Curr Trop Med Rep (2015) 2:161–170
DOI 10.1007/s40475-015-0057-8

PROTOZOA (L BARTELT, SECTION EDITOR)

Challenges and Innovative Strategies to Interrupt *Cryptosporidium* Transmission in Resource-Limited Settings

Amidou Samie[1] · **Ahmed Al-Qahtani**[2] · **Ali El Bakri**[3] · **Beeta Ehdaie**[4]

Published online: 23 July 2015

Abstract In addition to *Cryptosporidium* being recognized as a very important cause of morbidity and mortality among humans, it is also an important economical problem with hundreds of outbreaks reported throughout the world every year and in agriculture where it affects mostly young animals. Transmission of *Cryptosporidium* is often by oral route through water and food. Although *Cryptosporidium* is most prevalent in resource-constrained areas, the majority of studies on the disease transmission have been conducted in developed countries. The control of *Cryptosporidium* has progressed over time, and with the development of new and more powerful methods in diagnostics and genomics, it has become much easier to detect *Cryptosporidium* and conduct genotypic analysis which could help to identify the sources of infection via anthroponotic (*Cryptosporidium hominis*) or both anthroponotic and zoonotic (*Cryptosporidium parvum*) routes. While detection methods have improved, determining *Cryptosporidium* viability and, therefore, effectives of inactivation remains a challenge. Control measures are directed at reducing and/or preventing transmission of the infective oocysts to the humans and animals. The tough nature of the oocyst makes traditional water treatment processes such as coagulation/flocculation, sedimentation, filtration, and disinfection insufficient to remove the parasite. Nitazoxanide, the only FDA-approved drug for the treatment of cryptosporidiosis, may reduce oocyst shedding and can lead to both clinical and microbiological cure, but it is ineffective in HIV-positive and AIDS patients, and studies in malnourished children have mixed results. The investigation of the importance of traditional medicines used commonly in developing countries has undetermined efficacy, because of the lack of validated experimental models. Collaborative research between developed and developing countries is critical for the advancement of our understanding of the control measures to be used to stop the transmission of *Cryptosporidium*. An example of an international multidisciplinary partnership between two research institutions addressing innovative means toward interrupting *Cryptosporidium* transmission is discussed.

Keywords *Cryptosporidium* · Waterborne · Prevention · Transmission · Limited resource setting · Global partnerships

This article is part of the Topical Collection on *Protozoa*

✉ Amidou Samie
samie.amidou@univen.ac.za

Ahmed Al-Qahtani
ahmed.qahtani@khawarizmi.com

Ali El Bakri
aelbakri@sharjah.ac.ae

Beeta Ehdaie
be7v@virginia.edu

[1] Molecular Parasitology and Opportunistic Infections Program, Department of Microbiology, University of Venda, Private Bag X5050, Thohoyandou 0950, South Africa

[2] Health Sciences Department, Al Khawarizmi International College, Abu Dhabi, UAE

[3] Medical Laboratory Sciences Department, University of Sharjah, PO Box 27272, Sharjah, UAE

[4] Department of Civil and Environmental Engineering, University of Virginia, Charlottesville, VA, USA

Introduction

The first case of human cryptosporidiosis was seen in 1976 related in a 3-year-old girl from rural Tennessee who suffered severe gastroenteritis for 2 weeks [1]. *Cryptosporidium* was later recognized as a waterborne pathogen during an outbreak in Braun Station, Texas, where more than 2000 individuals

were afflicted with cryptosporidiosis [2]. The parasite continues to cause a number of waterborne disease outbreaks in the USA and other countries every year [2–4]. More recently, *Cryptosporidium* has emerged as an important enteric pathogen worldwide [5], and a growing population of immunocompromised persons and the continued outbreaks of cryptosporidiosis in recreational water have placed an even greater emphasis on this pathogen. The parasite defies water and health authorities by its ability to withstand chlorine disinfection and filtration [5, 6]. *Cryptosporidium* is primarily transmitted by either direct fecal oral route or ingestion of food or water contaminated with *Cryptosporidium* oocyst. Both the anthroponotic (*Cryptosporidium hominis* and *Cryptosporidium parvum*) and zoonotic (*C. parvum*) species present unique public health challenges. These two species show geographic differences (Table 1) in their distribution as a cause of human infection; for example, *C. parvum* is reported to be more common in the UK while *C. hominis* is more common in the USA [3]. *Cryptosporidium* infections have gained more attention in the past 20 years as clinically important human pathogens.

The taxonomic status of *Cryptosporidium* has been undergoing rapid changes because of new information that is supported primarily by molecular data [4]. Molecular data suggests that *Cryptosporidium* may be more closely related to gregarines, a group of apicomplexans in invertebrate that share a similar life cycle (see Fig. 1 Korpe et al., 2015) than other intestinal coccidians (such as *Cyclospora* and *Cystoisospora*) [2]. There are 26 species classified within the *Cryptosporidium* genus [7]. However, due to the similar spherical shape of the oocyst, microscopic size, and obscurity of internal structures, morphology is not reliable for delineating species within the *Cryptosporidium* genus [8]. In fact, the oocyst morphology trait is regarded as insufficient in the absence of biological and molecular data [9].

The infectivity of *Cryptosporidium* oocysts has shown to be very high although it is still not clear what the exact dose required to induce infection is. The probability of transmission from a small amount of contamination is fairly high as supported by DuPont et al. (1995) who determined that the infective dose of *Cryptosporidium* is only 132 oocysts for healthy individuals with no previous serological immunity to cryptosporidiosis [10]. In a comparative study by Haas and Rose (1994) of oocyst levels in treated water during identified waterborne outbreaks and conditions under which no outbreaks were detected, they suggested that action be taken at a level of 10–30 oocysts or higher per 100 L of treated water [11]. In a different study by DuPont et al. (1995), it was found that the median infective dose in 29 healthy volunteering subjects was 132 oocysts [12]. However, Haas and Rose (1994) further suggested that some individuals may develop cryptosporidiosis after ingestion of only one oocyst. The latter could be attributed to the autoinfection stages of the life cycles of *Cryptosporidium* leading to low of infective doses required to induce infection [11].

Propagation of *Cryptosporidium* in Reservoir Hosts

Cryptosporidium species affect primarily the ileum of humans and livestock with the potential to cause severe enteric diseases [13]. Humans and animals function as both definitive and reservoir hosts for *Cryptosporidium*. Figure 1 shows oocysts recovered from stool samples and identified using modified Ziehl-Neelson (MZN) acid-fast staining method, which is a commonly used differential staining method along with safranin-methylene blue stain, modified Kinyoun's acid-fast method, and DMSO-carbol fuchsin stain [14]. Infection begins when the ingested oocysts release sporozoites, which attach, adhere, and then invade the intestinal epithelial cells where replication occurs. The parasite possesses a number of surface glycoproteins, which are thought to play a role in the parasite pathogenesis including importance for attachment and invasion into the host cell (see accompanying article by Ludington and Ward).

