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Cryptosporidium in Humans and Animals - a One Health approach to prophylaxis

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SUMMARY

Cryptosporidium is a major cause of moderate to severe diarrhea in humans worldwide, second only to rotavirus. Due to the wide host range and environmental persistence of this parasite, cryptosporidiosis can be zoonotic and associated with foodborne and waterborne outbreaks. Currently, 31 species are recognized as valid and of these, *Cryptosporidium hominis* and *Cryptosporidium parvum* are responsible for the majority of infections in humans. The immune status of the host, both innate and adaptive immunity, has a major impact on the severity of the disease and its prognosis. Immunocompetent individuals

typically experience self-limiting diarrhea and transient gastroenteritis lasting up to 2 weeks and recover without treatment, suggesting an efficient host anti-parasite immune response. Immunocompromised individuals can suffer from intractable diarrhea, which can be fatal. Effective drug treatments and vaccines are not yet available. As a result of this, the close cooperation and interaction between veterinarians, health physicians, environmental managers and public health operators is essential to properly control this disease. This review focuses on a One Health approach to prophylaxis, including the importance of understanding transmission routes for zoonotic *Cryptosporidium* species, improved sanitation and better risk management, improved detection, diagnosis and treatment and the prospect of an effective anti-cryptosporidial vaccine.

Keywords: *Cryptosporidium*, *diagnosis, treatment, zoonotic, risk management, vaccines, prophylaxis*

INTRODUCTION

Cryptosporidium species are protozoan parasites that infect a broad range of hosts including humans, domestic and wild animals worldwide, causing asymptomatic or mild to severe gastrointestinal disease in their host species (1-6). Currently, 31 *Cryptosporidium* species have been recognised as valid and of these, by far the most common species reported in humans worldwide are *C. parvum* and *C. hominis* (7-12).

Human cryptosporidiosis is frequently accompanied by abdominal pain, fever, vomiting, malabsorption and diarrhea that may sometimes be profuse and prolonged (13, 14). The immune status of the host, both innate and adaptive immunity, has a major impact on the severity of the disease and its prognosis. Immunocompetent individuals typically experience self-limiting diarrhea and transient gastroenteritis lasting up to 2 weeks and recover without

treatment, suggesting an efficient host anti-parasite immune response. Immunocompromised individuals including HIV/AIDS patients (not treated with antiretroviral therapy), often suffer from intractable diarrhea, which can be fatal (15). An effective vaccine for cryptosporidiosis is not yet available.

The Global Enteric Multicenter Study (GEMS) study, which was a three year matched case-control study of moderate-to-severe diarrhea in over 22,000 infants and children at seven sites across Africa and Asia aged 0-59 months, found that *Cryptosporidium* was second only to rotavirus as a major cause of severe diarrhea (16, 17). More recent matched case-control studies of diarrhea have confirmed this (18). Similarly, a birth cohort study conducted by a Global Network for the Study of Malnutrition and Enteric Diseases (MAL-ED), has assessed pathogen-specific burdens in diarrheal and nondiarrheal stool specimens from 2,145 children aged 0-24 months, over five years at eight community sites in Africa, Asia and South America, and identified *Cryptosporidium* spp. as one of the five highest attributable burdens of diarrhoea in the first year of life (19). Globally, cryptosporidiosis is estimated to be responsible for the majority of deaths among children under 5 years of age (20-22) and *Cryptosporidium* infection in children is also associated with malnutrition, persistent growth retardation, impaired immune response and cognitive deficits (23, 24). The mechanism by which *Cryptosporidium* affects child growth seems to be associated with inflammatory damage to the small intestine (25). Undernutrition (particularly in children) is both a sequela of- and a risk factor for- cryptosporidiosis, particularly in low-income countries (26-31). FAO's executive summary of the State of Food Insecurity in the World (http://www.fao.org/docrep/018/i3458e/i3458e.pdf) indicates there are 842 million chronically malnourished persons worldwide, which significantly contributes to impaired immunity and thus increased susceptibility to infection with *Cryptopsporidium*, perpetuating

the cycle of chronic diarrhea and malnutrition. In developed countries, *Cryptosporidium* is less common (15) and accounts for ~9% of diarrheal episodes in children (32).

Cryptosporidiosis is a highly prevalent and extremely widespread disease (6) and several factors contribute to this. Infected individuals shed large numbers of oocysts, which are environmentally very robust, resistant to inactivation by commonly used drinking water disinfectants including chlorine treatment and are able to survive routine wastewater treatments (33, 34). *Cryptosporidium* oocysts are highly infectious; in human volunteer studies, as few as 10 or less *Cryptosporidium* oocysts can produce disease in healthy adults (35, 36). A quantitative risk assessment has estimated that ingestion of a single oocyst of the *C. parvum* IOWA isolate will result in clinical disease in 2.79% of immunologically normal persons (37). Another contributing factor to the high prevalence and widespread distribution of *Cryptosporidium* is the lack of treatment options. Only one drug, nitazoxanide (NTZ, Alinia; Romark Laboratories, Tampa, Florida, United States), has been approved by the United States (US) Food and Drug Administration (FDA). This drug, however, exhibits only moderate clinical efficacy in malnourished children and immunocompetent people, and none in immunocompromosed individuals like people with HIV (38, 39).

Because oocysts of *Cryptosporidium* species from humans and animals are ubiquitous in the environment, cryptosporidiosis can be acquired through multiple routes (reviewed by Robertson et al. 40). Transmission of oocysts is by the faecal-oral route, either directly or indirectly. For humans, direct transmission can be from person to person primarily due to poor hygiene among household members and attendees in day-care centers, aged care facilities and other institutions, or from animals to persons such as farm-workers and pet owners. Most indirect transmission is from contaminated drinking or recreational water. Contaminated food can also be a source of transmission and contamination can occur at every step throughout the food preparation process, from farm to table (41, 42). Findings from

animal models, human case reports, and a few epidemiological studies suggest that *Cryptosporidium* may also be transmitted via inhalation of aerosolized droplets or by contact with fomites contaminated by coughing (see 43).

The “One Health” approach to tackle zoonotic diseases defined as "One Medicine" by Schwabe (1984), is a worldwide strategy to improve health and well-being through the mitigation and prevention of disease risks that originate at the interface between humans, animals and their various environments (44). This review focuses on a One Health approach to prophylactic prevention of cryptosporidiosis including improved detection, diagnosis and treatment, the importance of understanding zoonotic transmission, better environmental and risk management and the prospect of an effective anti-cryptosporidial vaccine.

DETECTION, DIAGNOSIS AND TREATMENT

Cryptosporidium presents many challenges for detection and diagnosis. The use of different diagnostic methods and the inconsistent application of typing techniques can make direct comparisons difficult or even impossible between clinical, veterinary and environmental testing or

between different regions or countries (45). Detection of *Cryptosporidium* in clinical pathology laboratories is still based mainly on microscopic detection via stains and/or fluorescent antibodies (IFA) and other antigenic detection methods. Although microscopy needs relatively simple instruments and cheap consumables, it is labour intensive, requires a skilled operator and lacks sensitivity and specificity (45). Morphological characters for identifying *Cryptosporidium* are few (46, 47) and differential staining techniques are usually required due to the fact that oocysts are similar in size and shape to yeasts, fecal components and other debris (47, 48). Acid fast (AF) modified Ziehl–Neelsen staining is one of the most common differential staining techniques (45, 48). However, the detection limits of

conventional microscopy for *Cryptosporidium* have been reported to be as low as 50, 000 to 500, 000 oocysts per gram of human faeces (49), resulting in low levels of infection or sporadic shedding possibly going unnoticed when conventional methods of detection are used. Sporadic sheding is such that studies have shown that three separate faecal samples should be examined for immunocompetent patients and two samples for AIDS patients for confident diagnosis of cryptosporidial infections using acid-fast staining (50). IFA stains offer superior sensitivity; in some studies, about 97% sensitivity compared with only about 75% sensitivity for acid-fast staining (51). However, IFA is more expensive than acid-fast staining and requires a fluorescence microscope and trained staff (51). This is particularly problematic in resource-poor areas where cryptosporidiosis is a major health problem. A recent study proposed the use of phase contrast microscopy (PCM) as a specific and inexpensive method for detection of *Cryptosporidium*, however this method still lacks sensitivity (52).

Other antigen detection formats such as enzyme linked immunosorbent assays (ELISA's), enzyme immunoassays (EIA's) and immunochromatographic (dipstick) assay for *Cryptosporidium* are also commercially available and have the advantage of reducing assay times and being amenable to automation. However, diagnostic sensitivities are variable (70% to 100%) (51, 53-55); some rapid tests have reduced specificity and sensitivity for species other than *C. parvum* or *C. hominis* (56, 57) and confirmation of positive reactions is needed (55). Biosensor chips, that detect and quantitate *C. parvum* in real-time via anti-*C. parvum* IgM binding have also been developed (58, 59), however detection limits are relatively high (100 or more oocysts) and they have yet to be fully evaluated on water or faecal samples. Another major limitation of both conventional microscopy and antigen detection methods is that they cannot identify to species or subtype level, which is essential for understanding transmission dynamics and outbreaks, in particular for zoonotic species.

Polymerase chain reaction (PCR)-based techniques have permitted specific and sensitive detection and differentiation of *Cryptosporidium* spp. for clinical diagnosis and environmental monitoring (45). Real-time or quantitative PCR (qPCR) assays been developed to quantitate the numbers of *Cryptosporidium* oocysts present in human and animal faecal and water samples (60-63) with 100% specificity and sensitivies as low as 200 oocysts per gram of faeces, which equates to 2 oocysts per PCR (60). Multiplex qPCR assays have also been developed for the detection of *Cryptosporidium* and other common causes of diarrhoea such as *Giardia duodenalis* and *Entamoeba histolytica*, which have the advantage of identifying mixed infections (64-66). The most widely used molecular markers for identification and typing of *Cryptosporidium* species are the 18S ribosomal RNA (18S rRNA) gene and the 60-kDa glycoprotein (gp60) gene respectively (4, 10). Miniaturised fluidic devices, which can detect to species level have also been developed, mainly for the water industry, (reviewed by Bridle et al. 67), but as with antibody-based biosensor chips, have yet to be fully validated and are costly.

New drug targets for *Cryptosporidium* are urgently needed, as the only FDA-approved drug, nitazozanide, does not provide benefit for malnourished children and immunocompromised patients with cryptosporidiosis. However, *Cryptosporidium* has completely lost the plastid-derived apicoplast present in many other apicomplexans, and the remnant mitochondrion lacks the citrate cycle and cytochrome-based respiratory chain (68). Therefore, many classic drug targets are unavailable in *Cryptosporidium*. Progress in developing anti-cryptosporidial drugs has also been affected by the inability to generate large numbers of *Cryptosporidium* oocysts in vitro and an inability to genetically manipulate the organism (69, 70). The recent development of a hollow fiber in vitro culture system to generate large numbers of oocysts (up to 10^8 oocysts per day) (71) and advances in

genetically engineering *Cryptosporidium* (72), will transform the development of novel therapeutics.

