A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for cryptosporidium

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Cryptosporidium **spp are well recognised as causes of diarrhoeal disease during waterborne epidemics and in immunocompromised hosts. Studies have also drawn attention to an underestimated global burden and suggest major gaps in optimum diagnosis, treatment, and immunisation. Cryptosporidiosis is increasingly identified as an important cause of morbidity and mortality worldwide. Studies in low-resource settings and high-income countries have confirmed the importance of cryptosporidium as a cause of diarrhoea and childhood malnutrition. Diagnostic tests for cryptosporidium infection are suboptimum, necessitating specialised tests that are often insensitive. Antigen-detection and PCR improve sensitivity, and multiplexed antigen detection and molecular assays are underused. Therapy has some effect in healthy hosts and no proven efficacy in patients with AIDS. Use of cryptosporidium genomes has helped to identify promising therapeutic targets, and drugs are in development, but methods to assess the efficacy in vitro and in animals are not well standardised. Partial immunity after exposure suggests the potential for successful vaccines, and several are in development; however, surrogates of protection are not well defined. Improved methods for propagation and genetic manipulation of the organism would be significant advances.**

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Introduction

Cryptosporidium was identified as a cause of human infection in 1976.[1] During the early 1980s, cryptosporidiosis was recognised as the major cause of chronic diarrhoea in patients with AIDS, as a cause of zoonotic and waterborne outbreaks of diarrhoea, and as a cause of diarrhoea in children.[2–5] By the mid-1990s, cryptosporidium was known to be ubiquitous and was linked with childhood malnutrition and premature death in low-resource settings. A massive waterborne epidemic affected more than 400 000 people in Milwaukee, WI, USA, in 1993.[6] Despite this knowledge, cryptosporidiosis is substantially under-recognised and underdiagnosed, treatments are suboptimum, and preventive measures are incomplete. Even in settings such as the USA where modern diagnostics are widely available, estimates state that only about 1% of cases are diagnosed and reported.[7]

Recent advances in knowledge are shifting opinions of the epidemiology of cryptosporidiosis, and have increased estimates of the global burden of disease.[8] To identify potential gaps and opportunities for future studies, the US Foundation for the National Institutes of Health convened a group of experts to discuss advances in the epidemiology, diagnosis, therapeutics, and immunisation for cryptosporidiosis. In this Review, we summarise discussions of this meeting, and provide a more in-depth review of published research.

Epidemiology

Disease burden

Protozoa of the genus *Cryptosporidium* have a global distribution. Early studies suggested that cryptosporidium is in 1% of stools of hosts who are immunocompetent in high-income countries and in 5–10% of stools of hosts in low-resource settings.[9] Results of recent studies with PCR and antigen detection suggest that previous studies underestimated the frequency of infection, identifying cryptosporidium in 15–25% of children with diarrhoea.[9–13] Cryptosporidiosis is associated with longer duration of diarrhoea and greater childhood morbidity and mortality than are other causes,[14,15] and is particularly associated with prolonged diarrhoea (7–14 days) and persistent diarrhoea (≥14 days).[16,17] Results of a cross-sectional study in Uganda showed that mortality was higher among children with diarrhoeal disease with cryptosporidium than among those without.[12] Results of cohort studies have consistently shown that younger age was associated with high risk of infection. For example, in a multicentre study of children younger than 5 years in India,[18] 75% of cases were in children younger than 2 years. Many studies suggest that cryptosporidium infection is associated with malnutrition and growth deficits in children.[19–22] Results of a cohort study of children in Peru[23] showed that even asymptomatic infection was associated with poor growth. Symptomatic cryptosporidiosis stunted weight gain more than did asymptomatic infection, but asymptomatic infection was twice as common and might have a greater overall adverse effect on child growth.[23]

The Global Enteric Multicentre Study—which sought to assess the causes, burden, clinical syndromes, and adverse outcomes of moderate-to-severe diarrhoea in children at seven sites in sub-Saharan Africa and south Asia—identified cryptosporidium as one of the four major contributors to moderate-to-severe diarrhoeal diseases during the first 2 years of life at all sites.[24] Cryptosporidium was second only to rotavirus as a cause of moderate-to-severe diarrhoea in children younger than 2 years. At a follow-up visit 2–3 months after enrolment, cryptosporidiosis was associated with a 2–3 times higher

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risk of mortality among children aged 12–23 months with moderate-to-severe diarrhoea than in controls without diarrhoea.