Mechanisms whereby propagation and eventual shedding occur remain uncertain but have been investigated using in vitro systems. Cell damage in enterocyte monolayers is caused due to disruption of tight cell junctions, a loss of barrier function, the release of lactate dehydrogenase, and increased amount of cell death [15]. Several molecules may aid in direct tissue damage, such as phosholipases, proteases, and hemolysins [11, 15]. Proteases play an important role in the initial stages of *Cryptosporidium* infection [16, 17] and mediate protein degradation, invasion of host tissues, and evasion of host immunity. Distinct protease such as aminopeptidase, cysteine protease, and serine protease have been found in *Cryptosporidium* sporozoites, which are found during excystation process. Proteases Hemolysin H4 has similar sequence to the hemolysin of enterohemorrhagic *Escherichia coli* O157:H7. The main function of H4 is still unknown, but it disrupts cell membranes, suggesting that it aids in cellular invasion, which permit merozoites to exit the parasitophorous vacuole and spread to adjacent cells [15].

Global Epidemiology of *Cryptosporidium* in Humans and Animals

The prevalence of *Cryptosporidium* varies widely from country to country and from one region to another. Although cryptosporidiosis is prevalent in tropical countries, few studies have been conducted to accurately identify and characterize *Cryptosporidium* spp. using molecular tools. Enteric parasites are a very common source of diarrhea in many developing

Table 1 Worldwide distribution of some of the cases and surveillance data reported

Sample source	Country	Age group	Genotyping tool	Species/genotype/subgenotype	Ref.
Human stools	India	Children	18S rRNA, SSU, COWP, Cpgp40/15, TRAP-C1-based PCR	*C. hominis* (Ia, Id, Ie, Ib), *C. parvum* (Ic), *C. felis*	[77]
Environmental (water)	China	–	18S rRNA PCR-RFLP and sequence analyses; GP60	*C. hominis* (IbA19G2, IbA20G2, and IbA21G2), Ia, Id, Ie (IeA12G3T3), If (IfA22G1) *C. meleagridis*, *C. baileyi*, *C. parvum*, *C. suis*, *C. muris*, rat genotype, avian genotype 3	[78]
Human stools	UK	Adults and children	COWP and small sub-unit (SSU) rRNA gene PCR-RFLP	*C. parvum*, *C. hominis*, *C. meleagridis*, *C. felis*, *C. canis*, *Cryptosporidium* cervine, horse, skunk genotypes	[79]
Human stools	Haiti	Adults and children	18S rRNA PCR-RFLP	*C. hominis*, *C. parvum*, *C. felis*	[80]
Human and animal stools	Portugal	Adults and children	GP60	*C. hominis* (Ib, If), *C. parvum* (IIa, IIb, IIc, and IId)	[81]
Environmental (water)	France	–	IMS-IFA^2, 18S rRNA PCR-RFLP	*C. hominis*, *C. parvum*	[82]
Animal stools	Ireland	Neonatal calves	GP60	*C. parvum* (IIaA18G3R1), *C. bovis*, *Cryptosporidium* deer-like genotype	[83]
Human stools	MI (USA)	Adults and children	18S rRNA and COWPPCR-RFLP; GP60	*C. hominis*, *C. parvum* (cervine genotype, cervine genotype variant, human genotype W17)	[3]
Animal and human stools	Iran	Children and one adult	18S rRNA PCR-RFLP	*C. parvum*, *C. hominis* (anthroponotic and zoonotic genotype)	[84]
Human stools	UK	Adults	GP60	*C. hominis* (IbA10G2)	[30]
Human stools	Equatorial Guinea	Adults and children	COWP-based PCR-RFLP	*C. parvum*, *C. hominis*, *C. meleagridis*	[85]
Human stools	Sudan	–	ELISA	71 of the immunocompromised patients (91.0 %) 29 of immunocompetent patients (32.2 %)	
Cryptosporidium parvum	[86]				
Feces	Dubai	Birds (stone curlews)	Direct microscopy, PCR and RFLP, antigenic detection by immunochromatograpy	*Cryptosporidium parvum*	[87]
Feces	Iran	794 children	Ziehl-Neelson acid-fast stain and GP60-PCR sequencing	2.40 % crypto oocyst, GP60 showed 89.4 % *C. parvum*, 10.52 % *C. hominis*.	[88]
Feces	Jordan	44 children	18s rRNA	*C. parvum*, *C. hominis*, *C. canis*, *C. meleagridis*.	[89]
Feces	Palestine	30 fecal samples from hospital in Nabulus	Malachite green negative staining confirmed by PCR-RFLP	*C. parvum*	[90]
Feces	Saudi Arabia	408 immunocompromised patients	Kinyoun's acid-fast stain and monoclonal ELISA kit for *C. parvum* coproantigen detection.	*Cryptosporidium* infection 67 and 64 % infection rates.	[91]
Feces	Tunisia	1001 samples	Ziehl-Neelsen technique	Ten lambs and adult sheep (11.2 %) and nine chicken (45 %). *C. bovis* in three lambs, *C. meleagridis* in one chicken	[92]
Feces	Egypt	391 samples	RFLP analysis by PCR	40 % of *C. parvum*	[93]
Feces	Vhembe District, South Africa	Hospital and children samples		18 %	[14]

countries, and *Cryptosporidium* has been proved as one of the most persistent [14]. Human cryptosporidiosis is mainly caused by *C. hominis* and *C. parvum* which is found in both immunocompetent and immunocompromised individuals, but their occurrence differs in different parts of the world [18]. Macroepidemiological analyses depicted that *C. hominis* is more prevalent in North and South America, Australia, and Africa, while *C. parvum* causes more human infections in Europe, especially in the UK. *C. hominis* was more prevalent in infants under 1 year and in females aged 15 to 44 years. *C. parvum* occurrence peaks in spring, while *C. hominis* during the late summer and early autumn. Geographical patterns of these parasites were seen among the countries (Uganda, Serbia, Turkey, Israel, UK, USA, and New Zealand), due to various prevailing ecological determinants of transmission [18].

