Cryptosporidiosis in children in the Indian subcontinent

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Abstract

Cryptosporidiosis is a leading cause of diarrheal disease among children under two in developing countries. Previous estimates have shown a high burden of cryptosporidial diarrhea in children from Sub-Saharan Africa and South Asia. Asymptomatic cryptosporidial infections which go undetected and untreated have been shown to result in significant malnutrition. In this review, we carried out a literature search of studies published on cryptosporidiosis in children in the Indian subcontinent from 1983 to 2016. Of the 154 publications identified, 54 were included for final analysis with both hospital-based and community-based studies. There were wide variations in reported prevalence rates from hospital studies and highlight the need to be carry out these studies with uniform sampling and molecular tools for detection, especially in countries with a dearth of information. Community-based studies, however, showed similarities in spite of differences in when (the late 1990s up until recently) and where (South India or Bangladesh) they were conducted. When more sensitive detection methods were used, cryptosporidial diarrhea accounted for 7%–9% of all diarrhea episodes and 20%–30% of children in these cohorts experienced at least one cryptosporidial diarrheal episode. High rates of asymptomatic infections with increased detection by serology and multiple infections (symptomatic and asymptomatic) were also documented in all cohorts. This overview brings to light the high burden of disease associated with cryptosporidiosis in children in the subcontinent and the gaps in knowledge to be addressed.

Keywords: Children, cryptosporidiosis, diarrhea, Indian subcontinent, malnutrition

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INTRODUCTION

Diarrhea is a leading cause of mortality and morbidity in children under five accounting for 526,000 deaths annually or 9% of all deaths in children in this age group worldwide in 2015.[1] In Sub-Saharan Africa and South Asia which together account for nearly 80% of all deaths in children under five,[2] the number of deaths associated with diarrheal diseases is considerably higher than other regions (~400,000 and 230,000 deaths in 2010).[3] The five countries with the most deaths due to diarrhea were in these regions and included India, Nigeria, Democratic Republic of the Congo, Pakistan, and Ethiopia.[3]

In the Global Enteric Multicenter Study (GEMS) conducted in multiple sites in Sub-Saharan Africa and South Asia, *Cryptosporidium* spp. was found to be second to only rotavirus as the leading cause for moderate to severe diarrhea in children under five.[4,5] When these samples were retested using TaqMan array cards, *Cryptosporidium* spp. rates remained unchanged and this pathogen continued to be among the leading causes of diarrhea in children under

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two.[6] In the recently concluded malnutrition and enteric infections (MAL-ED) study conducted in South America, Sub-Saharan Africa, and South Asia, cryptosporidial diarrhea had an adjusted attributable fraction (AF) of 3.8% among children in the 2nd year of life.[7] Based on the results of this study, the annual burden of cryptosporidial diarrhea in children under two was estimated as 2.9 and 4.7 million cases in Sub-Saharan Africa and South Asia, respectively.[5] *Cryptosporidium* attributable deaths in children under two were estimated as 202,000 in both Sub-Saharan Africa and South Asia. In an estimate based on results from community-based studies in India, *Cryptosporidium* alone contributed to 3.9–7.1 million diarrheal episodes and 58,000–146,000 deaths each year in children under two.[8]

While it is increasingly evident that cryptosporidiosis is one of the most common causes of diarrhea in young children in developing countries, it is also important to realize that these children are trapped in a vicious cycle where they are both at increased risk and are also most affected by this pathogen.[4,9] In combination with poor sanitation, malnutrition has been found to be a major contributor to this cycle. Studies have shown that the mortality and morbidity of cryptosporidial infection and risk of acquiring cryptosporidiosis are higher in malnourished children.[10,11] In turn, several studies have shown that cryptosporidiosis in early childhood (symptomatic and asymptomatic) leads to increased risk of malnutrition, stunting, and cognitive deficits in these children.[10,11] These epidemiological findings have also been confirmed in mice models where malnourished mice were more susceptible to infection and infection led to further malnutrition.[12] More recent studies in mice provide a possible explanation for this interaction; protein malnutrition resulted in activation of a signaling pathway that inhibited caspase-dependent apoptosis, resulting in a slower turnover of intestinal epithelial cells and leading to persistence of the parasite.[13]