Microbiology

Molecular methods have enabled characterisation of *Cryptosporidium* species, which differ in epidemiology.[25] Although human infections have been noted with more than 15 species, most infections worldwide have been attributed to *Cryptosporidium hominis* and *Cryptosporidium parvum*. Genome sequences for both species[26,27] are available on CryptoDB.[28] *C hominis* was the main species causing childhood diarrhoea in studies from Peru, Brazil, Bangladesh, and India.[29–32] In a study in the UK, *C parvum* was more common in rural populations, associated with animal exposure, and peaked in the spring, whereas *C hominis* was more urban, associated with young children, and peaked in the late summer and autumn.[33] In Peru, infecting species did not differ with age of infection, socioeconomic status, or nutritional status. *C hominis,* especially subtype Ib, is associated with more oocyst shedding and symptoms including nausea, vomiting, and general malaise.[29,34]

Risk factors

Environmental factors associated with cryptosporidium infection also need to be better understood. Results of a longitudinal study in India[35] showed that the burden of infection was equally high in children who lived in households that used either bottled water or used municipal water for drinking, suggesting that most transmission does not involve drinking water. Seasonal patterns might also be associated with an increased transmission risk.[19] In Kenya, investigators detected a higher number of oocysts in surface waters at the end of the rainy season and at the beginning of the dry season compared with other times, consistent with the seasonal peak in human cryptosporidiosis in east Africa.[36] A meta-analysis examining the effects of seasonality[37] showed that both high ambient temperature (more important in temperate countries) and high rainfall (more important in the tropics) were associated with an increased risk of cryptosporidiosis. Results of a study from Uganda[38] suggested the possibility of respiratory transmission in immunocompetent children.

Malnutrition in early childhood also increases the risk of diarrhoea with cryptosporidium. In a birth cohort in Bangladesh, stunting at birth was associated with subsequent cryptosporidium infection.[39] Findings from a longitudinal study showed that children with a height-for-age *Z* score of more than –1 SD less than the mean (ie, HAZ scores < –1SD) before infection were more likely to have persistent growth deficits a year later than were children with HAZ scores of at least –1 SD before infection, in whom growth defecits were transient.[19] In Brazil, children infected with *C hominis* had a persistent decrease in HAZ score 3–6 months after infection.[30] The intestinal damage caused by cryptosporidium can result in long-term cognitive deficits, impaired immune response, and reduced vaccine efficacy.[40]

Pathogenesis of malnutrition in cryptosporidiosis

The mechanism by which cryptosporidium affects child growth seems be associated with inflammatory damage to the small intestine.[41] Impaired absorption and enhanced secretion might promote diarrhoeal disease and growth deficits. Mouse models further show a greater burden of infection and greater damage to the ileum in malnourished animals versus healthy animals.[42] Results of studies in animals, children, and HIV positive people with diarrhoeal disease also suggest that alanyl-glutamine might enhance intestinal repair and absorption and prevent further growth deficits.[43] The *ApoE E4* allele has been associated with protection against growth deficits in children with severe diarrhoeal disease, and results of studies in animals suggest possession of the *ApoE E4* allele is associated with reduction in parasitic burden and inflammatory damage.[44]

Diagnostics

Detection of cryptosporidium infection is based on analysis of stool samples by use of microscopy with tinctorial and fluorescent stains or via antigen and nucleic acid detection (table 1). In-vitro propagation of the organisms is not possible.[45] For epidemiological studies, serological tests might also be used. Microscopy is an important diagnostic method because of the low cost of reagents, but good staining and visual skills are necessary. The modified acid-fast staining has about 70% sensitivity compared with immunofluorescent antibody stains,[46] but could miss more than half of cases compared with molecular methods. Technical improvements and cost reductions in fluorescence microscopy, such as light-emitting diode light sources, enable testing with fluorescent stains such as auramine-rhodamine that are more sensitive than is the traditional modified acid-fast stain, but problems with specificity can arise.