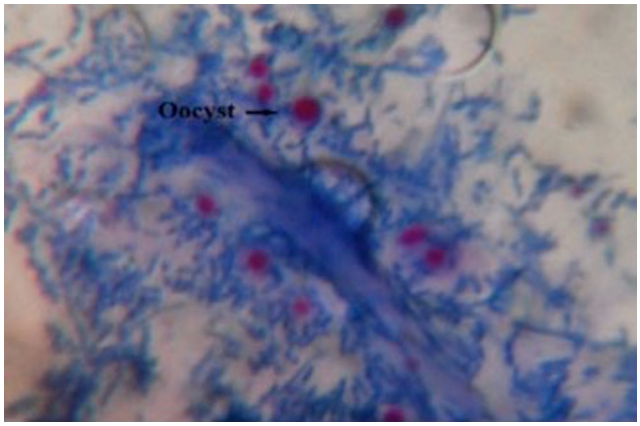

Fig. 1 Oocysts of *Cryptosporidium* in MZN staining (×100 objective)

Infections by *Cryptosporidium* parasites are estimated to be around 500 million annually in developing countries. In Africa, about 20–35 % people are infected with the organism and about 32.5 to 40 % are infected in the Sub-Saharan Africa [19]. A cryptosporidiosis prevalence survey done in Kenya over a year period reported a prevalence of 4 % in Kenya [20]. Animals, like humans, when infected, exhibit similar symptoms. According to Del Coco and colleagues, clinical illness and diarrhea caused by *Cryptosporidium* have been reported for different species of young animals including calves as young as 4 days. In Kwa-Zulu Natal, east coast of South Africa [16], *Cryptosporidium* was found to be endemic with a laboratory-confirmed prevalence ranging from 2.9 to 3.7 % and 2.9 to 3.0 % of cases submitted for analysis, respectively [21]. The Vhembe district of the Limpopo province in South Africa is reported to have cryptosporidiosis as the second most prevalent infection [15, 22]. Moreover, Obi and Bessong (2002) showed that the infection rate of *Cryptosporidium* was varying between 1.2 to 20.9 % according to season, with the highest prevalence in the summer months in the Venda region [15].

Prevalence of *Cryptosporidium* in Animals

Cryptosporidium infection is well known as a major cause of morbidity and mortality particularly in young animals [23]. Reported studies in the USA have estimated the incidence of cryptosporidiosis in cattle to be approximately 45.5 %, 24.5 % incidence in the UK, 26 % in Russia, 40 % in Germany, and 27 % in Hungary [24] whereas in India, the first encounter of cryptosporidiosis was among buffaloes and zebu cattle [25]. A study conducted in cattle farms in the Iringa and Tanga regions of Tanzania reported a prevalence rate of 20 and 21 %, respectively, in these regions. From their data, they further reported that the probability of cattle having *Cryptosporidium*-positive stools declined with increase in age, and this finding led them to conclude that *Cryptosporidium* is most prevalent in young animals [26]. To substantiate this finding, Ayinmode and Fagbeni (2010), in a study conducted in the south west Nigeria, reported a 27.4 % prevalence of *Cryptosporidium* in cattle younger than 6 months of age, 28.1 % prevalence for cattle between 7 and 12 months of age, and 19.9 % for cattle older than 12 months [27]. Interestingly, Ayinmode and Fagbeni (2010) further reported an infection rate of 38.1 % in female cattle compared to a 17.7 % in male cattle. Study surveys show that up to 38.5 % of cats and 44.8 % of dogs are infected with *Cryptosporidium* spp. [27]. A study done in China in the Shanghai and Shaoxing regions on the molecular epidemiology of *Cryptosporidium* in pigs reported a 14.3 and 25.0 %, respectively [28]. The prevalence of *Cryptosporidium* in sheep is also well documented; in Turkey in the Aydin province, a 46.5 % infection rate was reported among lambs [29]. The information communicated by Ulutas and Voyvoda (2004) [29] in their report resonate with other similar reports which concluded that cryptosporidial infections decrease with an increase in age [27].

Prevalence and Effects of *Cryptosporidium* in Children

Like in animals, cryptosporidiosis seems to be most prevalent in children mainly because of their immune system that is not fully developed [30]. A Yemeni study reported an overall prevalence rate of 43.7 % among children of ages between 1 and 12 months with a higher infection rate recorded in males (36.2 %) as compared to the lowest infection rate of 32.7 % which was observed in females [31]. In a reported study done among HIV-positive and HIV-negative children admitted to Uganda's Mulago National Referral Hospital for persistent diarrhea which lasted for over 2 weeks, *Cryptosporidium* infection was detected in only 6 % of HIV-negative children and 74 % of children who were HIV-positive [32]. In contrast to HIV, the immunological deficiencies that increase the risk of infection in malnourished children are unknown, but there exists a clear association between cryptosporidiosis and malnutrition [30]. In South Africa, studies conducted in Pretoria among children below 36 months and Johannesburg among children below 24 months reported a 25 and 20 % infection rate, respectively (Geyer et al., 1993) [33]. Another study done in the Vhembe region of the Limpopo province in South Africa reported a prevalence rate of 28.6 % among children between 2 and 5 years [14]. In Uganda, approximately 75 % of diarrheal cases are due to *C. hominis* and only 20 % is due to *C. parvum* [34]. *C. hominis* seems to be very dominant in the sub-Saharan Africa and America and Australia, whereas elsewhere, in European countries, *C. parvum* seems to be very predominant [35].

Prevalence of *Cryptosporidium* in HIV-Positive Patients

Cryptosporidium is reportedly the most frequent microorganism that causes chronic diarrhea in HIV-positive patients and

is coupled by manifestations such as nonbloody stools, abdominal pains, and loss of weight [36]. In a study conducted in southwest Nigeria, *Cryptosporidium* was found to have the highest prevalence (52.7 %) among HIV-positive patients compared to 11.3 % for *Ascaris*, 10 % for *Entamoeba histolytica*, 3.3 % for hookworm, and 2 % for *Trichuris* [37]. In Kenya, a 3.2 % prevalence of *Cryptosporidium* was reported with *C. hominis* being the most dominant genotype followed by *C. parvum* and, then, *Cryptosporidium meleagridis* [38]. In India, a 32.6 % infection rate of *C. parvum* was reported among HIV patients [27] whereas in Iran, a 26.7 % prevalence rate was reported among HIV-positive patients [39, 40].

Control and Prevention

Reservoir characteristics and environmental factors influence the epidemiology and transmissibility of *Cryptosporidium* spp. Although infectious following shedding by the host, oocysts are sensitive to inactivation by numerous unfavorable environmental conditions before an appropriate new host is found [41, 42]. Moreover, higher temperatures, age, and desiccation are other parameters that are critical in the survival and infectivity of the parasites' oocysts [43]. Exposure to sunlight for long hours renders oocysts entirely noninfective and nonviable [44, 45]. UV light has been reported to be germicidal for *Cryptosporidium* spp. oocysts, and recent advances in UV research have allowed for the application of UV disinfection technology in large-scale potable water treatment plants [46–48]. Solar photocatalytic disinfection (SPCDIS) using titanium-oxide-coated plastic inserts improves oocyst inactivation in water under overcast conditions [44].