To date, the best studied drug target is the bacterial derived inosine 5' - monophosphate dehydrogenase (IMPDH) gene, as *Cryptosporidium* does not contain guanine salvage enzymes and is totally dependant on this enzyme to convert adenosine salvaged from the host into guanine nucleotides (69, 74-76). This coupled with the parasite's high metabolic demand for nucleotides due to the complicated lifecycle of this parasite make IMPDH an important drug target (77-85).

Other drug targets include long chain fatty acyl-coenzyme A synthetases (LC-ACS) which are essential in fatty acid metabolism (68) and a recent study reported good efficacy of the ACS inhibitor triacsin C against cryptosporidial infection in mice (86). A parasite cysteine protease inhibitor was also effective in vitro and in an animal model (87). Other studies have focused on repurposing existing drugs to overcome the prohibitive costs of de novo drug development (estimated to be between $500 million and $2 billion per compound successfully brought to market) (88). For example, several compounds from the Medicines for Malaria Venture (MMV) Open Access Malaria Box have exhibited activity against *C. parvum* (89) and drugs such as the human 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitor, itavastatin and Auranofin (Ridaura®) initially approved for the treatment of rheumatoid arthritis and have been shown to be effective against *Cryptosporidium* in vitro (90, 91), which holds promise for further in vivo testing in animals and humans.

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ZOONOTIC *CRYPTOSPORIDIUM* SPECIES

Due to the morphological similarity of *Cryptosporidium* oocysts from different host species, initial findings of *Cryptosporidium* infections in both domestic and wild animals were assumed to be due to *C. parvum* leading to an overestimation of the potential role of animals as reservoirs of human disease (92). However, with the assistance of advanced molecular techniques, many of these species in wildlife particularly were identified as host-adapted genotypes (6, 10). Of the 31 *Cryptosporidium* species that have been recognised as valid, more than 20 species and genotypes have been identified in humans including *C. hominis, C. parvum, C. meleagridis, C. felis, C. canis, C. cuniculus, C. ubiquitum, C. viatorum, C. muris, C. suis, C. fayeri, C. andersoni, C. bovis, C. scrofarum, C. tyzzeri, C. erinacei* and *Cryptosporidium* horse, skunk and chipmunk I genotypes, with *C. hominis* and *C. parvum* most commonly reported (4, 6, 10). These *Cryptosporidium* spp. infect both immunocompetent and immunocompromised persons (6, 10). Of these, *C. parvum* and *C. hominis* are by far the most common species reported in humans worldwide (4, 93) and are responsible for most cryptosporidiosis outbreaks, with *C. hominis* responsible for more outbreaks than *C. parvum* in most regions (4). Although humans are the major host species for *C. hominis*, there have been isolated reports in domestic animals and wildlife hosts including sheep, goats, cattle, a dugong, non-human primates and kangaroos (94-102, Zahedi et al., unpublished) and in fish (103). *Cryptosporidium parvum* is primarily a parasite of artiodactyls and humans (4) but has also been frequently reported in wildlife, including various rodents, bovids, camelids, equids, canids, non-human primates and marine mammals (see 10) and in fish (103-106). *Cryptosporidium meleagridis*, although primarily a bird parasite, is the third most prevalent species infecting humans (4, 6, 93) and in some studies, *C. meleagridis* prevalence is similar to that of *C. parvum* (107, 108). *Cryptosporidium cuniculus* (previously known as rabbit genotype) was responsible for a drinking-water

associated outbreak of cryptosporidiosis in the UK (109-111) and has also been identified in many sporadic human cases of cryptosporidiosis (112-115). It is also the third most commonly identified *Cryptosporidium* species in patients with diarrhoea in the UK (112). Human infections with *C. canis* and *C. felis* have been reported mainly in studies conducted in children in developing countries (6) where they are responsible for as much as 3.3 and 4.4%, respectively, of overall cryptosporidiosis cases (116). *Cryptosporidium muris* is also considered a zoonotic species as there have been numerous reports of *C. muris* in humans and one report in human sewage (117-129). In a recent human infectivity study, *C. muris* was examined in six healthy adults (130). Volunteers were challenged with 10^5 *C. muris* oocysts and monitored for 6 weeks for infection and/or illness. All six patients became infected. Only two of the infected volunteers had a diarrheal illness (a 33% illness attack rate). Three other volunteers passed an occasional unformed stool or typically had a single soft stool per day without any accompanying gastrointestinal symptoms (130). Like *C. muris, C. andersoni* is also a gastric parasite and primarily infects the abomasum of cattle and to a lesser extent, sheep and goats (6, 93). It is occasionally detected in humans (cf. 10). Two studies in China by the same research group have reported that *C. andersoni* was the most prevalent *Cryptosporidium* species detected in humans (131-132). However, further research is required to better understand the zoonotic importance of *C. andersoni*.

Cryptosporidium ubiquitum is of public health concern because of its wide geographic distribution and broad host range (133). It has been frequently reported from drinking source water and wastewater in various geographic locations, and is considered an emerging zoonotic pathogen as it has been identified in many human cases of cryptosporidiosis (133). Subtyping at the gp60 locus has suggested that sheep and wild rodents are a key source of *C. ubiquitum* transmission to humans, through either direct human contact with infected animals or by contamination of drinking source water (133). It is thought that human encroachment

into wildlife territories has been responsible for the emergence of *C. ubiquitum* and other genotypes like chipmonk genotype I and to a lesser extent, skunk and mink genotypes in human populations (133-143). This highlights the importance of extensive molecular epidemiological studies of wildlife to better understand the public health risks.

RISK MANAGEMENT

A key part of a One Health approach to *Cryptosporidium* prophylaxis is a better understanding of environmental, epidemiological and aetiological factors associated with cryptosporidial infections to enable more targeted risk management. The far-reaching One Health strategy aims at integrating multidisciplinary knowledge and evidence, and at coordinating the intervations, in order to create a global synergism catering for all aspects of health-care for humans, animals and the environment (the One Health Triad).

Under an environmental perspective, the prophylaxis of waterborne cryptosporidiosis must consider optimal management (or design) of source, recycled– and recreational– waters. Protection of source water and swimming pools is a key element of *Cryptosporidium* prevention as contamination of drinking water and swimming pools is a major mode of transmission (see ref 33, 144, 145) and is often achieved by restricting the access to catchments and water bodies, while swimming pools are designed and monitored according to construction standards and guidelines. Infection prevention and management however, can only be achieved through a deep understanding of the routes of transmission, sources of contamination (human and animal), disease prevalence in the population, and the risk factors in the final host.

The link between *Cryptosporidium* in drinking water and sporadic infections is well documented (33, 144, 145), however, the association between drinking water contamination and endemic cryptosporidiosis is not well established. For example, some studies reporting

drinking unsafe water as a risk factor for endemic cryptosporidial infection (146, 147) and others report no association (148-150). Seasonal patterns are also thought to be associated with an increased transmission risk (151, 152), such as when recreational waters are more heavily utilised.

High precipitation events favor the transfer and survival of oocysts in surface waters and/or groundwater (153, 154). This may result in contamination of source water and increased risk of cryptosporidiosis depending on the source of contamination (154). Indeed, the average odds of identifying *Cryptosporidium* oocysts in fresh surface waters is 2.61 (95% CI = 1.63-4.21; I^2 = 16%) times higher during and after extreme weather events (155). Shifts in precipitation patterns (intensity and location) is one of the climate change predictions for the future (156) and this will clearly impact both waterborne and foodborne transmission of *Cryptosporidium* and therefore future human exposures may differ significantly from current patterns as the climate changes (157). Hydrodymanic modelling has been shown to represent a valid and cost-effective support, for decision making and understanding of events (158). Quantitative microbial risk assessment (QMRA), is another widely used tool to estimate health impacts from exposure to *Cryptosporidium* and other pathogens (155) and has been applied to climate change (159). The tool, called CC-QMRA (Climate Change Quantitative Microbial Risk Assessment), quantifies the anticipated impacts in terms of relative infection risks under climate change scenarios for *Cryptosporidium* and other pathogens and can be used to evaluate impacts of climate change on infection risks from waterborne and foodborne transmission of *Cryptosporidium* (160). For example, CC-QMRA can be used to evaluate the effectiveness of interventions such as upgrading wastewater and drinking water treatment and strengthening drinking water and bathing water regulations.

Quantitatification and identification of *Cryptosporidium* in wildlife excreta is an essential starting point for estimating catchment loads (161). Variables such as soil physico-chemical properties, hydrology, orography, meteorology etc. can all afftect oocyst viability, transport and fate. Source water contamination can be avoided or reduced by the implementation of management strategies like wildlife population control, revegetation, landscaping and soil conditioning. In addition, effective risk management cannot overlook the prevalence, infectivity and zoonotic potential of *Cryptosporidium* isolates in the animal populations within the catchment. Similarly, recreational waters like swimming pools, sauna, spas, aquatic parks etc. are also potential sources of outbreaks, depending on the age and health status of the users (and maintenance). Personal hygiene practices (e.g. showering before swimming in public swimming pools, washing hands before cooking, eating and after defecation and washing fruits and vegetables before consumption), are an essential part of any prevention strategy and can prevent/reduce disease transmission (162-165). Enforcing and encouraging similar practices, however, becomes absolutely crucial during outbreaks and in the presence of hyper-susceptible final hosts.

It has been shown that an important host risk factor includes HIV status. *Cryptosporidium* is an important pathogen regardless of HIV-prevalence (16), however HIV-positive children are between 3 and 18 times more likely to have *Cryptosporidium* than those who were HIV-negative (166-168). With the widespread availability of antiretroviral therapy, particularly in industrialised countries, the incidence of cryptosporidiosis has decreased among people living with AIDS (169). However, the increasing number of transplant recipients and those receiving immunosuppressive drugs may contribute significantly to the burden in the future (170, 171). Malnutrition is also a risk factor for both diarrhea and prolonged diarrhea caused by *Cryptosporidium*, with significantly higher rates of infection in malnourished children controlling for HIV status (172-175). An unknown number of

individuals experience asymptomatic *Cryptosporidium* infection (176). This clinically silent infection may remain undetected and untreated and therefore may contribute not only to parasite transmission but also to malnutrition and the associated clinical sequalae. Breast-feeding may provide some protection, as a recent study of Bangladeshi infants reported that protection from *Cryptosporidium* infection was associated with high anti-*Cryptosporidium* IgA in breast milk (177).