In spite of heavy burden of infection, therapeutic options for cryptosporidiosis remain suboptimal and no vaccine is available. The only drug approved by the US Food and Drug Administration for the treatment of cryptosporidial infection in healthy immune individuals aged >1 year is nitazoxanide. Nitazoxanide has been found to have efficacy ranging from 56% to 86% and has been found to be ineffective in immunocompromised children.[14] Other therapeutic agents (paromomycin, macrolides, rifabutin, rifaximin, and bovine immunoglobulin) have been investigated, but a systematic review showed no evidence for any chemotherapeutic benefit in the treatment of cryptosporidiosis among immunocompromised individuals.[15]

Cryptosporidium is an apicomplexan parasite with a waterborne infectious cyst stage. The ingested cysts contain sporozoites that exist in the small intestine and attach to and invade the intestinal epithelial cell remaining intracellular but extracytoplasmic within a parasitophorous vacuole. Several parasite surface/apical antigens (*O*-glycosylated mucin-like glycoproteins) associated with attachment and invasion have been identified including circumsporozoite-like glycoprotein, gp900, gp40, Cp23, gp15, and Muc4.[16] Antibodies to these antigens have been shown to prevent sporozoite attachment in *in vitro* models.[17] Although the immunogenicity (humoral and cell-mediated) and vaccine potential of these candidates have been explored using multiple approaches including DNA-based vaccines, bacterial vectors, and *in silico* models, none have progressed to the stage of human trials till date.[16]

In this review, we aimed to provide a comprehensive summary on cryptosporidiosis in children in the Indian subcontinent where children at risk in these low- to middle-income countries[18] live in similar socioeconomic conditions. We have assessed different study designs and populations and gathered data on prevalence, risk factors, and other relevant epidemiological data.

METHODS

We conducted a literature search of studies published on cryptosporidiosis among children in the Indian subcontinent from 1983 to 2016 with a National Center for Biotechnology Information Entrez search. Keywords used were *Cryptosporidium*, cryptosporidiosis, and diarrhea combined with additional keywords of infant, preschool, child, and the country names from the subcontinent (India, Pakistan, Bangladesh, Sri Lanka, Maldives, Nepal, Bhutan). The data were then imported to a Zotero database and duplicated entries were removed. We used the PRISMA guidelines [Figure 1] for screening and selection of articles.[19] Studies were evaluated based on the following inclusion criteria: (1) studies must include data from children (0–15 years), (2) studies must be generated in one of the countries listed above, and (3) full text was available. Studies could either be hospital-based, community-based, or cohort studies. Site-specific data from multi-site studies that included data from countries in the subcontinent were also included.

RESULTS

A total of 154 publications during the period 1983–2016 were identified by the literature search. After duplicates were removed, 140 were screened with our selection criteria.