Control measures need to be directed at reducing and/or preventing transmission of the infective oocysts to both humans and animals [49]. The tough nature of the oocyst makes traditional water treatment processes such as coagulation/flocculation, sedimentation, filtration, and disinfection insufficient to remove the parasite. Furthermore, chlorination has been found to be inadequate and not sufficient in the removal of the parasite species [50]. Recently, efforts are being directed toward developing vaccines for animals [51]. Moreover, Finch and Belosevic (2011) found that using modern microbial reduction process design techniques such as the integrated disinfection design framework (IDDF) ensured the provision of drinking water with a low risk of transmitting human pathogens to the public [52]. Separation of animal reservoir hosts from water supply provides an option for reducing and minimizing the oocyst burden and therefore reducing transmission. The construction of wetlands to treat wastewater and runoff from confined animal feeding operations has been found to be not only an efficient and effective process in the removal of pathogens like *Cryptosporidium* spp. but also very cost-effective [53]. People at risk should be made aware of activities such as swimming in community pools, consumption of freshwater plants, and unguarded contact with infected people and/or animals that may increase their likelihood of becoming infected with *Cryptosporidium* spp. and how to avoid those activities. Hand washing, education and point of use water treatment (mainly in developing countries), boiling of water before drinking, consumption of safe bottled water, and the use of filtration units can minimize and significantly reduce the risk of transmission of the infective oocysts and significantly improve diarrhea rates [54].

Anti-cryptosporidial pharmaceutical agents could reduce disease transmission. *Cryptosporidium* enteritis history of treatment has always been inadequate and unreliable. Against this background, relapses are often seen following treatment with agents such as paromomycin, nitazoxanide, and azithromycin. Thus, there is a great need to develop new anti-cryptosporidial agents (see accompanying article by Sparks et al.). Currently, nitazoxanide is the only FDA-approved drug for the treatment of the disease cryptosporidiosis. While reportedly used during outbreaks [55], the role of nitazoxanide or other chemotherapeutic agents to reduce transmission in resource-limited settings akin to anti-helminthic therapies is presently cost-prohibitive and has not been thoroughly studied. Furthermore, although nitazoxanide has shown improvements in both the clinical and microbiological cure rates, the drug is ineffective in HIV-positive and AIDS patients [37, 56] and studies in malnourished children are mixed. Abubakar et al. (2007) concluded from many different studies that current chemotherapy did not have a role for the management of cryptosporidiosis in immunocompromised individuals [22]. It is clear that restoring the immune status using anti-retroviral drugs is the most important therapy option in immunocompromised and HIV/AIDS individuals infected with *Cryptosporidium* spp. [57] and may therefore indirectly reduce transmission. In a murine model of *Cryptosporidium* infection, nitazoxanide did not decrease stool shedding during malnutrition [58], whereas supplementation with the di-peptide alanyl-glutamine did [59], suggesting similarly that supporting host defenses during malnutrition may be another strategy of reducing parasite shedding.

In addition to conventional pharmaceuticals, various trials are designed by numerous research groups worldwide investigating the potency of traditional medicinal plants and their extracts for treating cryptosporidiosis [60]. In a recent study, investigating the anti-parasitic effectiveness of *Allium sativum* (garlic) in the treatment of *Cryptosporidium* infections in experimentally infected immunocompetent and immunosuppressed mice, Gaffar (2012) concluded that garlic has good efficacy as a prophylactic and a promising therapeutic agent against *Cryptosporidium* [60]. Furthermore, Al-Hamairy et al. (2012) examined that the effect of six plant extracts on the shedding of oocysts in experimental mice was well illustrated

where there was a marked decrease of the shedding of *Cryptosporidium* oocysts in mice by using 250 mg/kg body weight. It was shown that *Peganum harmala* (68.6 %) showed highest anti-parasitic activity followed by *Artemesia herba-alba* (60.0 %), *Ricinus communis* (36.8 %), while *Allium sativum* and *Thymus vulgaris* show the lowest activity 18.6 and 20.5 %, respectively. Moreover, Al-Hamairy and his group showed that the efficacy of plant extracts at a dose of 500 mg/kg body weight was higher than 250 mg/kg body weight in shedding of the parasite oocysts [61]. Studies have also shown that garlic plant and indinavir are effective in treatment of cryptosporidiosis [62]. Toulah et al. (2012) treated 145 immunosuppressed infected Wister rats with garlic plant and indinavir. The rats aging 3 weeks were divided into five groups: (1) normal control, (2) indinavir-treated control, (3) immunosuppressed infected, (4) garlic-treated immunosuppressed infected, and (5) indinavir-treated immunosuppressed infected rats. All were subjected to clinical, parasitological, and histopathological examination at different days postinfection. The results showed that in immunosuppressed infected rats, all rats had diarrhea, loss of appetite, weakness, and limited movement, with 51.4 % death rate. In both treated groups, some rats regained activities, with death rate of 33.3 %. There was a significant decrease in the number of excreted oocysts at day 5 and 10 posttreatment in treated groups. One week posttreatment, the number of excreted oocysts had continued in decreasing among garlic-treated immunosuppressed infected rats while among Indinavir-treated immunosuppressed infected rats, it insignificantly increased. Since oocysts excreted till the end of the experiment, no cure rate was detected among both treated groups. The histopathological changes improved in treated groups in spite of the presence of some parasites on the epithelial surfaces of ileum [62]. In a separate recent study, Majeed et al. (2015) evaluated the effect of aqueous alcohol *Artemisia herba-alba* and *T. vulgaris* extract on the *Cryptosporidium parvum* and there compared with spiromycine drug in the infected white mouse (BALB/C). Results obtained showed more clear effects in aqueous alcohol *Artemisia herba-alba*- and *T. vulgaris*-extract-treated groups and continued till reaching negligible degree or no oocyst detection at 10th and 14th to 17 days posttreatment, but spiromycine drug continues oocyst excretion until the end of the experiment with a significant difference ($p>0.05$). Thus, it was concluded that administration of *Artemisia herba-alba* or *T. vulgaris* extract was protective against infections with *C. parvum* [63].

Currently, there are no vaccines against *Cryptosporidium* infections (see accompanying article by Ludington and Ward). For now, the lack of active therapy and vaccine prevention highlights the need to ensure that infection is avoided and prevented through public education, appropriate hygiene, and proper water management and treatment.

Determining *Cryptosporidium* Viability/Deactivation

Crucial in determining the effectiveness of new approaches to deactivating *Cryptosporidium* in water-treatment strategies is the accurate assessment of *Cryptosporidium* viability. The most common methods for testing *Cryptosporidium* viability are through in vitro fluorogenic vital assays and excystation assays. These methods evaluate oocyst viability by examining the integrity of the oocyst wall and metabolic activity of sporozites under conditions most favorable to infection [6, 64, 65]. To directly test viability and infectivity of *Cryptosporidium* oocysts, in vivo animal testing is the reference standard method. However, many in the field revert to in vitro viability assays because animal infectivity models require specialized laboratory facilities making them costly and time-consuming, which can be challenging in resource-limited settings. Furthermore, in the context of evaluating disinfection methods, viability assays are ideal because they enable researchers to study infectivity potential of an individual oocyst in environmental samples [66, 67].

The most common fluorogenic vital assays use 6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI) dyes to determine the integrity of the oocyst wall. DAPI is a permeable fluorescent stain that binds to nucleic acids, labeling intact sporozoites. PI is an impermeable fluorescent dye that stains the cytoplasm and DNA only when the cell wall is permeable or damaged [64, 65, 68, 69]. Therefore, DAPI staining will be present and PI staining will be absent among viable oocysts because their oocysts walls will be intact. Among nonviable oocysts, PI and DAPI staining will be present since the oocyst wall will have lost its integrity and the PI dye will be able to stain the cytoplasm.