VACCINES

The development of vaccines for cryptosporidiosis, particularly in vulnerable populations such as children and malnourished populations is urgent, but has been hampered by an incomplete understanding of the host immune response to *Cryptosporidium* (178, 179). Therefore, a better understanding of host-parasite interactions is crucial for the development of an effective vaccine (178). Given that adults in highly endemic areas are partly immune to reinfection; and human challenge studies have shown that previous infection or exposure leads to a higher infectious dose [ID_{50}] (180, 181), development of a successful vaccine should be possible. It is known that both innate and adaptive host response are important in the control of *Cryptosporidium* infection (182-184). Yet the nature of these responses, particularly in humans, is not completely understood (179, 185).

Early mediators of innate immune protection include the thick mucus layer of the small intestine, intestinal epithelial cells and chemokines, cytokines and antimicrobial peptides secreted into the intestinal lumen and/or underlying submucosa and bloodstream (179). Important cytokines include interferon gamma (IFN-γ), which is secreted early in infection by natural killer (NK) cells, macrophages and dendritic cells which are thought to play a major role in orchestrating both the innate and adaptive immune responses (179, 183). Th1 inflammatory response, cytokines, such as interleukin 12, 15 and 18 are also important in the

resistance and recovery to *Cryptosporidium* infection (186-191). Treatment of both immunocompetent and immunodeficient mice with IL-12 before infection prevented or greatly reduced the severity of infection and was attributed to a decrease in IFN-γ reduction (191). Data suggests that IL-15 has an important role in activating an NK cell-mediated pathway that leads to the elimination of *Cryptosporidium* from the intestine (187). IL-18 is produced by epithelial cells in the gut and a number of different immune cells and is upregulated in response to *C. parvum* infection and it has been proposed that one of the functions of IL-18 is to promote IFN-γ expression by macrophages (188). Toll-like receptors expressed by epithelial cells have been shown to be important in modulation of the host immune response and subsequent parasite clearance (192-198).

MicroRNA (miRNA) regulation also appears to play an important role in host cell protection against *Cryptosporidium* (199-204). miRNA are small RNA molecules of 23 nucleotides that result in gene silencing via translational suppression or mRNA degradation and are a mechanism to fine-tune cellular responses to the environment, and may be regulators of host anti-microbial immune responses (202). More than 700 miRNAs have been identified in humans and are postulated to control 20-30% of human genes. miRNA-mediated post-transcriptional gene regulation may regulate expression of genes critical to epithelial anti-microbial defense and one cellular miRNA (*let-7i*) has been shown to target Toll-like receptor 4 (TLR4) and regulate TLR4-mediated anti-*C. parvum* defense (199). Functional manipulation of select miRNA expression levels in epithelial cells has been shown to alter *C. parvum* infection burden in vitro (202, 203). The intercellular adhesion molecule-1 (ICAM-1; CD54) is a 90 kDa member of the Ig superfamily expressed by several cell types including endothelial cells and epithelial cells and is thought to facilitate adhesion and recognition of lymphocytes at infection sites as ICAM-1 is constitutively present on endothelial cells and epithelial cells, but its expression is increased by pro-inflammatory cytokines or following

microbe infection. Evidence has shown that miR-221-mediated translational suppression controls ICAM-1 expression through targeting the ICAM-1 3′-untranslated region (UTR), in epithelial cells in response to *C. parvum* infection, as transfection of an miR-221 precursor in an vitro model of human biliary cryptosporidiosis abolished *C. parvum*-stimulated ICAM-1 protein expression (202).

Mannose-binding lectin (MBL) is an evolutionarily conserved protein, secreted by hepatocytes, that functions in human innate immunity by binding to microbial surfaces and promoting opsonophagocytosis. MBL has been shown to be important in the protection against cryptosporidiosis, as children and HIV-infected adults with mannose-binding lectin deficiency have increased susceptibility to cryptosporidiosis and more severe disease (205-207). The genetic contribution to deficient or low serum levels of MBL results from polymorphisms in the MBL2 gene (MBL1 is a pseudogene), which create low MBL-producing MBL2 genotypes in ~5% of the world's population (207). In one study on a cohort of preschool children from Dhaka, Bangladesh, polymorphisms in the MBL2 gene (and corresponding haplotypes) and deficient serum levels of MBL were associated with increased susceptibility to infection with *Cryptosporidium*. MBL deficiency of <500 ng/mL was associated with single and multiple symptomatic episodes of *Cryptosporidium* infection, with an OR of 7.6 for children with multiple symptomatic infections with *Cryptosporidium* (207). The mechanism by which MBL controls *Cryptosporidium* infection and protects children from it is still not clearly understood.

Adaptive immunity creates immunological memory after an initial response to *Cryptosporidium* and leads to an enhanced response to subsequent encounters with *Cryptosporidium*. For example, antibodies to the parasite antigen gp15 were associated with protection against reinfection (208). The adaptive immune response to *Cryptosporidium* is characterized as a T-helper 1 (Th1) response (189) and the importance of the adaptive

immune response during *Cryptosporidium* infection is highlighted by the susceptibility of AIDS patients to cryptosporidiosis, as well as the resolution of infection observed following CD4+ T lymphocyte cell reconstitution in patients given antiretroviral therapy (185, 209). Low absolute CD4+ T cell counts in patients with HIV/AIDS were thought to be responsible for persistent and severe cryptosporidiosis, however research with Simian immunodeficiency virus (SIV)-infected macaques reported that persistent cryptosporidiosis was more dependant on SIV load and profound viral damage to gut lymphoid tissue and rapid depletion of mucosal CD4+ T cells during the acute phase of viral infection, than on declining circulating CD4+ T cell levels during chronic SIV infection (210). This suggests that depletion of local CD4+ T cells may be more predictive of disease severity than absolute CD4+ T cell numbers. The importance of other T cells such as CD8+ have not been extensively studied but do appear to play a role (179). The role of humoral immunity in protection from cryptosporidiosis is not well understood and no clear surrogate marker of protective immunity exists (reviewed in 46 and 179).

The ideal *Cryptosporidium* vaccine should provide rapid life-long immunity in all vaccinated individuals, be broadly protective against the most common species and subtypes of *Cryptosporidium*, prevent disease transmission, and be readily accessible, stable, and cheap (178, 179). Ensuring cross-reaction against the most common species infecting humans however will be difficult, as more than 20 *Cryptosporidium* species and genotypes can infect humans as discussed above. For example, a recent study showed that infection of gnotobiotic pigs with *C. hominis* resulted in complete protection against subsequent infection with *C. hominis*, but incomplete protection against infection with *C. parvum* (211), therefore multiple species will need to be targeted to provide sufficient cross-protection. In addition, as children, malnourished and immunocompromised individuals are the most important vaccine targets, they may not be able to develop a strong and sustained immune-mediated protection in

response to vaccination. Indeed, malnutrition has been cited as an important factor underlying limited efficacy of vaccines (212). It is therefore likely that adjuvants such as TLR ligands (213), will be required to enhance the immune-response in target populations (193, 196).

Several antigens, aimed at raising immunoglobulin G antibodies, are being developed as vaccine candidates (178). Some of the best studied are *gp*15 (214-219), *cp*15 (220-225) and *cp*23 (223, 226, 227). The *gp*15 antigen is derived from the glycoprotein *gp*60, which is cleaved by a parasite serine proteinase into two surface proteins - *gp*15 and *gp*40, both of which play an essential role in parasite motility and attachment to and invasion of host epithelial cells (228), and can stimulate interferon γ production by peripheral blood mononuclear cells of those previously infected (214), The *gp*15 antigen is relatively conserved between *C. parvum* and *C. hominis,* and studies in Bangladesh indicated that there is significant cross-reactivity between them and that antibodies to *gp*15 were associated with shorter duration of illness (217). Similarly, in a study in Kenya, AIDS patients without diarrhea had significantly higher serum IgG levels to *gp*15 than those with diarrhea (229).

*cp*15 is an immunodominant protein present on the oocyst surface and is associated with internal structures and bears no apparent similarity to *gp*15 (228). Immunisation of pregnant goats with *cp*15 vaccines protected off-spring (230). The impact of malnutrition however on vaccination was demonstrated in recent research on intranasal vaccination of nourished and malnourished mice, with the *cp*15 antigen primed with a live enteric bacterial vector (225). The authors reported that malnutrition blunted antigen-specific cell-mediated responses to *cp*15 and that vaccination resulted in only transient reduction in stool shedding of *Cryptosporidium* and was not protective against disease (225).

*cp*23 is an immunodominant protein, geographically conserved among *C. parvum* isolates, is present in both the sporozoite and merozoite stages (178), and antibodies to it are frequently detected following *Cryptosporidium* infection (231, 229). Serum antibodies to

both *gp*15 and *cp*23 are associated with protection from diarrhea in immunocompetent adult human volunteers infected with *Cryptosporidium* (208, 232-234). Thus, a multivalent vaccine, incorporating multiple antigens or antigenic epitopes, may enhance protection against infection. For example a divalent *cp*23 and *cp*15 vaccine prolonged the prepatent period and decreased oocyst shedding in mice vaccinated with the divalent vaccine compared with vaccination with *cp*23 alone (223). Similarly a reverse vaccinology approach based on genome mining that included three antigens; the well characterised *cp*15, a calcium-activated apyrase involved in the invasion process of *Cryptosporidium* and profilin, an agonist of the innate immune system through its recognition by Toll-like receptors, induced specific and potent humoral and cellular immune responses in mice, however, further studies are necessary to verify the protection induced by these antigens (224). The development of an effective vaccine against *Cryptosporidium* is still a challenge and a better understanding of which immune responses are necessary for protection are essential to the development of immune-based interventions.

CONCLUSIONS

Recent developments have improved our understanding of both the genetics of and immune response in cryptosporidial infections. However, many knowledge gaps remain. Current diagnostic tests each have their limitations in cost, performance, differentiation of clinical significance, and assessment of co-infections with other pathogens. Inaccessability of diagnostic testing in non-industrialised has meant that the knowledge of the epidemiology of *Cryptosporidium* infection in early infancy is scarce, and as a result, the burden of cryptosporidiosis is under-reported and under-estimated, which reinforces ineffective clinical and public health management of *Cryptosporidium*. Rapid, cost-effective and reliable diagnostic tests therefore need to be developed for non-industrialised countries to improve

detection, reporting and interpretation of results in the setting of multiple infections. With the recent improvements in cell culture and genetic manipulation, identification of novel or repurposed therapeutics should be radically transformed. Vaccines have the potential to reduce the significant burden of disease, but the extent and types of immunity necessary, and the methods by which to administer and induce protective immunity need further study.

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DISCLOSURES

The authors have no potential conflict of interest to disclose.