In the USA and Europe, reference laboratories often use immunofluorescence microscopy as a gold standard. Other antigen detection formats, such as enzyme immune assay or immunochromatographic methods, are also commercially available, have higher throughput, and are being increasingly used for diagnosis. However, diagnostic sensitivities are variable (70% to 100%).[46–48,52] Some rapid tests have reduced specificity and sensitivity for species other than *C parvum* or *C hominis,*[53,49] and confirmation of positive reactions is needed.[49]

PCR is increasingly used for detection of cryptosporidium and other enteric pathogens in research laboratories, and affords excellent sensitivity.[50,54] Amplification of cryptosporidium gene encoding 18S rRNA is widely used for this purpose, but other genes have also been targeted. Molecular analysis is essential to

discriminate *Cryptosporidium* species. PCR with sequencing of about 800 base-pair fragment of the gene encoding 18S rRNA is commonly used for speciation.[55,56] Real-time assays based on smaller fragments have been described.[50,53] Because *C hominis* and *C parvum* are similar (>96%) at the DNA sequence level,[57] sequencing of the *gp60* gene has been used for subtyping within species.[58] Multilocus methods are desirable but have not been standardised.[59] Disruption of oocysts by bead-beating, freeze-thaw, boiling, or chemical lysis is necessary before DNA extraction.[51,60] However, point-of-care molecular tests are in development that can use simplified extraction methods.[61] Multiplexed molecular diagnostics for enteropathogens often show that multiple infections are common in resource-poor settings both in individuals with diarrhoea and in healthy control individuals.[62] Some data suggest quantitative load of cryptosporidium[63] might correlate with increased disease severity, thus quantitative assays will be important for future studies and for assessment of drug regimens.

Serological assays for cryptosporidium are an important device for epidemiological studies because specific antibody responses develop after both symptomatic and asymptomatic infection. Whereas IgA responses are generally short-lived, IgG responses can persist for several months. Antibody to Cp23 seems to correlate with distant infection, whereas responses to Cp17 (also called gp15) suggest recent infection, and responses to P2 are associated with repeated infection.[64] These assays, adapted to a Luminex-based serodiagnostic platform, can be done with finger-prick blood collected on filter paper,[65] or with oral fluid.[66]

Therapeutics

Antiparasitic treatment for cryptosporidiosis is suboptimum.[67] For individuals who are immunocompromised, improvement in cellular immune function is a key priority for management of cryptosporidiosis (eg, combination antiretroviral therapy for cryptosporidiosis in AIDS).[67,68] However, substantial mortality occurs during initial treatment.[69]

Various drugs have been described with activity against cryptosporidium in vitro, in animal models, and in patients (table 2). Spiramycin, azithromycin, and immunoglobulin have not been efficacious in controlled trials in patients with AIDS.[67] Results of two randomised, placebo-controlled trials of paromomycin showed little effect on symptoms and oocyst shedding,[75,76] but the small sample sizes prohibited definitive conclusions.[67] Nitazoxanide is FDA-approved for treatment of cryptosporidiosis. Findings from randomised studies have shown a beneficial effect in adults and children without HIV,[70–72] with significant reduction in mortality in malnourished children treated with nitazoxanide.[72] However, cessation of diarrhoea was recorded in only 56% of patients receiving nitazoxanide compared with 23% of patients receiving placebo.[72] Moreover, results of three controlled trials involving patients with HIV not on effective antiretroviral therapy[72–74] did not show overall improvement. Findings from in-vitro and animal studies suggest that drug combinations might have some efficacy.[77] In the management of patients with HIV or AIDS, clinicians should consider symptomatic therapy, optimisation of antiretroviral therapy, and, perhaps, the inclusion of nitazoxanide or paromomycin.[67,68]