Often, fluorogenic assays are used in conjunction with in vitro excystation assays to evaluate viability [65, 66, 69]. As fluorogenic assays determine the integrity of the oocyst wall, excystation assays evaluate the ability of sporozites to excyst from the oocyst under conditions favorable to infectivity. Excystation assays are designed to recreate infectious environments, such as highly acidic environments found in the mammalian gastrointestinal system, under which oocysts release sporozoites for infection. If the oocyst wall is damaged or the sporozoites are not intact, sporozoites will not excyst and thus are no longer infective. However, if the oocyst wall is intact and the sporozoites are able to excyst under highly acidic conditions, the oocyst is considered viable and infectious [66, 68].

Previous studies have compared in vitro excystation assays to in vivo animal infectivity testing and demonstrated strong correlations between excystation and infectivity [64, 66, 68]. As a result, in vitro assays are used to evaluate oocyst viability and efficacy of novel disinfection methods. Since in vitro assays directly evaluate individual infectivity potential of oocysts, the disinfection mechanisms at the cellular level can

be investigated, while also being cost-effective and feasible assay to perform in resource-limited settings [66]. In this manner, molecular approaches, such as detection of inducible hsp70 mRNA from viable oocysts, could be further validated in field studies [70].

Developing Collaborations to Design Practical, Cost-Effective, Innovative Strategies

A key factor in addressing the challenges and furthering innovative strategies for interruption of *Cryptosporidium* transmission in resource-limited settings is through global partnerships among researchers across disciplines. Through the Water and Health in Limpopo Program (WHIL), researchers at the University of Virginia in Charlottesville, Virginia, USA, and the University of Venda in Limpopo Province, South Africa, have been working together to design practical, cost-effective innovative strategies to reduce incidences of waterborne illnesses. The WHIL program is an interdisciplinary program that has allowed a rich exchange of knowledge and expertise, resulting in development of innovative solutions to address some of the most challenging developing world challenges, including detection and disinfection of *C. parvum* oocysts in drinking water sources. From this collaborative program, researchers have discovered disinfecting capabilities of silver against *C. parvum* oocysts [69, 71]. They have also developed novel laboratory methods to quantify oocyst viability, which until now has only been measured through qualitative microscopic analysis of fluorogenic and excystation assays [69]. Finally, they have developed and field-tested a low-cost, novel porous ceramic tablet embedded with silver nanopatches for water purification at the household level in resource-limited settings [72, 73]

A study that emerged from the WHIL collaboration was that by Shawel et al. (2013), where the efficacy of ceramic water filters in reducing gastrointestinal infections among an HIV-positive cohort in Limpopo Province, South Africa, was evaluated [71]. Results demonstrated that ceramic water filters were effective in reducing rates of diarrheal diseases and *C. parvum* oocyst shedding in stool samples among participants in the intervention group [71]. These encouraging findings led to further investigation of the mechanisms by which ceramic water filters may be interrupting transmission of *Cryptosporidium*. Ceramic water filters purify drinking water by two mechanisms: (1) removal of pathogens through physical filtration and (2) chemical disinfection by silver nanoparticles embedded within the ceramic media. The results from Shawel et al. (2013) prompted further collaboration within the WHIL program, leading researchers to investigate the disinfection efficacy of silver on *C. parvum* oocysts. Studies conducted by Su et al. (2014) used in vitro excystation and fluorogenic assays to examine disinfection capabilities of silver nanoparticles on *C. parvum* oocysts [69]. They validated their findings through in vivo animal infectivity assays, where they found almost two orders of magnitude reduction in oocyst shedding among mice that had been fed silver-nanoparticle-treated oocysts relative to the untreated oocysts. These results correlated with excystation results, where a decrease in oocyst viability was observed in response to silver nanoparticle treatment. Prior to this, no published studies have attempted to quantify the antimicrobial effects of silver nanoparticles or ionic silver for *C. parvum.* Su et al. (2014) went on to also develop the first quantitative in vitro oocyst viability assay that applies dielectrophoretic sensing methodology (DEP) to detect subtle changes in the oocyst due to changes in cellular functions or oocyst wall integrity [69, 74].

These encouraging results led WHIL researchers to investigate practical and low-cost methods to embed silver in porous ceramic media in order to develop water purification technologies at the household level and thus preventing the transmission of *Cryptosporidium* through contaminated drinking water. Ehdaie et al. (2014) discovered a low-cost method to form metallic nanopatches in porous ceramic media in the form of a tablet for applications in water purification. The low-cost silver-embedded ceramic tablet purifies water at the household by slowly releasing silver ions into the water, providing residual disinfection. Laboratory tests have demonstrated a 3-log reduction in *E. coli* after 8 h for 10 L of water. Results also suggest the silver-embedded ceramic tablet is reusable for at least 6 months [73]. As a result, this novel purification method would only cost $0.002 per liter of treated water, making it the most inexpensive water purification technology currently available [73, 75, 76].

Currently, researchers in the WHIL program are completing laboratory testing to investigate disinfection efficacy of silver through in vitro viability assays, excystation, and DAPI/PI staining. Early results have been encouraging showing loss of viability among oocysts treated with the silver-embedded ceramic tablet. These results lend support to observations made by Su et al. (2014) of silver as potential chemical disinfectant agent against *C. parvum* oocysts and the novel silver-embedded ceramic tablet as an effective method to treat water in order to reduce transmission of *Cryptosporidium* [69].

Of course, additional research, such as a human health studies or animal infectivity models, needs to be conducted to directly measure efficacy against *Cryptosporidiosis* which researchers in the WHIL project are planning to do. However, preliminary studies have provided encouraging results. Due to the exchange of research and expertise across disciplines through the WHIL program, researchers have been able to discover novel disinfectant agents, develop innovative strategies to quantify oocyst viability, and interrupt transmission of *Cryptosporidium* through development of novel water purification technologies.

Conclusion

Cryptosporidium ssp., due to environmental hardiness, a unique life cycle and immune evasion within the host, resistance to available antimicrobial therapies in the most susceptible hosts, and diverse human and animal reservoirs, represent a major challenge in disease prevention—particularly in endemic settings where resources are often limited. To continue striving forward in overcoming the global challenges of *Cryptosporidium* transmissions, global partnership will be imperative. Overcoming global challenges requires global partnerships, similar to that of the WHIL program in order to develop innovative and effective solutions for resource-limited settings.