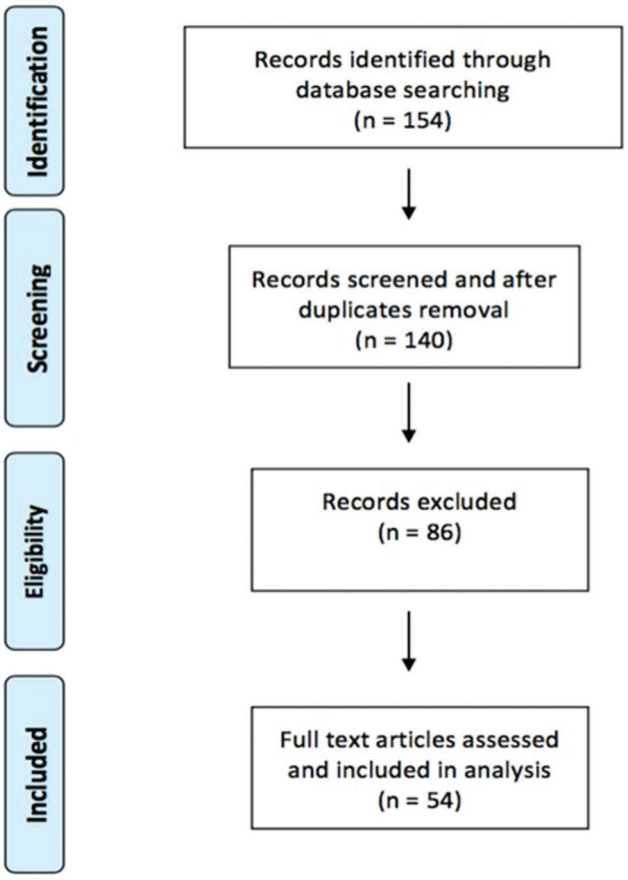

Figure 1: Flow diagram for literature search

Articles excluded ($n = 86$) for various reasons were full text unavailable ($n = 17$), case report ($n = 5$), environmental study ($n = 5$), not from the subcontinent ($n = 6$), no cryptosporidial data ($n = 37$), review articles ($n = 8$), no data from children ($n = 8$). After exclusion, 54 full-text articles available for review were included for qualitative synthesis [Figure 1]. Among these, 42 were further categorized based on the type of study for a comparative analysis [Tables 1-3]. While a majority of studies were from India (26/42), this analysis included studies from Bangladesh (7/42), Nepal (4/42), Pakistan (2/42), Sri Lanka (1/42), and multisite studies (2/42), but none from Bhutan and Maldives [Figure 2]. Two non-PubMed-indexed articles from Bhutan and Maldives were subsequently added.[39,51]

Hospital based studies

Among the 42 studies included, 21 were hospital-based, cross-sectional studies on cryptosporidial diarrhea with data from 17 centers [Table 1] and 10 were case–control studies from eight centers [Table 2]. Most studies reported using microscopy for detection. In India, prevalence of cryptosporidial diarrhea in hospitalized children ranged from 1.13% to 27.5%.[30,31] The wide range could possibly

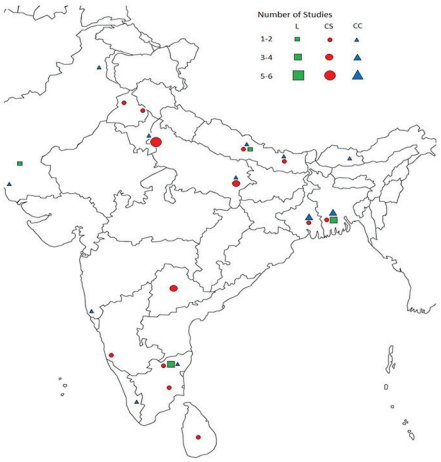

Figure 2: Geographical distribution of studies on cryptosporidiosis in the Indian subcontinent. L: Longitudinal studies, CS: Cross-sectional studies, CC: Case–control studies; Maldives not represented in map

be due to differences in populations studied (some studies had younger children under 3–5 years and others had older children), detection methods used (microscopy, enzyme immunoassay [EIA], and polymerase chain reaction [PCR]), and geographical variations. To illustrate this, studies that have used EIA or PCR showed much higher detection rates than when microscopy was carried out EIA 27.4% and microscopy 4% in a study from Delhi and PCR 13.3% and microscopy 4.4% in Vellore.[31,45] Prevalence of cryptosporidial diarrhea in hospitalized children with diarrhea was probably underestimated in studies when microscopy alone was used for diagnosis.

In studies reported from the same center, prevalence rates measured using microscopy showed a decreasing trend with time [Tables 1 and 2]. In Vellore, India, prevalence rates decreased from 13.1% in 1983 to 7.2% in 1990 in children under three and from 4.4% in 2003 to 2% in 2005 in children under five.[29,40,45,57] In Kolkata, studies from the 1980s showed prevalence rates of 5.6%[41] and 6.2%[43] that had decreased to ~3% by 2003[27] in children under five. Although not hospital-based, a community study in Dhaka showed 4.8% prevalence in 1985 which reduced to 1.4% another study in 1993.[46,47] Whether this was a true decrease in cryptosporidial diarrhea requiring hospitalization is uncertain, given high detection rates in the community from the same city.[6,45] Limited data were available from 1 to 2 hospital-based studies from Pakistan, Nepal, and Sri Lanka, and these studies showed similar rates as those seen in India. No data were available from Bhutan. A single study from Maldives in a nonindexed journal provided data for giardial and cryptosporidial diarrhea together. The review of available literature showed that studies with molecular or antigen detection methods in children hospitalized with diarrhea are

Table 1: Cross-sectional hospital-based studies on cryptosporidiosis among children in the Indian subcontinent