REFERENCES

1. Monis PT & Thompson RCA. *Cryptosporidium* and *Giardia* – zoonoses: fact or fiction? *Infect Genet Evol* 2003; **3**: 23-244.
2. Hunter PR, Hadfield SJ, Wilkinson D, et al. Subtypes of *Cryptosporidium parvum* in humans and disease risk. *Emerg Infect Dis*; 2007 **13**: 82-88.
3. Ryan U & Power M. *Cryptosporidium* species in Australian wildlife and domestic animals. *Parasitol* 2012; **139**: 1673-1688.
4. Xiao L. Molecular epidemiology of cryptosporidiosis: an update. *Exp Parasitol* 2010; **124**: 80-89.
5. Kváč M, Saková K, Květoňová D, et al. Gastroenteritis caused by the *Cryptosporidium* hedgehog genotype in an immunocompetent man. *J Clin Microbiol* 2014; **52**: 347-349.

6. Ryan U, Fayer R & Xiao L. *Cryptosporidium* species in humans and animals: current understanding and research needs. *Parasitol* 2014; **141**: 1667-1685.

7. Costa J, Cruz C, Eiras JC & Saraiva A, Characterization of a *Cryptosporidium scophthalmi*-like isolate from farmed turbot (*Scophthalmus maximus*) using histological and molecular tools. *Dis Aquat Organ* 2016; In press.

8. Li X, Pereira Md, Larsen R, Xiao C, et al. *Cryptosporidium rubeyi* n. sp. (Apicomplexa: Cryptosporidiidae) in multiple *Spermophilus* ground squirrel species. *Int J Parasitol Parasites Wildl* 2015; **4**: 343-350.

9. Ryan U, Paparini A, Tong K, et al. *Cryptosporidium huwi* n. sp. (Apicomplexa: Eimeriidae) from the guppy (*Poecilia reticulata). Exp Parasitol* 2015; **150**: 31-35.

10. Zahedi A, Paparini A, Jian F, Robertson I & Ryan U. Public health significance of zoonotic *Cryptosporidium* species in wildlife: critical insights into better drinking water management *Int J Parasitol Parasites Wildl* 2016; 5, 88e109.

11. Holubová N, Sak B, Horčičková M, et al. *Cryptosporidium avium* n. sp. (Apicomplexa: Cryptosporidiidae) in birds. *Parasitol Res* 2016; **115**:2243-2251.

12. Kváč M, Havrdová N, Hlásková L, et al. *Cryptosporidium proliferans* n. sp. (Apicomplexa: Cryptosporidiidae): Molecular and biological evidence of cryptic species within gastric *Cryptosporidium* of mammals. *PLoS One* 2016; **11**:e0147090.

13. Chalmers RM & Davies AP. Minireview: clinical cryptosporidiosis. *Exp Parasitol* 2010; **124**: 138-146.

14. Bouzid M, Hunter PR, Chalmers RM & Tyler KM. *Cryptosporidium* pathogenicity and virulence. *Clin Microbiol Rev* 2013; **26**: 115-134.

15. Current WL & Garcia LS. Cryptosporidiosis. *Clin Microbiol Rev* 1991; **4**: 325-358.

16. Kotloff KL, Nataro JP, Blackwelder WC, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet* 2013; **382**: 209-222.

17. Nasrin D, Wu Y, Blackwelder WC, et al. Health care seeking for childhood diarrhea in developing countries: evidence from seven sites in Africa and Asia. *Am J Trop Med Hyg* 2013; **89**: 3-12.

18. Breurec S, Vanel N, Bata P, et al. Etiology and Epidemiology of Diarrhea in Hospitalized Children from Low Income Country: A Matched Case-Control Study in Central African Republic. *PLoS Negl Trop Dis* 2016; **10**: e0004283.

19. Platts-Mills JA, Babji S, Bodhidatta L, et al. Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). *Lancet Glob Health* 2015; **3**:e564-75.

20. Shoultz DA, de Hostos EL & Choy RKM. Addressing *Cryptosporidium* infection among young children in low-income settings: The crucial role of new and existing drugs for reducing morbidity and mortality. *PLoS Negl Trop Dis* 2016; **10**: e0004242.

21. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; **380**: 2095-2128.

22. Striepen B, Parasitic infections: Time to tackle cryptosporidiosis. *Nature* 2013; **503**: 189-191.

23. Mølbak K, Andersen M, Aaby P, et al. *Cryptosporidium* infection in infancy as a cause of malnutrition: a community study from Guinea-Bissau, west Africa. *Am J Clin Nutr* 1997; **65**: 149-152.

24. Guerrant DI, Moore SR, Lima AA, et al. Association of early childhood diarrhea and cryptosporidiosis with impaired physical fitness and cognitive function four-seven years later in a poor urban community in northeast Brazil. *Am J Trop Med Hyg* 1999; **61**: 707-713.

25. Kirkpatrick BD, Daniels MM, Jean SS, et al. Cryptosporidiosis stimulates an inflammatory intestinal response in malnourished Haitian children. *J Infect Dis* 2002; **186**: 94-101.

26. Macfarlane DE & Horner-Bryce J. Cryptosporidiosis in well-nourished and malnourished children. *Acta Paediatr Scand* 1987; **76**: 474-477.

27. Sallon S, Deckelbaum RJ, Schmid II, Harlap S, Baras M & Spira DT. *Cryptosporidium,* malnutrition, and chronic diarrhea in children. *Am J Dis Child* 1988; **142**: 312-315.

28. Bushen OY, Kohli A, Pinkerton RC, et al. Heavy cryptosporidial infections in children in northeast Brazil: comparison of *Cryptosporidium hominis* and *Cryptosporidium parvum*. *Trans R Soc Trop Med Hyg* 2007;**101**: 378-384.

29. Checkley W, Gilman RH, Epstein LD, et al. Asymptomatic and symptomatic cryptosporidiosis: their acute effect on weight gain in Peruvian children. *Am J Epidemiol* 1997; **145**: 156-163.

30. Mondal D, Haque R, Sack RB, Kirkpatrick BD & Petri WA, Jr. Attribution of malnutrition to cause-specific diarrheal illness: evidence from a prospective study of preschool children in Mirpur, Dhaka, Bangladesh. *Am J Trop Med Hyg* 2009; **80**: 824-826.

31. Quihui-Cota L, Lugo-Flores CM, Ponce-Martínez JA & Morales-Figueroa GG. Cryptosporidiosis: a neglected infection and its association with nutritional status in schoolchildren in northwestern Mexico. *J Infect Dev Ctries* 2015; **9**: 878-883.

32. Fletcher S, Stark D, Harkness J & Ellis J. Enteric Protozoa in the Developed World: a Public Health Perspective. *Clin Microbiol Rev* 2012; **25**: 420-449.

33. Baldursson S & Karanis P. Waterborne transmission of protozoan parasites: 2004 2010. *Water Res* 2011; **45**: 6603-6614.

34. Burnet JB, Penny C, Ogorzaly L & Cauchie HM. Spatial and temporal distribution of *Cryptosporidium* and *Giardia* in a drinking water resource: implications for monitoring and risk assessment. *Sci Total Environ* 2014; **472**: 1023-1035.

35. Chappell CL, Okhuysen PC, Langer-Curry R, et al. *Cryptosporidium hominis*: experimental challenge of healthy adults. *Am J Trop Med Hyg* 2006; **75**: 851-857.

36. Okhuysen PC, Chappell CL, Crabb JH, Sterling CR & DuPont HL. Virulence of three distinct *Cryptosporidium parvum* isolates for healthy adults. *J Infect Dis* 1999; **180**: 1275-1281.

37. Pouillot R, Beaudeau P, Denis JB & Derouin F. A quantitative risk assessment of waterborne cryptosporidiosis in France using second-order Monte Carlo simulation. *Risk Anal* 2004; **24**: 1-17.

38. 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**: 387-393.

39. Amadi B, Mwiya M, Sianongo S, 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.

40. Robertson, LJ, Bjorkman, C., Axen C & Fayer R. Cryptosporidiosis in farmed animals. In *Cryptosporidium: parasite and disese*, ed. Caccio SM & Widmer G, Springer: New York; 2014; 145-236.

41. Nyachuba DG. Foodborne illness: is it on the rise? *Nutr Rev* 2010; **68**: 257-269.

42. Budu-Amoako E, Greenwood SJ, Dixon BR, Barkema HW & McClure JT. Foodborne illness associated with *Cryptosporidium* and *Giardia* from livestock. *J Food Prot* 2011; **74**: 1944-1955.

43. Sponseller JK, Griffiths JK & Tzipori S. 2014. The evolution of respiratory Cryptosporidiosis: evidence for transmission by inhalation. *Clin Microbiol Rev* 2014; **27**: 575-586.

44. Schwabe CW. Veterinary medicine and human health. 1984. 3rd ed. Baltimore (MD): Williams and Wilkins.

45. Chalmers RM & Katzer F. Looking for *Cryptosporidium*: the application of advances in detection and diagnosis. *Trends Parasitol* 2013; **29**: 237-251.

46. Checkley W, White AC Jr, Jaganath D, et al. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for *Cryptosporidium*. *Lancet Infect Dis* 2015; **15**: 85-94.

47. Fall A, Thompson RC, Hobbs RP & Morgan-Ryan U. Morphology is not a reliable tool for delineating species within *Cryptosporidium. J Parasitol* 2003; **89**: 399-402.

48. O'Donoghue PJ. *Cryptosporidium* and cryptosporidiosis in man and animals. *Int J Parasitol* 1995; **25**: 139-195.

49. Weber R, Bryan RT, Bishop HS, Wahlquist SP, Sullivan JJ & Juranek DD. Threshold of detection of *Cryptosporidium* oocysts in human stool specimens: evidence for low sensitivity of current diagnostic methods. *J Clin Microbiol* 1991; **29**: 1323-1327.

50. Clavel A, Arnal AC, Sánchez EC, et al. Evaluation of the optimal number of faecal specimens in the diagnosis of cryptosporidiosis in AIDS and immunocompetent patients. *Eur J Clin Microbiol Infect Dis* 1995; **14**: 46-49.

51. Chalmers RM, Campbell BM, Crouch N, Charlett A & Davies AP. Comparison of diagnostic sensitivity and specificity of seven *Cryptosporidium* assays used in the UK. *J Med Microbiol* 2011; **60**: 1598-1604.

52. Ignatius R, Klemm T, Zander S, et al. Highly specific detection of *Cryptosporidium* spp. oocysts in human stool samples by undemanding and inexpensive phase contrast microscopy. *Parasitol Res* 2016; **115**:1229-1234.

53. Garcia LS & Shimizu RY. Evaluation of nine immunoassay kits (enzyme immunoassay and direct fluorescence) for detection of *Giardia lamblia* and *Cryptosporidium parvum* in human fecal specimens. *J Clin Microbiol* 1997; **35**: 1526-1529.