The availability of genome sequence and functional genomics data for *C hominis*, *C parvum*, and other species has provided researchers with new devices with which to explore unique metabolic pathways as targets for chemotherapy.[26–28,78,79] For example, the calcium-dependent protein kinases are a conserved family of enzymes in plants and some apicomplexan parasites, including cryptosporidium.[80,81] Structural analysis shows that apicocomplexan calcium-dependent protein kinases have a glycine as a gatekeeper residue for the ATP binding site, which makes a hydrophobic region more available for inhibitors active against *C parvum* in human cell lines and SCID/beige mice.[81–83]

	Advantages	Disadvantages
Microscopy	Low technology Widely available	Low sensitivity (about 70–80% with modified acid-fast stain)[45,46] Requires special stains and skilled technicians
Antigen detection	Good sensitivity (70–100%)[45–48] Several commercially-available kits in enzyme immunoassay, immunofluorescence assay, and immunochromatography test formats	Costly for resource-poor country settings
Nucleic acid amplification	Excellent sensitivity[45,49] Can speciate, subtype, and quantify[50,51] Amenable to multiplexing for additional enteropathogen targets	Expensive instrumentation Technically demanding, requires skilled laboratory technicians for DNA extraction and amplification
Serological methods	Useful for surveillance purposes and discrimination of historical, recent, and repetitive infection	Research laboratory use only

Table 1: **Trade-offs of diagnostic methods for cryptosporidium**

	Status	Limitations
Nitazoxanide	Approved for use for cryptosporidiosis but not with HIV co-infection	Efficacy 56–96% in healthy hosts[70–72] Not effective in patients with advanced AIDS[72–74] High cost and availability limit widespread use
Paromomycin	Approved for use for other indications	Limited efficacy in patients with AIDS[75,76] No controlled data in other groups
Azithromycin	Approved for use for other indications	Not effective in patients with advanced AIDS[67] Anecdotes of efficacy in combination in patients with AIDS
Rifaximin	Approved for use for other indications	Anecdotes of responses in patients with AIDS[67,68]
Rifabutin	Approved for use for other indications	Effective at prevention of cryptosporidiosis in studies of *Mycobacterium avium* prophylaxis[67,68]
HIV protease inhibitors	Approved for use for HIV treatment	Associated with resolution of cryptosporidiosis in patients with AIDS[67,68] Partial efficacy against *Cryptosporidium parvum* in mouse models

Table 2: **Chemotherapy of cryptosporidiosis**

Microtubule formation is another potential drug target. Dinitroanilines, including trifluralin, are herbicides that block microtubule formation and inhibit cryptosporidial growth in vitro and in vivo.[84–86] Furthermore, the development of hybrid compounds based on albendazole and trifluralin led to the identification of analogues with excellent in-vitro efficacy and 79–81% reductions in oocyst shedding in mice (Thompson RCA, unpublished).

Cryptosporidium has little ability to synthesise nutrients de novo, including aminoacids, nucleosides, and fatty acids.[87] Many genes associated with metabolism have been lost, including apicoplast pathways, the mitochondrial respiratory chain, and hypoxanthine-xanthine-guanine phosphoribosyl transferase.[26] *Cryptosporidium* relies on glycolysis to produce ATP, producing lactate, ethanol, and acetate end products. Thus, inhibitors of hexokinase and lactate dehydrogenase have some efficacy.[88] Additionally, several proteins involved in fatty acid metabolism have been found on the parasitophorus vacuole membrane.[89–91] For example, Triacsin C and other drugs inhibit fatty acyl-CoA-binding protein and fatty acid-CoA synthetase, and show a reduction in *C parvum* oocyst production in vitro and in mice.[91–93] A parasite cysteine protease inhibitor was also effective in vitro and in an animal model.[94]