Compliance with Ethics Guidelines

Conflict of Interest Amidou Samie, Ahmed Al-Qahtani, Beeta Ehdaie, and Ali El Bakri declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Flanigan TP, Soave R. Cryptosporidiosis. Prog Clin Parasitol. 1993;3:1–20.
2. Hijjawi NS, Meloni BP, Ryan UM, Olsen ME, Thompson RCA. Successful in vitro cultivation of *Cryptosporidium andersoni*: evidence for the existence of novel extracellular stages in the life cycle and implications for the classification of *Cryptosporidium*. Int J Parasitol. 2002;32:1719–26.
3. Feltus DC, Giddings CW, Schneck BL, Monson T, Warshauer D, McEvoy JM. Evidence supporting zoonotic transmission of *Cryptosporidium* spp. in Wisconsin. J Clin Microbiol. 2006;44:4303–8.
4. Fayer R. Cryptosporidium: a water-borne zoonotic parasite. Vet Parasitol. 2004;126:37–56.
5. Checkley W, White Jr AC, Jaganath D, Arrowood MJ, Chalmers RM, Chen XM, et al. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for cryptosporidium. Lancet Infect Dis. 2015;15(1):85–94.
6. Guerrant RL. Cryptosporidiosis: an emerging, highly infectious threat. Emerg Infect Dis. 1997;3:51–7.
7. Tosini F, Drumo R, Elwin K, Chalmers RM, Pozio E, Cacciò SM. The CpA135 gene as a marker to identify *Cryptosporidium* species infecting humans. Parasitol Int. 2010;59:606–9.
8. Fall A, Thompson RCA, Hobbs RP, Morgan-Ryan UM. Morphology is not a reliable tool for delineating species within *Cryptosporidium*. J Parasitol. 2003;89:399–402.
9. Fayer R, Morgan U, Upton SJ. Epidemiology of *Cryptosporidium*: transmission, detection, and identification. Int J Parasitol. 2000;30:1305–22.
10. Haas CN, Rose JB. Reconciliation of microbial risk models and outbreak epidemiology: the case of the Milwaukee outbreak. In: Proceeding of the 1994 Annual Conference: Water Quality. Published in New York, American Water Works Assoc. 517–23
11. Okhuysen PC, Chappell CL. Cryptosporidium virulence determinants—are we there yet? Int J Parasitol. 2002;32:517–25.
12. DuPont HL, Chappell CL, Sterling CR, Okhuysen PC, Rose JB, Jakubowski W. The infectivity of *Cryptosporidium parvum* in healthy volunteers. New Engl J Med. 1995;332:855–9.
13. Borowski H, Thompson RC, Armstrong T, Clode PL. Morphological characterization of *Cryptosporidium parvum* life cycle stages in an in vitro model system. J Parasitol. 2010;137:13–26.
14. Samie A, Bessong PO, Obi CL, Sevilleja JE, Stroup S, Houpt E, et al. *Cryptosporidium* species: preliminary descriptions of the prevalence and genotype distribution among school children and hospital patients in the Venda region, Limpopo Province, South Africa. Exp Parasitol. 2006;114:314–22.
15. Obi CL, Bessong PO. Diarrhoeagenic bacterial pathogens in HIV-positive patients with diarrhoea in rural communities of Limpopo province, South Africa. J Health Popul Nutr. 2002;20:230–4.
16. Del Coco VF, Cordoba MA, Basualdo JA. *Cryptosporidium* infection in calves from a rural area of Buenos Aires, Argentina. Vet Parasitol. 2008;158:31–5.
17. Que X, Reed SL. Cysteine proteinases and the pathogenesis of amebiasis. Clin Microbiol Rev. 2000;13:196–206.
18. Putignani L., Menichella D. Global distribution, Public health and clinical impact of the protozoan pathogen *Cryptosporidium*, Hindawi Publishing Corporation: interdisciplinary perspectives on infectious diseases. 2010;1–39
19. Kfir R, Hilner C, Preez M, Bateman B. Studies on the prevalence of giardia cysts and *Cryptosporidium* oocysts in South African water. Water Sci Technol. 2000;31:435–66.
20. Gatei W, Ashford RW, Beeching NJ, Kamwati SK, Greensill J, Hart CA. *Cryptosporidium muris* infection in an HIV-infected adult, Kenya. Emerg Infect Dis. 2006;8:204–6.
21. Jarmey-Swan C, Bailey IW. Howgrave-Graham. Ubiquity of the water-borne pathogens, *Cryptosporidium* and *Giardia*, in KwaZulu-Natal populations. Water SA. 2001;27:57–64.
22. Abubakar I, Aliyu SH, Arumugam C, Usman NK, Hunter PR. Treatment of cryptosporidiosis in immunocompromised individuals. Systematic review and meta-analysis. Br J Clin Pharmacol. 2007;63(4):387–93.
23. Graff CE, Vanopdenbosch MO, Mora H, Peeters E. A review of the importance of cryptosporidiosis in farm animals. Int J Parasitol. 1999;29:1269–87.
24. Kumar D, Sreekrishnan R, Das SS. Cryptosporidiosis: an emerging disease of zoonotic importance. Proc Natl Acad Sci India. 2005;75:160–72.
25. Dubey JP, Fayer R, Rao JR. Cryptosporidial oocysts in faeces of water buffalo and Zebu Calves in India. J Vet Parasitol. 1992;6:55–6.
26. Swai ES, French NP, Karimuribo ED, Fitzpatrick JL, Bryant MJ, Kambarage DM, et al. Prevalence and determinants of *Cryptosporidium* spp infection in small holder dairy cattle in Iringa and Tanga regions of Tanzania. Onderstepoort J Vet Res. 2007;74:23–9.
27. Ayinmode AB, Ayinmode DB, Fagbemi BO. Prevalence of Cryptosporidium infection in cattle from South Western Nigeria. Veterinarski Arhiv. 2010;80(6):723–31.
28. Yin Y, Shen Y, Yuan Z, Lu WXY, Cao J. Prevalence of the *Cryptosporidium* pig genotype II in pigs from the Yangtze River Delta, China. Plos One. 2011;6:1–4.
29. Ulutaş B, Voyvoda H. Cryptosporidiosis in diarrhoeic lambs on a sheep farm. Türk Parazitol Derg. 2004;28:15–7.
30. Mor SM, Tzipori S. Cryptosporidiosis in children in Sub-Saharan Africa: a lingering challenge. Clin Infect Dis. 2008;47:915–92.
31. Al-Shamiri A, Al-Zubairy A, Al-Mamari R. The Prevalence of Cryptosporidium spp. in children, Taiz District, Yemen. Iran J Parasitol. 2010;5:26–32.