54. Johnston SP, Ballard MM, Beach MJ, Causer L & Wilkins PP. Evaluation of three commercial assays for detection of *Giardia* and *Cryptosporidium* organisms in fecal specimens. *J Clin Microbiol* 2003; **41**: 623-626.

55. Youn S, Kabir M, Haque R & Petri WA Jr. Evaluation of a screening test for detection of *Giardia* and *Cryptosporidium* parasites. *J Clin Microbiol* 2009; **47**: 451-452.

56. Robinson TJ, Cebelinski EA, Taylor C & Smith KE. Evaluation of the positive predictive value of rapid assays used by clinical laboratories in Minnesota for the diagnosis of cryptosporidiosis. *Clin Infect Dis* 2010; **50**: e53-55.

57. Agnamey P, Sarfati C, Pinel C, et al. Evaluation of four commercial rapid immunochromatographic assays for detection of *Cryptosporidium* antigens in stool samples: a blind multicenter trial. *J Clin Microbiol* 2011; **49**: 1605-1607.

58. Kang CD, Cao C, Lee J, Choi IS, Kim BW & Sim SJ. Surface plasmon resonance-based inhibition assay for real-time detection of *Cryptosporidium parvum* oocyst. *Water Res* 2008; **42**: 1693-1699.

59. Campbell GA & Mutharasan R. Near real-time detection of *Cryptosporidium parvum* oocyst by IgM-functionalized piezoelectric-excited millimeter-sized cantilever biosensor. *Biosens Bioelectron* 2008; **23**: 1039-1045.

60. Hadfield SJ, Robinson G, Elwin K & Chalmers RM. Detection and differentiation of *Cryptosporidium* spp. in human clinical samples by use of real-time PCR. *J Clin Microbiol* 2011; **49**: 918-924.

61. Elwin K, Robinson G, Hadfield SJ, Fairclough HV, Iturriza-Gómara M & Chalmers RM. A comparison of two approaches to extracting *Cryptosporidium* DNA from human stools as measured by a real-time PCR assay. J Microbiol Methods 2012; **89**: 38-40.

62. Yang R, Murphy C, Song Y, et al. Specific and quantitative detection and identification of *Cryptosporidium hominis* and *C. parvum* in clinical and environmental samples. *Exp Parasitol* 2013; **135**: 142-147.

63. Yang R, Jacobson C, Gardner G, et al. Longitudinal prevalence, oocyst shedding and molecular characterisation of *Cryptosporidium* species in sheep across four states in Australia. *Vet Parasitol* 2014; **200**: 50-58.

64. Taniuchi M, Sobuz SU, Begum S, et al. Etiology of diarrhea in Bangladeshi infants in the first year of life analyzed using molecular methods. *J Infect Dis* 2013; **208**: 1794-802.

65. Van Lint P, Rossen JW, Vermeiren S, et al. Detection of *Giardia lamblia, Cryptosporidium* spp. and *Entamoeba histolytica* in clinical stool samples by using multiplex real-time PCR after automated DNA isolation. *Acta Clin Belg* 2013; **68**: 188-192.

66. Nurminen N, Juuti R, Oikarinen S, et al. High-throughput multiplex quantitative polymerase chain reaction method for *Giardia lamblia* and *Cryptosporidium* species detection in stool samples. *Am J Trop Med Hyg* 2015; **92**: 1222-1226.

67. Bridle H, Kersaudy-Kerhoas M, Miller B, et al. Detection of *Cryptosporidium* in miniaturised fluidic devices. Water Res 2012; **46**: 1641-1661.

68. Abrahamsen MS, Templeton TJ, Enomoto S, et al. Complete genome sequence of the apicomplexan, *Cryptosporidium parvum*. *Science* 2004; **304**: 441-415.

69. Ryan U & Hijjawi N. New developments in *Cryptosporidium* research. *Int J Parasitol* 2015; **45**: 367-373.

70. Miyamoto Y & Eckmann L. Drug Development Against the major diarrhea-causing parasites of the small intestine, *Cryptosporidium* and *Giardia*. *Front Microbiol* 2015; **6**: 1208.

71. Morada M, Lee S, Gunther-Cummins L, et al. Continuous culture of *Cryptosporidium parvum* using hollow fiber technology. *Int J Parasitol* 2016; **46**: 21-29.

72. Vinayak S, Pawlowic MC, Sateriale A, et al. Genetic modification of the diarrhoeal pathogen *Cryptosporidium parvum*. *Nature* 2015; **523**: 477-480.

73. Striepen B, Pruijssers AJ, Huang J, et al. Gene transfer in the evolution of parasite nucleotide biosynthesis. *Proc Natl Acad Sci U S A* 2004; **101**: 3154-3159.

74. Kirubakaran S, Gorla SK, Sharling L, et al. Structure-activity relationship study of selective benzimidazole-based inhibitors of *Cryptosporidium parvum* IMPDH. *Bioorg Med Chem Lett* 2012; **22**: 1985-1988.

75. Mandapati K, Gorla SK, House AL, et al. Repurposing *Cryptosporidium* inosine 5'-monophosphate dehydrogenase inhibitors as potential antibacterial agents. *ACS Med Chem Lett* 2014; **5**: 846-850.

76. Umejiego NN, Gollapalli D, Sharling L, et al. Targeting a prokaryotic protein in a eukaryotic pathogen: identification of lead compounds against cryptosporidiosis. *Chem Biol* 2008; **15**: 70-77.

77. Maurya SK, Gollapalli DR, Kirubakaran S, et al. Triazole inhibitors of *Cryptosporidium parvum* inosine 5'-monophosphate dehydrogenase. *J Med Chem* 2009; **52**: 4623-4630.

78. Macpherson IS, Kirubakaran S, Gorla SK, et al. The structural basis of *Cryptosporidium*-specific IMP dehydrogenase inhibitor selectivity. *J Am Chem Soc* 2010; **132**: 1230-1231.

79. Sharling L, Liu X, Gollapalli DR, Maurya SK, Hedstrom L & Striepen B. A screening pipeline for antiparasitic agents targeting *Cryptosporidium* inosine monophosphate dehydrogenase. PLoS Negl Trop Dis 2010; **4**: e794.

80. Gorla SK, Kavitha M, Zhang M, et al. Selective and potent urea inhibitors of *Cryptosporidium parvum* inosine 5'-monophosphate dehydrogenase. J Med Chem. 2012; **55**: 7759-7771.

81. Gorla SK, Kavitha M, Zhang M, et al. Optimization of benzoxazole-based inhibitors of *Cryptosporidium parvum* inosine 5'-monophosphate dehydrogenase. *J Med Chem* 2013; **56**: 4028-4043.

82. Johnson CR, Gorla SK, Kavitha M, et al. Phthalazinone inhibitors of inosine-5'-monophosphate dehydrogenase from *Cryptosporidium parvum*. *Bioorg Med Chem Lett* 2013; **23**: 1004-1007.

83. Jefferies R, Yang R, Woh CK, et al. Target validation of the inosine monophosphate dehydrogenase (IMPDH) gene in *Cryptosporidium* using Phylomer$^{(®)}$ peptides. *Exp Parasitol* 2015; **148**: 40-48.

84. Kim Y, Makowska-Grzyska M, Gorla SK, et al. Structure of *Cryptosporidium* IMP dehydrogenase bound to an inhibitor with in vivo antiparasitic activity. *Acta Crystallogr F Struct Biol Commun* 2015; **71**: 531-538.

85. Li RJ, Wang YL, Wang QH, Huang WX, Wang J & Cheng MS. Binding mode of inhibitors and *Cryptosporidium parvum* IMP dehydrogenase: A combined ligand- and receptor-based study. *SAR QSAR Environ Res* 2015; **26**: 421-438.

86. Guo F, Zhang H, Fritzler JM, et al. Amelioration of *Cryptosporidium parvum* infection in vitro and in vivo by targeting parasite fatty acyl-coenzyme A synthetases. *J Infect Dis* 2014; **209**: 1279-1287.

87. Ndao M, Nath-Chowdhury M, Sajid M, et al. A cysteine protease inhibitor rescues mice from a lethal *Cryptosporidium parvum* infection. *AntimicrobAgents Chemother* 2013; **57**: 6063-6073.

88. Adams CP & Brantner, VV. Estimating the cost of new drug development: is it really $802 million? *Health Aff (Millwood)* 2006; **25**: 420-428.

89. Bessoff K, Spangenberg T, Foderaro JE, Jumani RS, Ward GE & Huston CD. Identification of *Cryptosporidium parvum* active chemical series by repurposing the open access malaria box. *Antimicrob Agents Chemother* 2014; **58**: 2731-2739.

90. Bessoff K, Sateriale A, Lee KK & Huston CD. Drug repurposing screen reveals FDA-approved inhibitors of human HMG-CoA reductase and isoprenoid synthesis that block *Cryptosporidium parvum* growth. *Antimicrob Agents Chemother* 2013; **57**: 1804-1814.

91. Debnath A, Ndao M & Reed SL. Reprofiled drug targets ancient protozoans: drug discovery for parasitic diarrheal diseases. *Gut Microbes* 2013; **4**: 66-71.

92. Appelbee AJ, Thompson RC & Olson ME. *Giardia* and *Cryptosporidium* in mammalian wildlife-current status and future needs. *Trends Parasitol* 2005; **21**: 370-376.

93. Ryan U & Xiao L. Taxonomy and molecular taxonomy. In *Cryptosporidium: parasite and disease*, ed. Caccio SM & Widmer G, Springer: New York; 2014; 3-42.

94. Morgan UM, Xiao L, Hill BD, et al. Detection of the *Cryptosporidium parvum* "human" genotype in a dugong (*Dugong dugon*). *J Parasitol* 2000; **86**: 1352-1354.

95. Smith HV, Nichols RA, Mallon M, et al. Natural *Cryptosporidium hominis* infections in Scottish cattle. *Vet Rec* 2005; **156**: 710-711.

96. Giles M, Chalmers R, Pritchard G, Elwin K, Mueller-Doblies D & Clifton-Hadley F. *Cryptosporidium hominis* in a goat and a sheep in the UK. *Vet Rec* 2009; **164**: 24-25.

97. Abeywardena H, Jex AR, Nolan MJ, et al. Genetic characterisation of *Cryptosporidium* and *Giardia* from dairy calves: discovery of species/genotypes consistent with those found in humans. *Infect Genet Evol* 2012; **12**: 1984-1993.

98. Ye J, Xiao L, Ma J, Guo M, Liu L & Feng Y. Anthroponotic enteric parasites in monkeys in public park, China. *Emerg Infect Dis* 2012; **18**: 1640-1643.