Location	Years of study		Age	Detection method	Number of children with diarrhea	Percentage of *Cryptosporidium*
India						
Calcutta[20]	1985	1986	<9	Microscopy	402	5.96
Varanasi[21]	1994	1996	<5	Microscopy	1179	7.4
Manipal[22]	1995	2000	<7	Microscopy	780	6.4
Varanasi[23]	1997	2000	<14	Microscopy	25	3
Chandigarh[24]	1998		NA	Microscopy	355	1.4
New Delhi[25]	1999	2000	<14	Microscopy	127	18.9
Hyderabad[26]	1999	2000	<3	Microscopy	202	2.99
Kolkata[27]	2003	2004	<5	Microscopy	1338‡	2.98
Hyderabad[28]	2003	2006	NA	Microscopy and EIA (Ridascreen)	681	7.6
Vellore[29]	2005	2008	<5	Microscopy	1018	2
New Delhi[29]	2005	2008	<5	Microscopy	970	3.5
Tiruchirappalli [29]	2005	2008	<5	Microscopy	591	2.7
Hyderabad[30]	2009	2012	<15	Microscopy	5123	1.1
New Delhi[31]	2012	2013	<5	EIA (DRG international Inc.)	175	27.4
Chandigarh[32]	1987*		<12	Microscopy	375	1.3
New Delhi[33]	1991*		<10	Microscopy	201	4.97
Amritsar[34]	1995*		<3	Microscopy	150	1.3
Bangladesh						
Dhaka[9]	1991	1994	<5	Microscopy	1949	3.5
Dhaka[35]	2001	2002	<5	EIA (in house)	1672	2.81
Nepal						
Kathmandu[36]	1992		<10	Microscopy	326	6.8
Eastern Nepal[37]	2007	2008	<15	Microscopy	863	4.1
Sri Lanka						
Kandy and Peradeniya[38]	2011	2013	<12	Microscopy	138	5.7
Maldives						
Maldives[39]	2007		3 months to 5 years	EIA (ProSpecT)	61	2†

*Study year not available, publication year mentioned, †2% denotes both giardia and *Cryptosporidium* infections, ‡Both diarrheal and nondiarrheal samples. NA: Not available, EIA: Enzyme immunoassay

necessary in countries with no or very little data to estimate the burden of cryptosporidial diarrhea.

Case–control studies

A total of 16 studies from the subcontinent have been carried out with a case–control study design both in the community (6 studies) and hospital (10 studies) setting [Table 2]. Community-based case–control studies have contributed to ascertaining the attributable risk (AR) of cryptosporidiosis by examining how much of the burden is associated with diarrheal infections. In addition, some studies have also determined risk factors associated with cryptosporidial diarrhea. AR is defined as proportion of disease cases or the proportion of the risk of disease that might theoretically be eliminated if the risk factors were eliminated.[58] Other synonyms for AR include AF, etiologic fraction, and attributable proportion.

The GEMS study mentioned earlier was an age-stratified, matched case–control study where children with moderate to severe diarrhea presenting at health centers along with randomly selected matched community controls were recruited at seven sites, three of which were in Asia.[4] In all Asian sites (India, Pakistan, and Bangladesh), *Cryptosporidium* was a leading cause of moderate to severe diarrhea in children under two with annual adjusted AF of 3.18 per 100 child years in children under one and 1.36 per 100 child years in children under two.[5] In case of less severe diarrhea, the adjusted AF in three Asian countries per 100 children-years in infants and toddlers was 4.88 and 4.71, respectively. *Cryptosporidium* attributable extended fatality risk was 0.9% in moderate to severe diarrhea cases and 1.4% in less severe diarrhea cases.[5]

We have calculated AR[59] and pathogenicity index (PI)[45] for cryptosporidial diarrhea using available data from case–control studies. Both AR and PI were high in hospital- and community-based studies, indicating that cryptosporidial infections were associated with diarrhea in children in these settings. However, it is important to note that most studies apply AR or AF to cohort data where confounding factors and infections with other pathogens can also be adjusted for as in the GEMS study.[4,5] In the studies when stratified by age, AR and PI decrease in children older than two indicating that most infections beyond that age were probably nondiarrheal.[5,48]

Table 2: Case control studies on cryptosporidiosis among children in the Indian subcontinent