Molecular evidence suggests lateral gene transfer from bacteria, providing potential targets for cryptosporidium chemotherapy.[95,96] The catalysis of inosine monophosphate to xanthosine monophosphate via inosine-5′-monophosphate dehydrogenase (IMPDH) is a key rate-limiting step in guanine nucleotide synthesis.[97] By contrast with other apicomplexans, cryptosporidium *IMPDH* genes are prokaryotic.[98] High-throughput screening identified selective potential inhibitors of cryptosporidium IMPDH by targeting the highly divergent cofactor binding site.[99] A subsequent optimisation yielded single-digit nanomolar inhibitors with six different frameworks, with greater than 10^3-fold selectivity for *Cryptosporidium* IMPDH.[99–103] Two compounds reduced the oocyst burden in an interleukin-12 knockout mouse model of cryptosporidiosis. One compound surpassed paromomycin in a multiple-dosing regimen.[103] Possible future directions include ensuring of increased drug concentrations in the gut, and improvement of animal models to investigate the efficacy of potential compounds.[103]

Drug repurposing is the novel use of approved drugs. Cell-based screening assays, followed by in-vitro methods to prioritise leads, were developed.[104] Automated imaging and image analysis were then used to identify potential leads in vitro for future in-vivo studies. A screen of 727 compounds[104] yielded 16 confirmed selective inhibitors, including HMG-CoA reductase inhibitors that target the host enzyme. Further screening with and without low-dose nitazoxanide for synergistic drug combinations is underway.

Immune response and vaccine development

Several strands of evidence suggest that development of a vaccine to prevent cryptosporidiosis is feasible:[105] increased susceptibility and severity of disease in immunocompromised hosts; adults in highly endemic areas are partly immune to reinfection; and human challenge studies show that previous infection or exposure leads to a higher infectious dose [ID_{50}].[106,107] However, the protective immune responses necessary for an efficacious vaccine are incompletely understood.[108] The human immune response of clearing infection and preventing reinfection seems to involve separate innate and adaptive immune responses.

The innate immune response is crucial to provide an early response while activating the adaptive immune system.[109] Mannose-binding lectin has a key role in the innate response. Children and HIV-infected adults with mannose-binding lectin deficiency have increased susceptibility to cryptosporidiosis and more severe disease.[110–112] Polymorphisms in the mannose-binding lectin gene were strongly associated with cryptosporidium infections, especially recurrent infection.[110] Mannose-binding lectin might activate complement to mediate parasite clearance.[113] Toll-like receptors on the host cell surface trigger key responses to the organism. *C parvum* infection increases production of antimicrobial peptides (LL-37 and human β-defensin 2).[114,115] Results of in-vitro and in-vivo studies show that knockout of *TLR/MyD88* genes results in reduced production of defensins and greater parasite burden.[116] Results from in-vivo studies showed the presence of exosomes in the gut lumen, and exosomes carrying antimicrobial peptides from the epithelial surface help eliminate cryptosporidium.[117] MicroRNAs (miRNAs) have an important role in post-transcriptional regulation and modulation of the innate immune response to cryptosporidium.[118–121] For example, variation in miRNA expression has shown an association with changes in *C parvum* burden.[121,122]

Natural killer cells contribute to clearance of infection in some murine models. In mice, interferon γ is crucial for both the innate and acquired immune responses.[123] By contrast, human infection in naive hosts is associated with production of interleukin 15, which can activate natural killer cells to clear infection in vitro.[124,125] In other models, macrophages seem important for the innate host response.[126,127] The CD154-CD40 ligand receptor pair also has a key role in clearance of infections. Severe, chronic infection with biliary involvement is common in human hyper-IgM syndrome, associated with mutations in CD40 ligand.[128]

CD4 cells are crucial for the acquired immune response in both human beings and animals.[108] In a longitudinal cohort, children who became infected with cryptosporidium were more likely to carry the HLA class II DQB1*0301 allele (which presents antigen to CD4 cells) and the HLA class I B*15 allele (which presents antigen to CD8 cells) than were children who were not infected.[129] In patients

with AIDS, the risk and severity of infection are associated with the CD4 cell count. Interferon γ is associated with acquired immunity in human infection, and interferon γ knockout mice have increased susceptibility to infection.[130] Interferon treatment reduces susceptibility to infection in cell lines, but not in primary epithelial cells[131]—CD8 cells assist in clearance of human infection.[132]

The role of humoral immunity in protection from cryptosporidiosis is unclear.[108] In murine models, hyperimmune globulin controlled infection, but elimination of β cells had no significant effect.[108] Secretory IgA has not correlated with protection in healthy volunteers or patients with AIDS.[108] By contrast, high concentrations of specific antibody were associated with short duration of illness in children in Bangladesh.[15] Similarly, cryptosporidium antibody in breast milk was associated with immune-protection of breast-feeding infants.[133] The antibody to the parasite surface antigen gp15/17 was associated with protection against reinfection;[134] however, this antibody could also be a marker for a stronger cellular immune response. Thus no clear surrogate marker of protective immunity exists in cryptosporidiosis.