99. Connelly L, Craig BH, Jones B & Alexander CL. Genetic diversity of *Cryptosporidium* spp. within a remote population of Soay Sheep on St. Kilda Islands, Scotland. *Appl Environ Microbiol* 2013; **79**: 2240-2246.

100. Karim MR, Zhang S, Jian F, et al. Multilocus typing of *Cryptosporidium* spp. and *Giardia duodenalis* from non-human primates in China. *Int J Parasitol* 2014; **44**: 1039-1047.

101. Koinari M, Lymbery AJ & Ryan UM. *Cryptosporidium* species in sheep and goats from Papua New Guinea. *Exp Parasitol* 2014; **141**: 134-137.

102. Parsons MB, Travis D, Lonsdorf EV, et al. Epidemiology and molecular characterization of *Cryptosporidium* spp. in humans, wild primates, and domesticated animals in the Greater Gombe Ecosystem, Tanzania. *PLoS Negl Trop Dis* 2015; **9**: e0003529.

103. Koinari M, Karl S, Ng-Hublin J, Lymbery AJ & Ryan UM. Identification of novel and zoonotic *Cryptosporidium* species in fish from Papua New Guinea. *Vet Parasitol* 2013; **198**: 1-9.

104. Reid A, Lymbery A, Ng J, Tweedle S & Ryan U. Identification of novel and zoonotic *Cryptosporidium* species in marine fish. *Vet Parasitol* 2010; **168**: 190-195.

105. Gibson-Kueh S, Yang R, Thuy NT, Jones JB, Nicholls PK & Ryan U. The molecular characterization of an *Eimeria* and *Cryptosporidium* detected in Asian seabass (*Lates calcarifer*) cultured in Vietnam. *Vet Parasitol* 2011; **181**: 91-96.

106. Certad G, Dupouy-Camet J, Gantois N, et al. Identification of *Cryptosporidium* Species in Fish from Lake Geneva (Lac Léman) in France. *PLoS One* 2015; **10**: e0133047.

107. Gatei W, Suputtamongkol Y, Waywa D, et al. Zoonotic species of *Cryptosporidium* are as prevalent as the anthroponotic in HIV-infected patients in Thailand. *Ann Trop Med Parasitol* 2002; **96**: 797-802.

108. Cama VA, Ross JM, Crawford S, et al. Differences in clinical manifestations among *Cryptosporidium* species and subtypes in HIV-infected persons. *J Infect Dis* 2007; **196**: 684-691.

109. Chalmers RM, Robinson G, Elwin K, et al. *Cryptosporidium* sp. rabbit genotype, a newly identified human pathogen. *Emerg Infect Dis* 2009; **15**: 829-830.

110. Robinson G, Chalmers RM, Stapleton C, et al. A whole water catchment approach to investigating the origin and distribution of *Cryptosporidium* species. *J Appl Microbiol* 2011; **111**: 717-730.

111. Puleston RL, Mallaghan CM, Modha DE, et al. The first recorded outbreak of cryptosporidiosis due to *Cryptosporidium cuniculus* (formerly rabbit genotype), following a water quality incident. *J Water Health* 2014; **12**: 41-50.

112. Chalmers RM, Elwin K, Hadfield SJ & Robinson G. Sporadic human cryptosporidiosis caused by *Cryptosporidium cuniculus*, United Kingdom, 2007-2008. *Emerg Infect Dis* 2011; **17**: 536-538.

113. Elwin K, Hadfield SJ, Robinson G & Chalmers RM. The epidemiology of sporadic human infections with unusual cryptosporidia detected during routine typing in England and Wales, 2000-2008. *Epidemiol Infect* 2012; **140**: 673-683.

114. Koehler AV, Whipp MJ, Haydon SR & Gasser RB. *Cryptosporidium cuniculus*-new records in human and kangaroo in Australia. *Parasit Vectors* 2014; **7**: 492.

115. Martínez-Ruiz R, de Lucio A, Fuentes I, Carmena D. Autochthonous *Cryptosporidium cuniculus* infection in Spain: First report in a symptomatic paediatric patient from Madrid. *Enferm Infecc Microbiol Clin* 2016; In press.

116. Lucio-Forster A, Griffiths JK, Cama VA, Xiao L & Bowman DD. Minimal zoonotic risk of cryptosporidiosis from pet dogs and cats. *Trends Parasitol* 2010; **26**: 174-179.

117. Guyot K, Follet-Dumoulin A, Lelièvre E, et al. Molecular characterization of *Cryptosporidium* isolates obtained from humans in France. *J Clin Microbiol* 2001; **39**: 3472-3480.

118. Gatei W, Ashford RW, Beeching NJ, Kamwati SK, Greensill J & Hart CA. *Cryptosporidium muris* infection in an HIV-infected adult, Kenya. *Emerg Infect Dis* 2002; **8**: 204-206.

119. Tiangtip R & Jongwutiwes S. Molecular analysis of *Cryptosporidium* species isolated from HIV-infected patients in Thailand. *Trop Med Int Health* 2002; **7**: 357-364.

120. Gatei W, Greensill J, Ashford RW, et al. Molecular analysis of the 18S rRNA gene of *Cryptosporidium* parasites from patients with or without human immunodeficiency virus infections living in Kenya, Malawi, Brazil, the United Kingdom, and Vietnam. *J Clin Microbiol* 2003; **41**: 1458-1462.

121. Palmer CJ, Xiao L, Terashima A, et al. *Cryptosporidium muris*, a rodent pathogen, recovered from a human in Perú. *Emerg Infect Dis* 2003; **9**: 1174-1176.

122. Gatei W, Wamae CN, Mbae C, et al. Cryptosporidiosis: prevalence, genotype analysis, and symptoms associated with infections in children in Kenya. *Am J Trop Med Hyg* 2006; **75**: 78-82.

123. Leoni F, Amar C, Nichols G, Pedraza-Díaz S & McLauchlin J. Genetic analysis of Cryptosporidium from 2414 humans with diarrhoea in England between 1985 and 2000. *J Med Microbiol* 2006; **55**: 703-707.

124. Muthusamy D, Rao SS, Ramani S, et al. Multilocus genotyping of *Cryptosporidium* sp. isolates from human immunodeficiency virus-infected individuals in South India. *J Clin Microbiol* 2006; **44**: 632-634.

125. Al-Brikan FA, Salem HS, Beeching N & Hilal N. Multilocus genetic analysis of *Cryptosporidium* isolates from Saudi Arabia. *J Egypt Soc Parasitol* 2008; **38**: 645-658.

126. Neira O P, Muñoz S N, Wilson L G, Barthel M ME, Rosales L MJ & Henríquez R C. *Cryptosporidium* species in immunodeficient and immunocompetent patients of Valparaíso: a descriptive study. *Rev Chilena Infectol* 2012; **29**: 63-71.

127. Hasajová A, Valenčáková A, Malčeková B, et al. Significantly higher occurrence of *Cryptosporidium* infection in Roma children compared with non-Roma children in Slovakia. *Eur J Clin Microbiol Infect Dis* 2014; **33**: 1401-1406.

128. Spanakos G, Biba A, Mavridou A & Karanis P. Occurrence of *Cryptosporidium* and *Giardi*a in recycled waters used for irrigation and first description of *Cryptosporidium parvum* and *C. muris* in Greece. *Parasitol Res* 2015; **114**: 1803-1810.

129. Petrincová A, Valenčáková A, Luptáková L, et al. Molecular characterization and first report of *Cryptosporidium* genotypes in human population in the Slovak Republic. *Electrophoresis* 2015; **36**: 2925-2930.

130. Chappell CL, Okhuysen PC, Langer-Curry RC, Lupo PJ, Widmer G & Tzipori S. *Cryptosporidium muris*: infectivity and illness in healthy adult volunteers. *Am J Trop Med Hyg* 2015; **92**: 50-55.

131. Jiang Y, Ren J, Yuan Z, et al. *Cryptosporidium andersoni* as a novel predominant *Cryptosporidium* species in outpatients with diarrhea in Jiangsu Province, China. *BMC Infect Dis* 2014; **14**: 555.

132. Liu H, Shen Y, Yin J, et al. Prevalence and genetic characterization of *Cryptosporidium, Enterocytozoon, Giardia* and *Cyclospora* in diarrheal outpatients in China. *BMC Infect Dis* 2014; **14**:25.

133. Li N, Xiao L, Alderisio K, et al. Subtyping *Cryptosporidium ubiquitum*, a zoonotic pathogen emerging in humans. *Emerg Infect Dis* 2014; **20**: 217-224.

134. 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**: 4303-4308.

135. Feng Y, Alderisio KA, Yang W, et al. *Cryptosporidium* genotypes in wildlife from a New York watershed. *Appl Environ Microbiol* 2007; **73**: 6475-6483.

136. Robinson G, Elwin K & Chalmers RM. Unusual *Cryptosporidium* genotypes in human cases of diarrhea. *Emerg Infect Dis* 2008; **14**: 1800-1802.

137. ANOFEL *Cryptosporidium* National Network. Laboratory based surveillance for *Cryptosporidium* in France, 2006-2009. *Euro Surveill* 2010; **15**: 19642. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19642.

138. Elwin K, Hadfield SJ, Robinson G & Chalmers RM. The epidemiology of sporadic human infections with unusual cryptosporidia detected during routine typing in England and Wales, 2000-2008. *Epidemiol Infect* 2012; **140**: 673-683.

139. Insulander M, Silverlås C, Lebbad M, Karlsson L, Mattsson JG & Svenungsson B. Molecular epidemiology and clinical manifestations of human cryptosporidiosis in Sweden. *Epidemiol Infect* 2013; **141**: 1009-1020.

140. Lebbad M, Beser J, Insulander M, et al. Unusual cryptosporidiosis cases in Swedish patients: extended molecular characterization of *Cryptosporidium viatorum* and *Cryptosporidium* chipmunk genotype I. *Parasitology* 2013; **140**: 1735-1740.

141. Ng-Hublin JS, Combs B, Mackenzie B & Ryan U. Human cryptosporidiosis diagnosed in Western Australia: a mixed infection with *Cryptosporidium meleagridis*, the *Cryptosporidiu*m mink genotype, and an unknown *Cryptosporidium* species. *J Clin Microbiol* 2013; **51**: 2463-2465.

142. Ebner J, Koehler AV, Robertson G, et al. Genetic analysis of *Giardia* and *Cryptosporidium* from people in Northern Australia using PCR-based tools. *Infect Genet Evol* 2015; **36**: 389-395.

143. Guo Y, Cebelinski E, Matusevich C, et al. Subtyping novel zoonotic pathogen *Cryptosporidium* chipmunk genotype I. *J Clin Microbiol* 2015; **53**: 1648-1654.