Location	Years of study		Study setting	Age	Detection method	Diarrheal cases		Controls			AR%	PI
						n	Percentage of *Cryptosporidium*	*n*	Location	Percentage of *Cryptosporidium*		
India												
Vellore[40]	1983		Hospital	<3	Microscopy	682	13.1	418	Hospital	9.8	27.9	1.3
Calcutta[41]	1985	1987	Hospital	<5	Microscopy	566	5.6	167	Hospital	1.2	79.5	4.7
Idukki[42]	1987		Community	<10	Microscopy	266	6	294	Community	3	51.6	2.0
Varanasi[21]	1988	1989	Community	<5	Microscopy	607	3.8	529	Community	1.3	66.7	2.9
New Delhi[25]	1988	1989	Hospital	<2	Microscopy	100	5	50	NA	0	-	-
Calcutta[43]	1989	1991	Hospital	<5	Microscopy	244	6.15	226	Hospital	1.33	79.4	4.6
Mumbai[44]	1989*		Hospital	<13	Microscopy	180	4.4	100	NA	0	-	-
Vellore[45]	2003		Hospital	<5	Microscopy	158	4.4	99	Hospital	0	-	-
					PCR	158	13.3	99		2.04	86.4	6.5
Kolkata† (GEMS)[5]	2007	2011	Community	<1	EIA (TechLab)	878	15.3	892	Community	6.7	60.3	2.3
				1–<2		752	14.4	778		8.2	46.9	1.8
				2–<5		477	1.7	923		12	−688.5	0.1
Bangladesh												
Dhaka[46]	1985		Community	<5	Microscopy	805	4.8	146	Hospital	0	-	-
				5–15		122	1.6	37		0	-	-
Dhaka[47]	1993	1994	Community	<5	Microscopy	814	1.4	814	Community	0.4	71.7	3.5
Dhaka[48]	2004	2006	Hospital	<1	EIA (Techlab)	1088	5.2	485	OP clinics	2.9	45.6	1.8
				1–5		672	4.8	660		3.3	32.3	1.5
Mirzapur† (GEMS)[5]	2007	2011	Community	6–14	EIA (TechLab)	279	1.8	457	Community	1.1	39.3	1.6
				<1		672	8.2	1122		3.4	60.6	2.4
				1–<2		579	6	967		3.3	46.5	1.8
				2–<5		463	4.5	1111		5.3	−18.8	0.9
Pakistan												
Islamabad[49]	1996		Hospital	<5	Microscopy	475	10.3	150	Hospital	3.3	70.3	3.1
Karachi† (GEMS)[5]	2007	2011	Community	<1	EIA (TechLab)	788	14.1	788	Community	9.1	39.0	1.6
				1–<2		512	10.9	902		5.7	50.6	1.9
				2–<5		298	4.7	745		3.2	33.0	1.5
Nepal												
Eastern Nepal[50]	1999	2000	Hospital	<5	Microscopy	160	5.6	50	Hospital	0	-	-
Kathmandu and Bharatpur[51]	2006	2009	Hospital	<5	EIA (ProSpecT)	1200	2.2	1200	Hospital	0.8	64.2	2.8
Bhutan												
Thimphu and Mongar[51]	2011	2015	Community	<5	EIA (ProSpecT)	1716	2.6	1644	Hospital	1.6	39.1	1.6

*Study year not available, publication year mentioned, †Data for moderate to severe diarrhea. NA: Not available, AR%: Attributable risk, PI: Pathogenicity index, EIA: Enzyme immunoassay, PCR: Polymerase chain reaction, GEMS: Global Enteric Multicenter Study

Table 3: Longitudinal cohort studies on cryptosporidiosis among children in the Indian subcontinent

Location	Years of follow up	Age	Detection method	Enrolled children				Diarrheal episodes	
				n	Percentage of *Cryptosporidium*			*n*	Percentage of *Cryptosporidium*
					All	Diarrhe a	Asymptomatic		
Dhaka, Bangladesh[45]	1999-2002	2-5	EIA (TechLab)	289	42.5	25.7	22.6	893	8.4
Dhaka, Bangladesh[47]	2008-2014	<1 year <2 years	Real time PCR	392	77	25.5	51.5	990	5.6
								763	9
Vellore, India[39]	2002-2006	0-3	Microscopy	452	-	11.7	-	-	-
Vellore, India[42]	2008-2011	0-2	PCR and serology	176	67	23.8	43.2	781	8.5
Vellore, India[44]	2009-2013	0-3	PCR and serology	410	97	30	59.9	2121	9.4