32. Tumwine JK, Kekitiinwa A, Bakeera-Kitaka S, Ndeezi G, Downing R, Feng X, et al. Cryptosporidiosis and microsporidiosis in Ugandan children with persistent diarrhoea with and without concurrent infection with the human immunodeficiency virus. Am J Trop Med Hyg. 2005;73:921–5.
33. Geyer A, Crewe-Brown HH, Greeff AS, Fripp PJ, Steele AD, Van Schalkwyk TV, et al. The microbial aetiology of summer paediatric gastroenteritis at Ga-Rankuwa Hospital in South Africa. East Afr Med J. 1993;70:78–81.
34. Tumwine JK, Kekitiinwa A, Nabukeera N, Akiyoshi DE, Rich SM, Widmer G, et al. *Cryptosporidium Parvum* in children with diarrhea in Mulago Hospital, Kampala, Uganda. Am J Trop Med Hyg. 2003;8:710–5.
35. Xiao L, Ryan UM. Cryptosporidiosis: an update in molecular epidemiology. Curr Opin Infect Dis. 2004;17:483–90.
36. Kotler DP. Gastrointestinal manifestations of HIV infection. Adv Intern Med. 1995;40:197–242.
37. Adesiji YO, Lawal RO, Taiwo SS, Fayemiwo SA, Adeyeba OA. Cryptosporidiosis in HIV patients with diarrhoea in Osum state southwestern Nigeria. Eur J Gen Med. 2007;4:119–22.
38. Nyamwange C, Mkoji GM, Mpoke S. Cryptosporidiosis among HIV positive patients in the North Rift region of Kenya. Afr J Health Sci. 2012;21:92–106.
39. Taherkhani H, Fallah H, Jadidian K, Vaziri S. A study on the prevalence of *Cryptosporidium* in HIV positive patients. J Res Health Sci. 2007;7:20–4.
40. Bartelt LA, Sevilleja JE, Barrett LJ, Warren CA, Guerrant RL, Bessong PO, et al. High anti-Cryptosprodium parvum IgG seroprevalence in HIV-infected adults in Limpopo, South Africa. Am J Trop Med Hyg. 2013;89(3):531–4.
41. Miller WA, Lewis DJ, Pereira MD, Lennox M, Conrad PA, Tate KW, et al. Farm factors associated with reducing Cryptosporidium loading in storm runoff from dairies. J Environ Qual. 2008;37: 1875–82.
42. Robertson LJ. Giardia and Cryptosporidium infections in sheep and goats: a review of the potential for transmission to humans via environmental contamination. Epidemiol Infect. 2009;137:913–21.
43. King BJ, Keegan AR, Monis PT, Saint CP. Environmental temperature controls Cryptosporidium oocyst metabolic rate and associated retention of infectivity. Appl Environ Microbiol. 2005;71:3848–57.
44. Mendez-Hermida F, Ares-Mazas E, McGuigan KG, Boyle M, Sichel C, Fernandez-Ibanez P. Disinfection of drinking water contaminated with Cryptosporidium parvum oocysts under natural sunlight and using the photocatalyst TiO2. J Photochem Photobiol B. 2007;88:105–11.
45. King BJ, Monis PT. Critical processes affecting Cryptosporidium oocyst survival in the environment. Parasitology. 2007;134:309–23.
46. Huffman DE, Slifko TR, Arrowood MJ, Rose JB. Inactivation of bacteria, virus, and Cryptosporidium by a point-of-use device using pulsed broad spectrum white light. Water Res. 2000;34:2491–8.
47. King BJ, Hoefel D, Daminato DP, Fanok S, Monis PT. Solar UV reduces Cryptosporidium parvum oocyst infectivity in environmental waters. J Appl Microbiol. 2008;104:1311–23.
48. Connelly SJ, Wolyniak EA, Williamson CE, Jellison KL. Artificial UV-B and solar radiation reduce in vitro infectivity of the human pathogen Cryptosporidium parvum. Environ Sci Technol. 2007;41: 7101–6.
49. Roberson JA, Bruno J. The latest and greatest Cryptosporidium research. Position paper of the American Water Works Association. J Water Supply. 1997;2:339–46.
50. Betancourt WQ, Rose JB. Drinking water treatment processes for removal of Cryptosporidium and Giardia. Vet Parasitol. 2004;126: 219–34.
51. Innes EA, Bartley PM, Rocchi M, Benavidas-Silvan J, Burrells A, et al. Developing vaccines to control protozoan parasites in ruminants: dead or alive? Vet Parasitol. 2011;180:155–63.
52. Finch GR, Belosevic M. Controlling Giardia spp. and Cryptosporidium spp. in drinking water by microbial reduction processes. Can J Civ Eng. 2011;28:67–80.
53. Graczyk TK, Lucy FE, Tamang L, Mashinski Y, Broaders MA, Connolly M, et al. Propagation of human enteropathogens in constructed horizontal wetlands used for tertiary wastewater treatment. Appl Environ Microbiol. 2009;75:4531–8.
54. Fewtrell L, Kaufmann RB, Kay D, Enanoria W, Haller L, Colford Jr JM. Water, sanitation, and hygiene interventions to reduce diarrhoea in less developed countries: a systematic review and meta-analysis. Lancet Infect Dis. 2005;5:42–52.
55. Vandenberg O, Robberecht F, Dauby N, Moens C, Talabani H, Dupont E, et al. Management of a *Cryptosporidium hominis* outbreak in a day-care center. Pediatr Infect Dis J. 2012;31(1):10–5.
56. Amadi B, Mwiya M, Sianongo S, Payne L, Watuka A, Katubulushi M, et al. High dose prolonged treatment with nitazoxanide is not effective for cryptosporidiosis in HIV positive Zambian children: a randomised controlled trial. BMC Infect Dis. 2009;9:195.
57. Cabada MM, White Jr AC. Treatment of cryptosporidiosis: do we know what we think we know? Curr Opin Infect Dis. 2010;23:494–9.
58. Costa LB, JohnBull EA, Reeves JT, Sevilleja JE, Freire RS, Hoffman PS, et al. Cryptosporidium-malnutrition interactions: mucosal disruption, cytokines, and TLR signalling in a weaned murine model. J Parasitol. 2011;97(6):1113–20.
59. Costa LB, Noronha FJ, Roche JK, Sevilleja JE, Warren CA, Oria R, et al. Novel in vitro and in vivo models and potential new therapeutics to break the vicious cycle of Cryptosporidium infection and malnutrition. J Infect Dis. 2012;205(9):1464–71.
60. Gaafar MR. Efficacy of *Allium sativum* (garlic) against experimental cryptosporidiosis. Alex J Med. 2012;48:59–66.
61. Al-Hamairy A, Mugheer AH, Al-Dulaimi FA. Prevalence of Cryptosporidium sp. and treatment by using some plant extracts in Al-Hilla City, Babylon Province of Iraq. Egypt J Exp Biol (Zoo). 2012;8:287–93.
62. Toulah FH, El-Shafei AA, Al-Rashid HS. Evaluation of garlic plant and indinavir drug efficacy in the treatment of cryptosporidiosis in experimentally immunosuppressed rats. J Egypt Soc Parasitol. 2012;42:321–8.
63. Majeed S, Al-Taai NA, Al-Zubaidi IA. The effect of aqueous alcohol Artemisia herba-alba and Thymus vulgaris extract on the Cryptosporidium parvum in the white mouse (BALB/C). Int J Adv Res. 2015;3:31–5.
64. Campbell AT, Robertson LJ, Smith HV. Viability of *Cryptosporidium parvum* oocysts: correlation of in vitro excystation with inclusion or exclusion of fluorogenic vital dyes. Appl Environ Microbiol. 1992;58:3488–93.
65. Campbell AT, Robertson LJ, Smith HV. Effects of preservatives on viability of *Cryptosporidium* parvum oocysts. Appl Environ Microbiol. 1993;59:4361–2.
66. Clancy JL, AWWA Research Foundation, Great Britain. Drinking water inspectorate cryptosporidium viability and infectivity methods. USA: American Water Works Association; 2000. p. 137.
67. Bukhari Z, Smith HV, Humphreys SW, Kemp JS, Wright SE. In *comparison of excretion and viability patterns in experimentally infected animals: potential for release of viable c. parvum oocysts into the environment*. In: Betts WB, Casemore DP, Fricker CR, Smith HV, Watkins J, editors. Protozoan parasites and water. Cambridge: The Royal Society of Chemistry; 1995.
68. Jenkins MB, Anguish LJ, Bowman DD, Walker MJ, Ghiorse WC. Assessment of a dye permeability assay for determination of inactivation rates of *Cryptosporidium* parvum oocysts. Appl Environ Microbiol. 1997;63:3844–50.

