium parasite. Cryptosporidium oocysts are approximately 4µm× 6µm in diameter, spheric to ovoid shape, have a residual body, and four banana-like or comma-shaped sporozoites with a pointed front end and a stubbed hind end, where the nucleus is localized. The host cell surrounds the sporozoites with membrane protrusions and forms a parasitophorous vacuole in the brush border of the enterocyte. Interestingly, the localization of the parasitophorous vacuole by Cryptosporidium spp. is different from that of the other Apicomplexa; thus, Cryptosporidium spp. localization is described as intracellular, but extracytoplasmic. Additionally, the feeder organelle develops at the sporozoite and host cell membrane contact point. They supply the maturing parasite with nutrients and facilitate internalization (Helmy and Hafez, 2022).

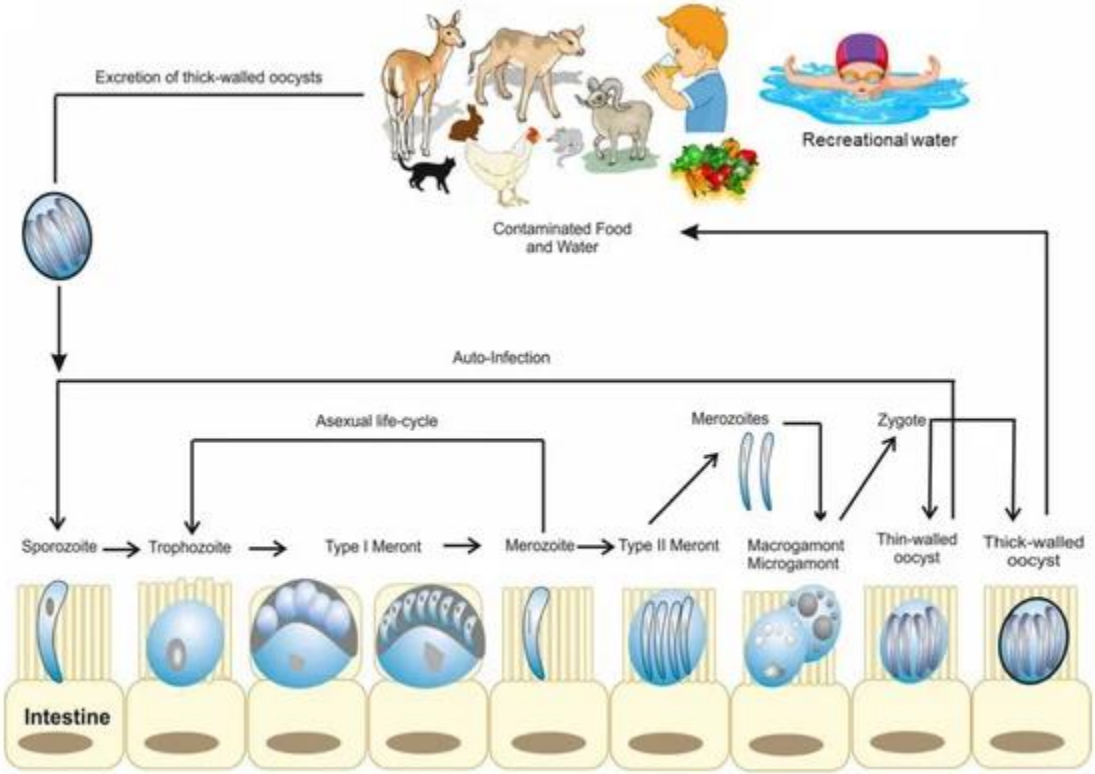

Figure 1: Life cycle and developmental stages of Cryptosporidium in animals and humans (Mohamed, 2014).

The infectious stage (sporulated oocyst) of Cryptosporidium was reported to be excreted in large numbers in the feces of experimentally infected calves (up to 4 × 107 oocysts per gram of feces), or excreted with the bronchial exudates in the case of respiratory cryptosporidiosis and which immediately contaminated the environment (Sponseller et al., 2014). The sporulated oocysts are very resistant to environmental factors and only a few chemical disinfectants show efficacy against the sporulated oocysts due to their thick wall. Therefore, it is difficult to completely remove the Cryptosporidium oocysts from contaminated drinking water. The thick wall oocysts are sporulated and are infectious when shedding, which can result in immediate infection of new hosts. The infectious dose of Cryptosporidium oocysts for humans is about nine oocysts per Cryptosporidium isolate and about 50 oocysts for calves. However, it was reported that 1 to 10 oocysts of Cryptosporidium caused infection for some individuals during the Milwaukee outbreak (Helmy and Hafez, 2022).