Several antigens have been explored for use in a vaccine. Results of studies in gnotobiotic pigs showed incomplete cross-protection between *C parvum* and *C hominis*.[135] Similarly, results of cohort studies of children in low-resource countries showed frequent re-infections.[29] Reinfections are more likely to be by different species and subtypes of cryptosporidium, but cases also exist of reinfection with the same subtypes.

Several antigens are being developed as vaccine candidates.[105] For example, gp60 (also called gp40/15) is a polyprotein cleaved by a parasite serine proteinase into two surface proteins—gp15 and gp40, the latter is variable and used for speciation and subtyping of strains. Both gp15 and gp40 can stimulate interferon γ production by peripheral blood mononuclear cells of those previously infected.[136] Among children in Bangladesh, IgA antibody to gp15 was not species specific, and was associated with shorter duration of illness.[137] Vaccines based on gp15 alone or in combination with other antigens are in development.[138]

A recombinant DNA vaccine consisting of a second 15 kDa antigen termed Cp15 was immunogenic, and immunisation of pregnant goats protected offspring.[139–143] Studies have expressed this antigen in attenuated *Salmonella*, recombinant vaccinia, and DNA vectors. Vaccination with Cp15 in a *Salmonella* vector protected mice from infection, but the effect was not significantly greater than with the vector alone.[142]

Results of a study in Bangladesh also showed that patients with infection had greater serum IgG, IgM, and IgA to Cp23 than did healthy patients, and the responses again were conserved across several subtypes and associated with shorter disease.[144] Studies in animals indicate that Cp23 plasmids can promote activation of both antibody and CD4 concentration, with reduced parasitic burden, and long-term immunity with parasitic challenge.[145] Other vaccine vectors include DNA, *Lactobacillus*, and *Salmonella* expressing Cp23.[146] Other antigens being explored for vaccine use include P2 antigen, profilin, *Cryptosporidium* apyrase, Muc4, and Muc5.[143,147,148]

Discussion

Growing evidence shows a high global burden of cryptosporidiosis, especially among children and people who are immunocompromised or malnourished. Data that we highlight in this Review emphasise the underappreciated role of cryptosporidium as an important childhood diarrhoeal pathogen. Moreover, results of the Global Enteric Multicentre Study[24] showed the association between cryptosporidium infection and subacute mortality. More detailed studies are needed to elucidate the mechanisms of injury and the resultant health effects of cryptosporidium infection. Further longitudinal studies that use advanced molecular methods are crucial to characterise the pathogenesis of infection, host, and environmental factors in susceptibility, immune response, and clinical outcomes. Better characterisation is needed of worldwide variations and effect in community-based settings. The effects of different *Cryptosporidium* genotypes on disease, growth, and development are poorly understood and need to be better defined. Better methods to define genotypes are needed to enhance understanding of parasite strains. Finally, although asymptomatic cryptosporidium has been associated with poor growth in single-site studies, well designed longitudinal studies are needed to improve our understanding of the role and adverse effects of asymptomatic infections on growth and development.

Diagnosis of cryptosporidium infection at the point of care in low-resource settings is a challenge. How to interpret multiple enteropathogens in a child with diarrhoea is unclear.[62,149] Microscopy and antigen detection assays are useful for clinical diagnosis at the genus level. Species differentiation and subtyping are important for outbreak investigations, epidemiology, burden assessment, and risk-factor and transmission studies, and might ultimately enable refined clinical diagnosis. Species and subtype information is not necessary for selection of clinical care and therapeutic options, but might need to be taken into account in drug investigations and clinical trials. Novel stool diagnostics, serodiagnostics, and biomarkers for cryptosporidium disease could enable more accurate identification of active cryptosporidiosis than do present methods, which could be used for accurate case ascertainment, and therapeutic or vaccine trials.