Location	Years of follow up	Age	Detection method	*n*	Diarrheal episodes	
					n	Percentage AF of *Cryptosporidium*
MALED[7]	2009-2012	0-2	EIA (TechLab)			
Vellore, India		<1 year and <2 years		251	749	0 and 6.9%
Dhaka, Bangladesh		<1 year and <2 years		265	1591	0 and 2.5%
Naushero Feroze, Pakistan		<1 year and <2 years		277	2272	3.6% and 5.5%
Bhaktapur, Nepal		<1 year and <2 years		240	976	0 and 3.2%

AF: Attributable fraction, PCR: Polymerase chain reaction, EIA: Enzyme immunoassay

Longitudinal cohort studies

The insights provided by longitudinal cohort studies include understanding the burden of disease and identifying the correlates of a protective immune response which can in turn lead to development of appropriate therapy and vaccines.[60] These studies are resource intensive to conduct but yield an in-depth understanding of epidemiology and natural history of disease including transmission patterns and the role of repeated and asymptomatic infections that would not be evident in even the most meticulously planned cross-sectional studies.[60]

Community-based cohort studies on cryptosporidiosis have been carried out in Vellore, India ($n = 3$), and Dhaka, Bangladesh ($n = 2$), from the late 1990s along similar lines to birth cohort studies in Peru, Brazil, and Africa[58,61,62] In addition, four sites from the subcontinent (Vellore, India; Dhaka, Bangladesh; Naushahro Feroze, Pakistan; and Bhaktapur, Nepal) were also a part of the multicenter MAL-ED cohort study [Table 3].

Studies in South India have been carried out in an urban slum in Vellore, Tamil Nadu. The first birth cohort study was carried out between 2002 and 2006 with 452 children recruited at birth and followed up till the age of three. Although the cohort was originally recruited for studies on rotavirus, *Cryptosporidium* was found to be the third most common pathogen associated with diarrhea (~3% of a total of 1949 diarrheal samples). Fifty-eight episodes in 53 children were identified by microscopy.[54] This rate was similar to a community-based, cross-sectional study carried out in Varanasi.[63] When a subset of children had all samples (diarrheal and bi-monthly surveillance samples) screened by PCR, the rates were found to be much higher with 35 episodes in 20 children and 40% of these children experiencing multiple episodes. Asymptomatic episodes were also found to be associated with prolonged oocyst shedding.[64]

The second study in Vellore was a quasi-experimental study conducted on 176 children living in the same urban slum, about half of whom were given protected drinking water and followed up till the age of two between 2008 and 2011.[55] While the protected drinking water did not reduce the incidence of cryptosporidial infections or delay the time of onset, the study used molecular and serological techniques to detect infections and found that 67% of all children had at least one episode of cryptosporidiosis and a total of 186 cryptosporidial episodes were detected. Cryptosporidial diarrhea accounted for 8.5% of all diarrheal episodes and was found to be more severe and of longer duration than noncryptosporidial diarrhea. This increased severity has also been described in a previous study comparing cryptosporidial and noncryptosporidial diarrhea.[35] A majority of children who were infected experienced only asymptomatic infections (64.4%), and the median time to infection was 12.6 months. Among children with multiple episodes (40%), the proportion associated with diarrhea increased with increasing order of infection, indicating the lack of acquisition of immunity or a potential genetic susceptibility.

The third study, published recently, was a birth cohort with 497 children recruited between 2009 and 2010 and followed up till the age of 3 years in the same urban slum area in Vellore.[56] In this study, an extremely high level of cryptosporidial infections was detected using a combination of molecular tools and serological assays. Nearly, all children (97%) had cryptosporidiosis with 60% of children experiencing only asymptomatic infections and 57% experiencing multiple infections. Cryptosporidial diarrhea was associated with 9.4% of all diarrheal episodes in the cohort, but unlike the previous study, it was not more severe than noncryptosporidial diarrhea. The median age of incidence was also significantly less at 9 months compared to other studies. Similar to the previous study, multiple infections did not provide good protection from subsequent infections. This and the probability of increased infections among those with symptomatic infections indicate a possible genetic susceptibility to be explored.

Studies in Bangladesh have been carried out in an urban slum, Mirpur. The first cohort enrolled 289 children aged 2 to 5 years and followed them up for 3 years between 1999 and 2002 (~220 children completed follow-up). Although the children were enrolled for studies on amoebiasis, high rates of cryptosporidial diarrhea were detected (8.4%).[52] In total, 142 episodes of cryptosporidiosis in 96 children were identified, of which 25.7% were diarrheal and 22.6% were asymptomatic (identified from monthly surveillance samples). Recurrent episodes were detected in 29.3%.[65] As children in this cohort were older than 2 years of age, no difference in rates of symptomatic and asymptomatic episodes was seen.