3. Pathogenesis of Cryptosporidium

After ingestion of the thick-walled oocyst with food or water by the host, many signaling molecules are expressed on the sporozoite surface that mediate their attachment and invasion to the host cells. Calcium-dependent protein kinases (CDPKs) were reported to be involved in the regulation of the invasion process of the sporozoite to the host cell (Etzold et al., 2014). After attachment and invasion of Cryptosporidium, the host–parasite interactions play an important role in pathogenesis. In calves, C. parvum causes acute to chronic catarrhal enteritis that begins in the distal ileum; however, different Cryptosporidium developmental stages were also detected in the duodenum, colon, and part of the cecum. The affected mucosa is hyperemic and edematous and the mesenteric lymph nodes are partially enlarged and edematous Histologically, mild to moderate villus atrophy associated with occasional villus fusion was observed. The affected crypts are partially dilated and contain neutrophil granulocytes. The lamina propria mucosa also had neutrophil granulocytes and a large mononuclear cell infiltration In the infected host, epithelial cell degeneration, metaplasia of physiological high prismatic to isoprismatic villus epithelial cells, hyperplastic crypt epithelium, displacement of microvilli in the area of the intracellular parasite stages' attachment zone, and long microvilli can be seen in the vicinity of the parasite stage (Helmy and Hafez, 2022). These pathological alterations result in the reduction of the intestinal absorption surface and, consequently, malabsorption. Damage to the intestinal epithelium may also have an impact on the activity of brush border membrane enzymes (glucoamylase, alpha-dextrinase, saccharase, lactase), resulting in a reduction in the small intestine's carbohydrate digestion ability. As a result, osmotically active particles persist in the intestinal lumen, osmotic diarrhea develops, and water resorption is impeded. Several causes can lead to increased chloride secretion into the gut lumen, including immune response to membrane injury, prostaglandins secreted by enterocytes of intra- and sub-epithelial lymphocytes, and plasma cells and macrophages that enhance blood vessel permeability (Helmy and Hafez, 2022).

4. Epidemiology

The first waterborne cryptosporidiosis outbreak was reported in 1993 in Milwaukee, Wisconsin (USA), with an estimated 403,000 people affected, 4400 hospitalizations, and more than 100 deaths. The zoonotic transmission of Cryptosporidium can take place via direct contact with an infected person and/or consumption of contaminated drinking water or food and/or inhalation of oocysts from contaminated air with aerosolized droplets or fomites (Karanis, 2018). There are multiple factors leading to human cryptosporidiosis and the occurrence of outbreaks, such as [1] contaminated drinking water, and unclean recreational/swimming pool water, [2] contaminated foods such as raw fruits and vegetables that were fertilized with contaminated effluent, [3] contact with infected people (hospitals, daycare centers, schools), [4] contact with infected animals (especially calves), and [5] anal sexual contact (Helmy and Hafez, 2022). Even though

cryptosporidiosis is primarily a water-based illness, the risk of foodborne transmission is well known. Food contamination with Cryptosporidium oocysts can occur during food (vegetables, fruits, seafood, and meat) manufacturing, processing, and preparation. The oocysts' resistance can help them survive various processing procedures, such as chlorine baths and blast freezing (Duhain et al., 2012). Calves usually become infected with cryptosporidiosis by ingestion of oocysts from the contaminated environment. There are many possible sources of infection including [1] shedding of infected neighbor animals, [2] contaminated stables, [3] dirty udders and teats of cows, and [4] contaminated water. The subclinical infected adult cattle act as oocysts shedders (Helmy and Hafez, 2022). Therefore, they are considered a potential reservoir for infection. Furthermore, Cryptosporidium infection can be also transmitted by animal handling personnel through dirty shoes and clothes as well as via infected dogs, cats, rodents, wild animals, insects (flies, cockroaches, and beetles), and free-living amoeba (Pumipuntu and Piratae, 2018). There is variation in the tendency of Cryptosporidium species to infect calves in an age-dependent manner. For example, C. parvum is the most prevalent species in calves up to 8 weeks old, while C. bovis is dominant in calves ranging between 2 to 11 months of age (Helmy and Hafez, 2022).