Many obstacles exist to the development of drugs for cryptosporidiosis, including difficulty in propagation of these organisms in vitro. Novel in-vitro methods could enable propagation and might also improve in-vitro screening for novel treatments and vaccines.[150] Animal models for drug assessments are poorly standardised,

***Panel:* Key recommendations**

- The effects of different cryptosporidium genotypes on disease, growth, and development are poorly understood and need to be better defined. Further longitudinal studies that use advanced molecular methods are needed to better characterise the pathogenesis and burden of disease from cryptosporidium infection.
- Several diagnostic methods for cryptosporidium are available, but infection is significantly underdiagnosed. In low-resource settings with high rates of mixed infections, quantitative assays will be important for future studies of burden, and assessment of drug regimens and point-of-care tests need to be developed and used more widely.
- An urgent need exists for better treatments for cryptosporidiosis, and for better-standardised methods for screening compounds in vitro and in animals.
- Drug development has been hampered by limitations of methods to propagate the organisms in vitro and to genetically manipulate the parasites.
- Development of a vaccine to prevent cryptosporidiosis is feasible, but further studies are needed to define mechanisms of protection from human disease and, perhaps, to develop live-attenuated strains through genetic engineering.

Search strategy and selection criteria

We searched PubMed, Web of Science, and Google Scholar with the search terms "cryptosporidium", "Epidemiology", "Diagnosis", "Immunology", "Treatment", and "Vaccine", from Jan 1, 1946, to April 1, 2014. We included relevant articles and citations in English only and identified knowledge gaps, research opportunities, and key recommendations.

and the target responses that correlate with efficacy in people are poorly characterised. Gnotobiotic piglets and immunosuppressed gerbils are the only animal models available for *C hominis*, although neither has been widely adopted. Whereas *C parvum* can be propagated in calves and lambs, cross-strain contamination has been a problem. Most in-vivo screening has been done in immunosuppressed rodents, however developments include a malnourished mouse model and natural murine infection with *Cryptosporidium tyzzeri*. Animal models need to be better standardised for pharmacological and efficacy studies and for comparison with results from studies in people.

The availability of several genome sequences draws attention to many potential targets for chemotherapy. Genetic manipulation could provide useful strategies for target prioritisation, but no methods are available. Funding to support development of molecular methods could enable development of more effective drugs. Incentives are needed to convince the pharmaceutical industry that a market for new therapeutics exists. Because people at highest risk of severe sequelae (eg, malnourished children) live in low-resource settings, government and non-governmental organisation support will be necessary for drug development and implementation of widespread treatment. In addition to development of novel drugs, a focus on delivery and financing is necessary.

Although there is cause for optimism about the potential development of a vaccine to prevent cryptosporidiosis, major barriers include poor understanding of the human protective immune response—including which antigens are crucial, which responses are associated with protective immunity, and which delivery routes are optimum. These obstacles could be overcome by a well funded vaccine development programme with clear benchmarks for success.

Conclusion

Despite advances in our understanding of the genetics and immunology of cryptosporidium, several important knowledge gaps and challenges exist. The panel lists the key messages of this Review. Diagnostic tests each have their limitations in cost, performance, differentiation of clinical significance, and assessment of co-infections with other pathogens. New methods need to be developed to improve interpretation of results in the setting of multiple infections, relevance of species subtypes, and in surveillance studies. In identification of novel or repurposed therapeutics, more efficient use of genomic databases, improved culture methods, and development of standardised assays is necessary to screen potential targets. We also need to optimise animal models for in-vivo studies that can better replicate human disease. 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 are unclear. Ultimately, progress in cryptosporidium research on diagnostic and therapeutic product development will need greater appreciation of the public health effect of this disease, with commitment from funding bodies to establish mechanisms to support this crucial work.

Contributors

WC and ACWJr were joint first authors. All authors participated in the meeting and contributed to discussions about and the writing of the Review.

Declaration of interests

We declare no competing interests.

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