The next study in Bangladesh was a birth cohort with 392 children followed up till the age of two and was also carried out in Mirpur slum from 2008 to 2014.[53] This study used quantitative PCR (qPCR) for detection and found that 77% (302) of children had at least one episode of cryptosporidiosis, of which 25.5% had at least one diarrheal episode and 72.2% had at least one asymptomatic episode, indicating that a majority of infections in the community were asymptomatic. The

total number of diarrheal episodes associated with *Cryptosporidium* (7%) did not increase when PCR was used compared to the previous study by Haque *et al*. but led to the identification of a large number of asymptomatic infections. The age of onset of cryptosporidial diarrhea was 13.9 months, which was significantly later than noncryptosporidial diarrhea (11.3 months). Higher parasite burden (as evidenced by lower Ct values in qPCR) was associated with diarrheal samples as compared to the surveillance samples. Nearly, 40% of children who were found to have cryptosporidial infection had repeated infections.

In the recently completed multicenter study on diarrheal disease (MAL-ED) with four sites from the subcontinent, approximately 250 children were enrolled at birth in 2009–2012 and followed up till the age of 2 years.[7] The study aimed to estimate pathogen-specific burden and collected both diarrheal and surveillance stool. EIA was used for detection and the adjusted AF for each pathogen was calculated, and *Cryptosporidium* spp. was among those with highest adjusted AF in all sites both in the 1st and 2nd years of life [Table 3] and was also associated with a higher diarrheal severity score.

Species and genotypes

In four cohort studies and a few case–control studies, *Cryptosporidium* species and genotypes were identified.[5,29,48,53-56,66] In India, *Cryptosporidium hominis* predominated in all studies and accounted for 70%–80% of all samples typed while it was associated with over 90% of samples typed in Bangladesh.[48,66] *Cryptosporidium parvum* was second most common and ranged from 12% to 17% in India and 3%–7% in Bangladesh. Other zoonotic isolates identified include *Cryptosporidium felis*, *Cryptosporidium meleagridis*, and *Cryptosporidium muris*.[5,54-56,66] Some studies also identified a few mixed *C. hominis* and *parvum* infections.[5,48,53,54] In one study, *C. hominis* diarrhea was found to be more severe than non-*C. hominis* diarrhea.[54] Other cohorts did not find any differences in between species.

Cryptosporidium isolates are genotyped based on polymorphisms of the gp60 locus and trinucleotide repeats encoding for serine.[67] In a study from Bangladesh, the common gp60 subtyped among *C. hominis* isolates were Ie (A11G3T3), Ib (A9G3R2), Id (A15G1), If (A13G1), and Ia (A14R1) with no subtype diversity in trinucleotide repeats,[53] while in India, If (A13G1) and Ie (A11G3T3) isolates were similar to Bangladesh, but diversity was seen in the Ia subtype.[54] A subset of isolates from all sites in the GEMS study was also typed, and the *C. hominis* subtypes found were Ia (diverse), Ie (A11G3T3), Id (A14), and If (A14G1). The authors mention that most of the *C. parvum* isolates were anthroponotic strains (IIc and IIe), which is similar to gp60 subtypes of *C. parvum* in India. Interestingly, the only three zoonotic strains identified were from Pakistan (IIdA15G1).[53] The presence of zoonotic subtypes warrants a more detailed study in countries with no data available as the transmission patterns may differ.

Risk factors

Some of the risk factors for cryptosporidiosis examined in community-based studies include sociodemographic factors such as household income, maternal age and education, and family size and environmental factors such as living conditions, source of drinking water, sanitation, and presence of animals. Living in poverty,[53] crowded living conditions, and presence of an older sibling in the household[68] increased the risk of cryptosporidial infections, increased risk of both symptomatic and asymptomatic cryptosporidiosis. Maternal age above 23, on the other hand, was found to be protective against asymptomatic infections.[68] Most cryptosporidial diarrhea reported in the Indian subcontinent both in the hospital and the community was acute rather than persistent.[52,69]