5. Diagnosis

Given the broad spectrum of susceptibility and the significant morbidity and mortality rates associated with Cryptosporidium in immunosuppressed patient populations, the development of efficient and effective screening criteria and robust testing algorithms is vital in clinical laboratories. However, there remains no international standard methods for the diagnosis of cryptosporidiosis. In some countries Cryptosporidium testing is limited to known HIV/AIDS patients, however, testing of adult samples is usually reliant upon stipulating factors such as watery or persistent diarrhoea, and when clinically suspected (Chalmers, 2008). In many countries, such as the United States and France, Cryptosporidium screening is not a routine component of standard "ova plus parasite" examinations carried out in clinical laboratories regardless of patient age, clinical and epidemiological evidence, unless specifically requesting by a clinician or recommended by the laboratory directorate. Thus, further contributing to the under reporting of cases, Expertise in the field of stool microscopy is declining among the modern clinical laboratory workforce, particularly in areas of decreasing prevalence of faecal parasites. While a number of studies have reported that molecular methods are employed by only a minority of routine clinical microbiology laboratories in Europe and the US (O'Leary et al., 2021). A summary of the advantages and disadvantages associated with the various diagnostic methods discussed herein are outlined in Table 1.

Diagnostic Test	Advantages	Disadvantages
Microscopy	Relatively low cost Widely available	Poor sensitivity Time consuming Skilled microscopist essential
Immunoassay based methods	Good sensitivty Wide variety of kits available Convenient adjunct to microscopic analysis	Not widely available in developing countries due to cost constraints and limited detection spectrum of kits False positives
Molecular/Nucleic acid amplification methods	Exceptional sensitivity Capable of species and subspecies identification Option to mulitiplex detection of several enteric pathogens	Expensive reagents and instrumentation required Requires skilled technician

Table 1: Advantages and disadvantages of microscopic, immunological and molecular diagnostic methods for Cryptosporidium spp. (O'Leary et al., 2021).

5.1. Brightfield and fluorescent microscopy

Faecal investigation for the presence of shed oocysts or antigens is the diagnostic mainstay in Cryptosporidium detection (Manser et al., 2014). Conventional clinical diagnosis has largely relied on microscopic examination of tinctorially or fluorescently stained faecal smears. The acid-fast properties of Cryptosporidium were demonstrated in 1981, with the development of a modified Ziehl-Neelson (mZN) stain for differential staining. Prior to this Cryptosporidium was largely identified through Giemsa staining of histological preparation of intestinal biopsy samples, with iodine, trichrome and iron haematoxylin stained faecal specimens yielding poor results, a variety of stains including the acid-fast Kinyoun's stain and differential stains such as the hot safrinin-methylene blue stain have also been employed by clinical laboratories , acid-fast staining, particularly the mZN technique, predominates in clinical laboratories . However, although there is a marked contrast between the red stained oocyst against the green background counterstain of the mZN, yeasts, fungal and bacterial spores may be erroneously identified as oocysts, Cryptosporidium targeting, immunofluorescent monoclonal antibodies (MAb) were initially introduced almost three decades ago following the advent of hybridoma technology, which allowed for the generation of highly specific antibodies (O'Leary et al., 2021). Comparative studies have found the sensitivity and specificity of immunofluorescent techniques to outweigh the sensitivity and specificity exhibited by conventional bright field staining techniques. Additionally, indirect immunofluorescent techniques, although requiring an additional incubation step, have been reported to possess similar levels of sensitivity and specificity to those of their direct counterparts (O'Leary et al., 2021).

5.2. Enzyme immunoassays (EIA), ELISA and immunochromatographic methods

Faecal-antigen diagnostic techniques have been developed in order to obviate the need for skilled microscopists, laborious methodologies and specialised equipment, such as fluorescent microscopes, while also accommodating batch testing requirements (Helmy et al., 2014). Comparative studies investigating the diagnostic utility of EIA and ELISA kits have found that they provide significantly improved sensitivity (94 - 100%) and specificity (93 -100%) over conventional acid-fast staining methods (Parghi et al., 2014). Immunochromatographic kits provide a detection system that surpasses enzyme-based methods in terms of rapidity by eliminating the need for additional reagent additions, washing steps and incubations, Antigen migration via capillary action allows detection of Cryptosporidium antigens by a discrete, colloidal dye labelled antibody

impregnated in a line assay, permitting objective antigen detection (O'Leary et al., 2021). Immunoassay based kits also offer a reduced diagnostic spectrum, as many are tailored solely for the detection of C. parvum and C. hominis. Therefore the clinical utility of such kits is limited in regions where alternative Cryptosporidium species are attributable to a significant number of cryptosporidiosis cases (O'Leary et al., 2021).