144. Widerström M, Schönning C, Lilja M, et al. Large outbreak of *Cryptosporidium hominis* infection transmitted through the public water supply, Sweden. *Emerg Infect Dis* 2014; **20**: 581-589.

145. Jacob P, Henry A, Meheut G, Charni-Ben-Tabassi N, Ingrand V, Helmi K. Health risk assessment related to waterborne pathogens from the river to the tap. *Int J Environ Res Public Health* 2015; **12**: 2967-2983.

146. Goh S, Reacher M, Casemore DP, et al. Sporadic cryptosporidiosis, North Cumbria, England, 1996-2000. Emerg Infect Dis 2004; **10**: 1007-1015.

147. Leach CT, Koo FC, Kuhls TL, et al. Prevalence of *Cryptosporidium parvum* infection in children along the Texas-Mexico border and associated risk factors. *Am J Trop Med Hyg* 2000; **62**: 656-661.

148. Khalakdina A, Vugia DJ, Nadle J, Rothrock GA & Colford JM Jr. Is drinking water a risk factor for endemic cryptosporidiosis? A case-control study in the immunocompetent general population of the San Francisco Bay Area. *BMC Public Health* 2003; **3**: 11.

149. Roy SL, DeLong SM, Stenzel SA, et al. Risk factors for sporadic cryptosporidiosis among immunocompetent persons in the United States from 1999 to 2001. *J Clin Microbiol* 2004; **42**: 2944-2951.

150. Sarkar R, Ajjampur SS, Prabakaran AD, et al. Cryptosporidiosis among children in an endemic semiurban community in southern India: does a protected drinking water source decrease infection? *Clin Infect Dis* 2013; **57**: 398-406.

151. Checkley W, Epstein LD, Gilman RH, Black RE, Cabrera L & Sterling CR. Effects of *Cryptosporidium parvum* infection in Peruvian children: growth faltering and subsequent catch-up growth. *Am J Epidemiol* 1998; **148**: 497-506.

152. Muchiri JM, Ascolillo L, Mugambi M, et al. Seasonality of *Cryptosporidium* oocyst detection in surface waters of Meru, Kenya as determined by two isolation methods followed by PCR. *J Water Health* 2009; **7**: 67-75.

153. Bridgman SA, Robertson RM, Syed Q, Speed N, Andrews N & Hunter PR. Outbreak of cryptosporidiosis associated with a disinfected groundwater supply. *Epidemiol Infect* 1995; **115**: 555-566.

154. Jagai JS, Castronovo DA, Monchak J & Naumova EN. Seasonality of cryptosporidiosis: A meta-analysis approach. *Environ Res* 2009; **109**: 465-478.

155. Young I, Smith BA & Fazil A. A systematic review and meta-analysis of the effects of extreme weather events and other weather-related variables on *Cryptosporidium* and *Giardia* in fresh surface waters. *J Water Health* 2015; **13**: 1-17.

156. Pachauri RK. Climate change 2007. In Pachauri RK, Reisinger A. (eds). Synthesis report. Contribution of Working Groups I, II and III to the Fourth Assessment Report. Geneva: IPCC, 2008.

157. Schijven J, Bouwknegt M, de Roda Husman AM, et al. A decision support tool to compare waterborne and foodborne infection and/or illness risks associated with climate change. *Risk Anal* 2013; **33**: 2154-2167.

158. Hoyer AB, Schladow SG & Rueda FJ. A hydrodynamics-based approach to evaluating the risk of waterborne pathogens entering drinking water intakes in a large, stratified lake. *Water Res* 2015 **83**, 227-236.

159. Haas CN, Gerba CP. Quantitative Microbiological Risk Assessment. New York: Wiley and Sons, 1999.

160. Schijven JF & de Roda Husman, AM. Effect of climate changes on waterborne disease in the Netherlands. *Water Sci Technol* 2005; **51**: 79-87.

161. Davies CM, Kaucner C, Deere D & Ashbolt NJ. Recovery and enumeration of Cryptosporidium parvum from animal fecal matrices. *Appl Environ Microbiol* 2003; **69**: 2842-2847.

162. Chaidez C, Soto M, Gortares P & Mena K. Occurrence of *Cryptosporidium* and *Giardia* in irrigation water and its impact on the fresh produce industry. *Int J Environ Health Res* 2005; **15**: 339-345.

163. Huang DB & Zhou J. Effect of intensive handwashing in the prevention of diarrhoeal illness among patients with AIDS: a randomized controlled study. *J Med Microbiol* 2007; **56**: 659-663.

164. Lee MB & Greig JD. A review of gastrointestinal outbreaks in schools: effective infection control interventions. *J Sch Health* 2010; **80**: 588-598.

165. Hill A, Nally P, Chalmers RM, Pritchard GC & Giles M. Quantitative risk assessment for zoonotic transmission of *Cryptosporidium parvum* infection attributable to recreational use of farmland. *Zoonoses Public Health* 2011; **58**: 323-333.

166. Tumwine JK, Kekitiinwa A, Bakeera-Kitaka S, et al. Cryptosporidiosis and microsporidiosis in Ugandan children with persistent diarrhea with and without

concurrent infection with the human immunodeficiency virus. *Am J Trop Med Hyg* 2005; **73**: 921-925.

167. Mbae CK, Nokes DJ, Mulinge E, Nyambura J, Waruru A & Kariuki S. Intestinal parasitic infections in children presenting with diarrhoea in outpatient and inpatient settings in an informal settlement of Nairobi, Kenya. *BMC Infect Dis* 2013; **13**: 243.
168. Tellevik MG, Moyo SJ, Blomberg B, et al. Prevalence of *Cryptosporidium parvum/hominis, Entamoeba histolytica* and *Giardia lamblia* among young children with and without diarrhea in Dar es Salaam, Tanzania. *PLoS Negl Trop Dis* 2015; **9**: e0004125.
169. Kaplan JE, Hanson D, Dworkin MS, et al. Epidemiology of human immunodeficiency virus-associated opportunistic infections in the United States in the era of highly active antiretroviral therapy. *Clin Infect Dis* 2000; **30**: S5-14.
170. Bonatti H, Barroso LF 2nd, Sawyer RG, Kotton CN & Sifri CD. *Cryptosporidium* enteritis in solid organ transplant recipients: multicenter retrospective evaluation of 10 cases reveals an association with elevated tacrolimus concentrations. *Transpl Infect Dis* 2012; **14**: 635-648.
171. Desoubeaux G, Caumont C, Passot C, et al. Two cases of opportunistic parasite infections in patients receiving alemtuzumab. *J Clin Pathol* 2012; **65**: 92-95.
172. Amadi B, Kelly P, Mwiya M, et al. Intestinal and systemic infection, HIV, and mortality in Zambian children with persistent diarrhea and malnutrition. *J Pediatr Gastroenterol Nutr* 2001; **32**: 550-554.
173. Haque R, Mondal D, Karim A, et al. Prospective case-control study of the association between common enteric protozoal parasites and diarrhea in Bangladesh. *Clin Infect Dis* 2009; **48**: 1191-1197.

174. Moore SR, Lima NL, Soares AM, et al. Prolonged episodes of acute diarrhea reduce growth and increase risk of persistent diarrhea in children. *Gastroenterology* 2010; **139**: 1156-1164.

175. Mondal D, Minak J, Alam M, et al. Contribution of enteric infection, altered intestinal barrier function, and maternal malnutrition to infant malnutrition in Bangladesh. *Clin Infect Dis* 2012; **54**: 185-192.

176. Houpt ER, Bushen OY, Sam NE, et al. Short report: asymptomatic *Cryptosporidium hominis* infection among human immunodeficiency virus-infected patients in Tanzania. *Am J Trop Med Hyg* 2005; **73**: 520-522.

177. Korpe PS, Liu Y, Siddique A, et al. Breast milk parasite-specific antibodies and protection from amebiasis and cryptosporidiosis in Bangladeshi infants: a prospective cohort study. *Clin Infect Dis* 2013; **56**: 988-992.

178. Mead JR. Prospects for immunotherapy and vaccines against *Cryptosporidium*. *Hum Vaccin Immunother* 2014; **10**: 1505-1513.

179. Ludington JG & Ward HD. Systemic and mucosal immune responses to *Cryptosporidium* vaccine development. *Curr Trop Med Rep* 2015; **2**: 171-180.

180. Okhuysen PC, Chappell CL, Crabb J, Valdez LM, Douglass ET, DuPont HL. Susceptibility and serologic response of healthy adults to reinfection with *Cryptosporidium parvum*. *Infect Immun* 1998; **66**: 441-443.

181. Chappell CL, Okhuysen PC, Sterling CR, Wang C, Jakubowski W & Dupont HL. Infectivity of *Cryptosporidium parvum* in healthy adults with pre-existing anti-*C. parvum* serum immunoglobulin G. *Am J Trop Med Hyg* 1999; **60**: 157-164.

182. Petry F, Jakobi V & Tessema TS. Host immune response to *Cryptosporidium parvum* infection. *Exp Parasitol* 2010; **126**: 304-309.

183. McDonald V, Korbel DS, Barakat FM, Choudhry N & Petry F. Innate immune responses against *Cryptosporidium parvum* infection. *Parasite Immunol* 2013; **35**: 55-64.

184. Takeuchi D, Jones VC, Kobayashi M, Suzuki F. Cooperative role of macrophages and neutrophils in host anti-protozoan resistance in mice acutely infected with *Cryptosporidium parvum*. *Infect Immun* 2008; **76**:3657-63.

185. Borad A & Ward H. Human immune responses in cryptosporidiosis. *Future Microbiol* 2010; **5**: 507-519.

186. Robinson P, Okhuysen PC, Chappell CL, et al. Expression of IL-15 and IL-4 in IFN-gamma-independent control of experimental human *Cryptosporidium parvum* infection. *Cytokine* 2001; **15**: 39-46.

187. Dann SM, Wang HC, Gambarin KJ, et al. Interleukin-15 activates human natural killer cells to clear the intestinal protozoan *Cryptosporidium*. *J Infect Dis* 2005; **192**: 1294-302.

188. McDonald V, Pollok RC, Dhaliwal W, Naik S, Farthing MJ & Bajaj-Elliott M. A potential role for interleukin-18 in inhibition of the development of *Cryptosporidium parvum*. *Clin Exp Immunol* 2006; **145**: 555-562.

189. Ehigiator HN, McNair N & Mead JR. *Cryptosporidium parvum*: the contribution of Th1-inducing pathways to the resolution of infection in mice. *Exp Parasitol* 2007; **115**: 107-113.

190. Choudhry N, Petry F, van Rooijen N & McDonald V. A protective role for interleukin 18 in interferon gamma-mediated innate immunity to *Cryptosporidium parvum* that is independent of natural killer cells. *J Infect Dis* 2012; **206**: 117-124.