69. Su YH, Tsegaye M, Varhue W, Lia KT, Abebe LS, Smith JA, et al. Quantitative dielectrophoretic tracking for characterization and separation of persistent subpopulations of *Cryptosporidium* parvum. Analyst. 2014;139:66–73.
70. Graces-Sanchez G, Wilderer PA, Horn H, Munch JC, Lebuhn M. Assessment of the viability of *Cryptosporidium parvum* oocysts with the induction ration of hsp70 mRNA production in manure. J Microbiol Methods. 2013;94(3):280–9.
71. Shawel AL, Sophia N, Vinka O, Mark C, Alukhethi S, Samie A, et al. Ceramic water filters impregnated with silver nanoparticles as a point-of-use water-treatment intervention for HIV-positive individuals in Limpopo Province. South Africa: A pilot study of technological performance and human health benefits; 2013.
72. Ehdaie B. Development of a porous ceramic tablet embedded with silver nanopatches for low-cost point-of-use water purification, University of Virginia, 2015
73. Ehdaie B, Krause C, Smith JA. Porous ceramic tablet embedded with silver nanopatches for low-cost point-of-use water purification. Environ Sci Technol. 2014;48:13901–8.
74. Pethig R. Review article-dielectrophoresis: status of the theory, technology, and applications. Biomicrofluidics 2010*, 4*, 10.1063/1.3456626
75. Program for Appropriate Technology in Health (PATH). Project brief: price-performance model: household water treatment and safe storage devices, critical opportunities for product improvement. PATH 2011, 1–14.
76. Program for Appropriate Technology in Health (PATH). Project brief: global landscape of household water treatment and safe storage products. PATH 2010, 1–4.
77. Ajjampur SSR, Gladstone BP, Selvapandian D, Muliyil JP, Ward H, Kang G. Molecular and spatial epidemiology of cryptosporidiosis in children in a semiurban community in South India. J Clin Microbiol. 2007;45:915–20.
78. Feng Y, Li N, Duan L, Xiao L. *Cryptosporidium* genotype and subtype distribution in raw wastewater in Shanghai, China: evidence for possible unique *Cryptosporidium hominis* transmission. J Clin Microbiol. 2009;47:153–7.
79. Chalmers RM, Elwin K, Thomas AL, Guy EC, Mason B. Long-term *Cryptosporidium* typing reveals the aetiology and species-specific epidemiology of human cryptosporidiosis in England and Wales, 2000 to 2003. Eurosurveillance. 2009;14:1–9.
80. Raccurt CP, Brasseur P, Verdier RI, et al. Human cryptosporidiosis and *Cryptosporidium* spp. in Haiti. Trop Med Int Health. 2006;11:929–34.
81. Alves M, Xiao L, Antunes F, Matos O. Distribution of *Cryptosporidium* subtypes in humans and domestic and wild ruminants in Portugal. Parasitol Res. 2006;99:287–92.
82. Coupe S, Delabre K, Pouillot R, Houdart S, Santillana-Hayat M, Derouin F. Detection of *Cryptosporidium*, *Giardia* and *Enterocytozoon bieneusi* in surface water, including recreational areas: a one-year prospective study. FEMS Immunol Med Microbiol. 2006;47:351–9.
83. Thompson HP, Dooley JSG, Kenny J, et al. Genotypes and subtypes of *Cryptosporidium* spp. in neonatal calves in Northern Ireland. Parasitol Res. 2007;100:619–24.
84. Pirestani M, Sadraei J, Dalimi Asl A, Zavvar M, Vaeznia H. Molecular characterization of *Cryptosporidium* isolates from human and bovine using 18s rRNA gene in Shahriar county of Tehran, Iran. Parasitol Res. 2008;103:467–72.
85. Blanco MA, Iborra A, Vargas A, Nsie E, Mbá L, Fuentes I. Molecular characterization of *Cryptosporidium* isolates from humans in Equatorial Guinea. Trans R Soc Trop Med Hyg. 2009;103:1282–4.
86. Musa HA, Salim GA, Ismael AA, Elfadil A, Kafi SK. Diarrhea due to Cryptosporidium parvum in immunocompromised and immunocompetent patients in Khartoum State. Sudan J Med Sci. 2011;6:39–42.
87. Zylan K, Bailey T, Smith HV, Silvanose C, Kinne J, Schuster RK, et al. An outbreak of cryptosporidiosis in a collection of Stone curlews (Burhinus oedicnemus) in Dubai. Avian Pathol. 2008;37:521–6.
88. Taghipour N, Nazemalhosseini-Mojarad E, Haghighi A, Rostami-Nejad M, Romani S, Keshavarz A, et al. Molecular epidemiology of cryptosporidiosis in Iranian children, Tehran, Iran. Iran J Parasitol. 2011;6:41–5.
89. Hijjawi N, Ng J, Yang R, Atoum MF, Ryan U. Identification of rare and novel Cryptosporidium GP60 subtypes in human isolates from Jordan. Exp Parasitol. 2010;125:161–4.
90. Hussein AS. Cryptosporidium parvum causes gastroenteritis epidemics in the Nablus region of Palestine. Trop Med Int Health. 2011;16:12–7.
91. Sanad MM, Al-Malki JS. Cryptosporidiosis among immunocompromised patients in Saudi Arabia. J Egypt Soc Parasitol. 2007;37:765–74.
92. Soltane R, Guyout K, Dei-Cas E, Ayadi A. Prevalence of Cryptosporidium spp. (Eucoccidiorida: Cryptosporiidae) in seven species of farm animals in Tunisia. Parasite. 2007;14:335–8.
93. Abd El Kader NM, Blanco MA. Ali-Tammam M, Abdel Ghaffar R, Osman A, El Sheikh N, Rubio JM, de Fuentes I. Detection of Cryptosporidium parvum and Cryptosporidium hominis in human patients in Cairo, Egypt. Parasitol Res. 2012;110:161–6.