5.3. Molecular approaches

PCR detection of Cryptosporidium has been proven to be more sensitive than conventional microscopic and immunological methods, while also permitting batch testing, species and sub-species identification of detected organisms, A sequence survey identifying >250 kb of the C. parvum genome heralded the beginning of the genomic era of Cryptosporidium research in the late 1990s (O'Leary et al., 2021). Given the clinical significance and the dearth of epidemiological and molecular Cryptosporidium data at the time, the National Institute of Allergy and Infectious Diseases (NIAID) subsequently allocated funding to a consortium of three American universities, which were tasked with sequencing both C. parvum and C. hominis genomes (Widmer and Sullivan, 2012). The C. parvum and genome sequencing projects enabled the identification of a number of highly polymorphic micro- and minisatellite loci and conserved loci flanking sequences (O'Leary et al., 2021). These sequences permitted the development of microsatellite and minisatellite locus-specific PCR assays for both species regions, thereby permitting genotyping superior to that of RFLP, and ultimately the development of a technique that is also capable of identifying the subtleties of intra-species differentiation (Xiao and Feng, 2017). Within the Cryptosporidium genus and more specifically among the predominant human-pathogenic species, C. parvum and C. hominis, asexual and sexual life cycle stages, genetic recombination and selective pressures, such as parasite-host coevolution, host adaptation and geographic segregation, have led to generation of new subtype families and diverse genetic populations (Garcia-R and Hayman, 2017). gp60, which is firmly established as a key marker of genetic variation within Cryptosporidium spp. is subject to selective pressure which has resulted in a lack of global sub-structuring, with the same gp60 alleles emerging in different locations globally. Thus, gp60 is not a sufficient descriptor of population structure to enable single locus typing. Multi-locus genotyping (MLG) is necessary to adequately assess genetic variation and population structures within Cryptosporidium spp. (O'Leary et al., 2021).

6. Prevention and Treatment of Cryptosporidium Infection

The “One Health” approach is a worldwide strategy that is used to mitigate zoonotic diseases and improve health by preventing infection occurrence at the human–animal–environment interface. Collaboration between all health sectors (veterinarians, occupational health physicians, and public health operators) can help in infection control by enhancing the educational system, status of thinking, legislation, and administrative structures. The One Health approach has been previously proposed to tackle cryptosporidiosis as well as other zoonotic diseases (Fawzy and Helmy, 2019).

6.1. Preventive Measures for Cryptosporidium Infection

Due to the absence of effective treatment, the prevention of cryptosporidiosis relies mainly on the elimination and/or reduction of contamination of the environment with infectious oocysts. In general, several physical stresses can affect Cryptosporidium oocysts including irradiation, heat, cold, pressure, and desiccation. The infectivity of C. parvum oocysts at different temperatures is due to the carbohydrate energy reserve of the sporozoites, and the residual bodies including amylopectin (which helps in the excavation process and the host–cell invasion) granules which are used quickly at higher temperatures (Helmy and Hafez, 2022). Increasing the temperature to 64.2 °C or more for 5 min and 72.4 °C for 1 min renders the oocysts non-infectious. Even in the presence of cryoprotectants, C. parvum oocysts can survive at −20 °C for prolonged periods, but not at −70 °C or below (Helmy and Hafez, 2022). However, ultraviolet (UV) irradiation can render Cryptosporidium oocysts non-infectious, the most effective disinfectants against Cryptosporidium oocysts are those that contain chlorine dioxide, hydrogen peroxide, or ammonia. Although high concentrations and longer exposure to chlorine-, bromine-, and iodine-related compounds can decrease the infectivity of the oocysts, they are limiting their practical applications. Ozone is one of the most effective chemical disinfectants against Cryptosporidium and can be used against Cryptosporidium oocysts in water (Fayer and Xiao, 2007). It has also been reported that rotifers, which occupy rivers, lakes, seawater, and ponds, and predacious protozoa, can ingest oocysts of C. parvum. Some rotifers were found to discharge oocysts in boluses containing a mixture of other eaten components, and therefore they can be used for Cryptosporidium oocyst control in water (Helmy and Hafez, 2022).