191. Bedi B, McNair NN, Forster I & Mead JR. IL-18 cytokine levels modulate innate immune responses and cryptosporidiosis in mice. *J Eukaryot Microbiol* 2015; **62**: 44-50.

192. Chen XM, O'Hara SP, Nelson JB, et al. Multiple TLRs are expressed in human cholangiocytes and mediate host epithelial defense responses to *Cryptosporidium parvum* via activation of NF-kappaB. *J Immunol* 2005; **175**: 7447-7456.

193. Barrier M, Lacroix-Lamandé S, Mancassola R, et al. Oral and intraperitoneal administration of phosphorothioate oligodeoxynucleotides leads to control of *Cryptosporidium parvum* infection in neonatal mice. *J Infect Dis* 2006; **193**: 1400-1407.

194. Costa LB, JohnBull EA, Reeves JT, et al. *Cryptosporidium*-malnutrition interactions: mucosal disruption, cytokines and TLR signaling in a weaned murine model. *J Parasitol* 2011; **97**: 1113-1120.

195. O'Hara S, Tietz Bogert PS, Trussoni CE, Chen X & Larusso NF. Tlr4 Promotes *Cryptosporidium parvum* clearance in a mouse model of biliary cryptosporidiosis. *J Parasitol* 2011; **97**: 813-821.

196. Lantier L, Drouet F, Guesdon W, et al. Poly(I:C)-induced protection of neonatal mice against intestinal *Cryptosporidium parvum* infection requires an additional TLR5 signal provided by the gut flora. *J Infect Dis* 2014; **209**: 457-467.

197. Perez-Cordon G, Yang G, Zhou B, et al. Interaction of *Cryptosporidium parvum* with mouse dendritic cells leads to their activation and parasite transportation to mesenteric lymph nodes. *Pathogens Dis* 2014; **70**: 17-27.

198. Yang Z, Fu Y, Gong P, et al. Bovine TLR2 and TLR4 mediate *Cryptosporidium parvum* recognition in bovine intestinal epithelial cells. *Microb Pathog* 2015; **85**: 29-34.

199. Chen XM, Splinter PL, O'Hara SP & LaRusso NF. A cellular micro-RNA, let-7i, regulates Toll-like receptor 4 expression and contributes to cholangiocyte immune responses against *Cryptosporidium parvum* infection. *J Biol Chem* 2007; **282**: 28929-2838.

200. Zhou R, Hu G, Liu J, Gong AY, Drescher KM & Chen XM. NF-kappaB p65-dependent transactivation of miRNA genes following *Cryptosporidium parvum* infection stimulates epithelial cell immune responses. *PLoS Pathog* 2009; **5**: e1000681.

201. Hu G, Zhou R, Liu J, Gong AY & Chen XM. MicroRNA-98 and let-7 regulate expression of suppressor of cytokine signaling 4 in biliary epithelial cells in response to *Cryptosporidium parvum* infection. *J Infect Dis* 2010; **202**: 125-35.

202. Gong AY, Zhou R, Hu G, et al. MicroRNA-221 controls expression of intercellular adhesion molecule-1 in epithelial cells in response to *Cryptosporidium parvum* infection. Int J Parasitol. 2011; **41**: 397-403.

203. Zhou R, Gong AY, Eischeid AN & Chen XM. miR-27b targets KSRP to coordinate TLR4-mediated epithelial defense against *Cryptosporidium parvum* infection. *PLoS Pathog* 2012; **8**: e1002702.

204. Xie H, Lei N, Gong AY, Chen XM & Hu G. *Cryptosporidium parvum* induces SIRT1 expression in host epithelial cells through downregulating let-7i. *Hum Immunol* 2014; **75**: 760-765.

205. Kelly P, Jack DL, Naeem A, et al. Mannose-binding lectin is a component of innate mucosal defense against *Cryptosporidium parvum* in AIDS. *Gastroenterology* 2000; **119**: 1236-1242.

206. Kirkpatrick BD, Huston CD, Wagner D, et al. Serum mannose-binding lectin deficiency is associated with cryptosporidiosis in young Haitian children. *Clin Infect Dis* 2006; **43**: 289-294.

207. Carmolli M, Duggal P, Haque R, et al. Deficient serum mannose-binding lectin levels and MBL2 polymorphisms increase the risk of single and recurrent *Cryptosporidium* infections in young children. *J Infect Dis* 2009; **200**: 1540-1547.

208. Moss DM, Chappell CL, Okhuysen PC, et al. The antibody response to 27-, 17-, and 15-kDa *Cryptosporidium* antigens following experimental infection in humans. *J Infect Dis* 1998; **178**: 827-833.

209. O'Connor RM, Shaffie R, Kang G & Ward HD. Cryptosporidiosis in patients with HIV/AIDS. *AIDS* 2011; **25**: 549-560.

210. Singh I, Carville A & Tzipori S. Cryptosporidiosis in rhesus macaques challenged during acute and chronic phases of SIV infection. *AIDS Res Hum Retroviruses* 2011; **27**: 989-997.

211. Sheoran A, Wiffin A, Widmer G, Singh P & Tzipori S. Infection with *Cryptosporidium hominis* provides incomplete protection of the host against *Cryptosporidium parvum*. *J Infect Dis* 2012; **205**: 1019-1023.

212. Savy M, Edmond K, Fine PE, et al. Landscape analysis of interactions between nutrition and vaccine responses in children. *J Nutr* 2009; **139**: 2154S-218S.

213. Steinhagen F, Kinjo T, Bode C & Klinman DM. TLR-based immune adjuvants. Vaccine. 2011; **29**: 3341-3355.

214. Preidis GA, Wang HC, Lewis DE, et al. Seropositive human subjects produce interferon gamma after stimulation with recombinant *Cryptosporidium hominis* gp15. *Am J Trop Med Hyg* 2007; **77**: 583-585.

215. Egorov AI, Montuori Trimble LM, Ascolillo L, et al. Recent diarrhea is associated with elevated salivary IgG responses to *Cryptosporidium* in residents of an eastern Massachusetts community. *Infection* 2010; **38**: 117-123.

216. Ajjampur SS, Sarkar R, Allison G, et al. Serum IgG response to Cryptosporidium immunodominant antigen gp15 and polymorphic antigen gp40 in children with cryptosporidiosis in South India. *Clin Vaccine Immunol* 2011; **18**: 633-639.

217. Allison GM, Rogers KA, Borad A, et al. Antibody responses to the immunodominant *Cryptosporidium* gp15 antigen and gp15 polymorphisms in a case-control study of cryptosporidiosis in children in Bangladesh. *Am J Trop Med Hyg* 2011; **85**: 97-104.

218. Sarkar R, Ajjampur SS, Muliyil J, Ward H, Naumova EN & Kang G. Serum IgG responses and seroconversion patterns to *Cryptosporidium* gp15 among children in a birth cohort in south India. *Clin Vaccine Immunol* 2012; **19**: 849-854.

219. Lazarus RP, Ajjampur SS, Sarkar R, et al. Serum anti-cryptosporidial gp15 antibodies in mothers and children less than 2 years of age in India. *Am J Trop Med Hyg* 2015; **93**: 931-938.

220. Jenkins MC & Fayer R. Cloning and expression of cDNA encoding an antigenic *Cryptosporidium parvum* protein. *Mol Biochem Parasitol* 1995; **71**: 149-152.

221. Hong-Xuan H, Lei C, Cheng-Min W, et al. Expression of the recombinant fusion protein CP15-23 of *Cryptosporidium parvum* and its protective test. *J Nanosci Nanotechnol* 2005*;* **5**: 1292-1296.

222. Wang C, Luo J, Amer S, et al. Multivalent DNA vaccine induces protective immune responses and enhanced resistance against *Cryptosporidium parvum* infection. *Vaccine* 2010; **29**: 323-328.

223. Liu K, Zai D, Zhang D, et al. Divalent Cp15-23 vaccine enhances immune responses and protection against *Cryptosporidium parvum* infection. *Parasite Immunol* 2010; **32**: 335-344.

224. Manque PA, Tenjo F, Woehlbier U, et al. Identification and immunological characterization of three potential vaccinogens against *Cryptosporidium* species. *Clin Vaccine Immunol* 2011; **18**: 1796-1802.

225. Roche JK, Rojo AL, Costa LB, et al. Intranasal vaccination in mice with an attenuated *Salmonella enterica* Serovar 908htr A expressing Cp15 of *Cryptosporidium*: impact of malnutrition with preservation of cytokine secretion. *Vaccine* 2013; **31**: 912-918.

226. Ehigiator HN, Romagnoli P, Priest JW, Secor WE & Mead JR. Induction of murine immune responses by DNA encoding a 23-kDa antigen of *Cryptosporidium parvum. Parasitol Res* 2007; **101**: 943-950.

227. Benitez AJ, McNair N & Mead JR. Oral immunization with attenuated *Salmonella enterica* serovar Typhimurium encoding *Cryptosporidium parvum* Cp23 and Cp40 antigens induces a specific immune response in mice. *Clin Vaccine Immunol* 2009; **16**: 1272-1278.

228. Boulter-Bitzer JI, Lee H & Trevors JT. Molecular targets for detection and immunotherapy in *Cryptosporidium parvum*. *Biotechnol Adv* 2007; **25**: 13-44.

229. Wanyiri JW, Kanyi H, Maina S, et al. Cryptosporidiosis in HIV/AIDS patients in Kenya: clinical features, epidemiology, molecular characterization and antibody responses. *Am J Trop Med Hyg* 2014; **91**: 319-328.

230. Sagodira S, Buzoni-Gatel D, Iochmann S, Naciri M & Bout D. Protection of kids against *Cryptosporidium parvum* infection after immunization of dams with CP15-DNA. *Vaccine* 1999; **17**: 2346-2355.

231. Priest JW, Li A, Khan M, et al. Enzyme immunoassay detection of antigen-specific immunoglobulin g antibodies in longitudinal serum samples from patients with cryptosporidiosis. *Clin Diagn Lab Immunol* 2001; **8**: 415-243.

232. Chappell CL, Okhuysen PC, Sterling CR, Wang C, Jakubowski W & Dupont HL. Infectivity of *Cryptosporidium parvum* in healthy adults with pre-existing anti-*C. parvum* serum immunoglobulin G. *Am J Trop Med Hyg* 1999; **60**: 157-164.

233. Riggs MW. Recent advances in cryptosporidiosis: the immune response. *Microbes Infect* 2002; **4**: 1067-1080.

234. Frost FJ, Tollestrup K, Craun GF, Fairley CK, Sinclair MI & Kunde TR. Protective immunity associated with a strong serological response to a *Cryptosporidium*-specific antigen group, in HIV-infected individuals. *J Infect Dis* 2005; **192**: 618-621.