6.2. Treatment of Cryptosporidium Infection

Vaccines Development

Currently, there are no available vaccines to control Cryptosporidium infection in humans and animals (Innes et al., 2020). There is a critical need to develop vaccines, particularly for high-risk groups such as young children, malnourished populations, and immunosuppressed persons. It has been reported that vaccinating mother cows against other diarrhea-causing pathogens such as rotavirus, coronavirus, and E. coli may protect against Cryptosporidium infection in calves via colostrum, thus helping the calf to resist the infection during the first weeks of age. To develop an effective vaccine, there is a need to understand the host immune response to infection and the host–parasite interactions as well as understand the innate and adaptive host response (Ryan et al., 2016). Several trials to produce effective vaccines against cryptosporidiosis have been carried out. It was reported that miRNA plays a crucial role in the protection of the host cell against Cryptosporidium and the regulation of miRNA expression levels in epithelial cells (Helmy and Hafez, 2022). the vaccine that contains multiple dominant antigens may enhance protection against the infection. For example, it was reported that cp23 plus cp15 divalent vaccine prolonged the prepatent period and reduced the shedding of the oocyst compared to vaccination with cp23 alone in mice (Liu et al., 2010). Furthermore, serum antibodies to both cp23 and gp15 protected diarrhea in immunocompetent persons infected with Cryptosporidium (Frost et al., 2005). Collectively, the ideal vaccine should (1) provide lifelong immunity in the vaccinated population, (2) protect against species and subtypes of Cryptosporidium to assure cross-protection against the most common species infecting humans, and (3) prevent Cryptosporidium transmission (Mead, 2014).

7. Discussion

Apicomplexa undergo a cascade of developmental changes as they transition through their life cycles. A complex succession of morphological types is specifically adapted to the tasks of invasion and intracellular replication in different hosts, organs, and tissues. As apicomplexans are single celled organisms, differentiation is not terminal or rigidly inherited, but rather a continuous flow, in which each generation elaborates a transient fate. Edward Tyzzer in his initial description of the Cryptosporidium muris life cycle identified 3 intracellular stages: microgamonts that produced 16

microgametes, macrogamonts that produced single macrogametes, and asexual schizonts. He commented that "the number of merozoites produced in this process of schizogony is almost invariably eight" (English et al., 2022); he also described fertilization and oocyst formation resulting in parasite stages containing 4 sporozoites. The concept of the tetraploid type II meront as a developmental intermediate between the asexual and sexual reproduction was introduced by John Vetterling in 1971 after studying Cryptosporidium wrairi in guinea pigs. This concept might have been inspired by his extensive work on Eimeria in various animals where distinct meront types occur. At the time, Cryptosporidium and Eimeria were seen as closely related members of the Coccidia (a phylogenetic view no longer held (English et al., 2022). Vetterling's 2 meront model has been cited widely since and has become the text book life cycle for Cryptosporidium. The core of the argument between these authors was how to interpret the tetraploid intracellular parasites found in infected animals and cultures. Are they mature meronts that will yield 4 merozoites committed to sexual differentiation, or are these immature stages that will undergo further nuclear divisions or form oocysts? This question was difficult to resolve using fixed samples, and we therefore chose to study living cells. We documented the fate of more than a thousand tetraploid parasites by time-lapse microcopy, and our observations are entirely consistent with Tyzzer's original assertion that all meronts produce 8 merozoites—we find no evidence for a type II meront. Molecular markers that report on parasite cell cycle progression further refute type II meronts in culture and in infected animals. We note that we have not tested C. wrairi, the guinea pig parasite Vetterling used in his original work; however, for C. parvum, the most widely studied species of this parasite genus, we demonstrate a simple and direct life cycle of only 3 morphologically distinct intracellular stages: meronts that yield 8 merozoites, male gamonts, and female gametes (English et al., 2022).

8. Conclusion

Cryptosporidium is one of the water- and foodborne pathogens with socioeconomic and public health importance worldwide. The infection is characterized by high morbidity and high mortality. Cryptosporidium infection is ubiquitous and has a high prevalence in animals and humans. Children under 5 years of age and immunocompromised individuals are the most susceptible groups to infections. Cryptosporidiosis in animals has become more common because of environmental contamination in livestock production. Cryptosporidium infection can be transmitted directly via drinking/ingestion of contaminated water or food with sporulated oocysts. Most of the foodborne outbreaks associated with Cryptosporidium are zoonotic. To prevent disease outbreaks, routine surveillance systems and the application of the One Health approach are required. Food safety and water sanitation are required to prevent and/or reduce future outbreaks worldwide. Each of the available diagnostic tools has its limitations in terms of isolation, detection of co-infections with other pathogens, and cost. In developing countries, the true burden of cryptosporidiosis is underestimated and underreported due to the limitation of diagnostic tools, which results in ineffective clinical and public health management of the disease. Therefore, there is a critical need to develop rapid, reliable, and cost-effective diagnostic tests to improve the detection, reporting, and interpretation of results. Cryptosporidium infection prevention and control can be achieved via understanding the sources of the infection (humans and animals), the routes of transmission, the oocyst survival in the environment, and the risk factors. Currently, no effective drugs or vaccines are available to treat and/or prevent infection in animals and humans. There is also a critical need for further studies for the development of effective vaccines.

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