Stunting at birth[70] and at 6 months[68] predisposed to cryptosporidial infections. In turn, as seen in studies from other parts of the world, cryptosporidial infections increased the risk for stunting at 24 months. There was a 2.6–3-fold increased risk of stunting[9,53,68] as compared to children with no cryptosporidial infections, and in Bangladesh, in addition, there was an absence of catch up growth leading to prolonged stunting. An additive effect was seen with increasing number of episodes of cryptosporidiosis and the risk increased in children from lower socioeconomic levels. In India, cryptosporidial infection also led to a slightly increased risk of being underweight at the age of 2 years.[68] *Cryptosporidium* was also seen in 14% of children with malabsorption syndrome, but whether there is an increased risk is uncertain.[71] Effect of cryptosporidiosis on cognitive function and physical fitness has been documented in studies from South America but is yet to be fully investigated in the Indian subcontinent.[10,54,72]

When drinking water was assessed, although providing bottled drinking water did not decrease cryptosporidial infection rates, a history of "drinking boiled water always" was protective against multiple infections and asymptomatic infections while "use of a toilet by all family members" was protective against multiple infections and cryptosporidial diarrhea.[55] Studies on water and sanitation are especially relevant as a high burden of infection has been shown in these communities. In rural Orissa, India, the sanitation trial

had shown high rates of *Cryptosporidium* oocysts in water sources including from tube wells and in community ponds with no decrease when latrine coverage was improved.[73] The group also found a link between use of flush pour latrines and contamination of tube wells nearby.

Although cryptosporidiosis has strong zoonotic potential, very little evidence of animal-human transmission was seen in these communities. In one study, there was a slightly increased risk of multiple infections in children living in households with a cow or handling of cow dung by the primary caregiver.[68] This absence of a link with animal contact has been thought to be due to the mostly anthroponotic infections detected, and unlike in the West, there is nearly constant exposure to domestic and stray animals in these settings. Studies on prevalence in animals in Orissa have found up to 30% positivity depending on the animal species with high levels of "environmental loading" by cattle.[74]

Genetic susceptibility and immune response

When human leukocyte antigen (HLA) alleles were studied, *Cryptosporidium*-infected children in Bangladesh were more likely to carry HLA Class II DQB1*0301 allele and Class I B*15 HLA allele. Associated with carriage was also the DQB1*0301/DRB1*1101 haplotype.[65] When children in India were HLA typed, however, no association with any HLA allele was found.[56] Polymorphisms in the MBL2 gene at the − 221 promoter region and the YO/XA MBL2 haplotype have also been found to be associated with *Cryptosporidium* infections in Bangladeshi children. Symptomatic infections with *Cryptosporidium* were associated with a deficiency in serum MBL levels (<500 ng/mL) in these children.[75] Whether MBL controls *Cryptosporidium* by a direct binding to sporozoites in the lumen thereby promoting phagocytosis or triggering of the complement system or whether it has a more immunomodulatory role and decreases local inflammation associated with cryptosporidiosis needs to be explored.

Studies in animal models and high rates of cryptosporidiosis in HIV-positive patients as well as resolution of symptoms with elevated CD4 counts indicate the importance of cell-mediated immune response in protection. However, no studies on cell-mediated immune response from children in this region have been carried out.[16] Most studies on immune response have focused on humoral response to oocyst lysate or specific immunodominant antigens. In the first study, Khan *et al*. compared antibody response to *C. parvum* oocyst lysate between children with and without cryptosporidial diarrhea.[35] Baseline IgM and follow-up IgG levels were higher in children with cryptosporidial diarrhea (cases) than noncryptosporidial diarrhea (controls). Children with persistent cryptosporidial diarrhea also had a decrease in IgA levels at follow-up as compared to children with acute cryptosporidial diarrhea, indicating that possible mucosal immunity could be associated with persistence. In follow-up studies, these sera were also tested for antibody response to gp15, Cp23, and Muc4.[76-78] Cases had higher levels of anti-gp15 IgM than controls at baseline and higher levels of IgG and IgA at follow-up.[76] This was also seen in India, where a rise in anti-gp15 IgG was seen following diarrhea in children and peaked at 9 weeks.[79] Similar to oocyst lysate responses, children with persistent diarrhea had a decrease in IgA levels at follow-up compared to acute diarrhea.[76] In the case of cp23, higher anti IgG, IgA, and IgM were seen at follow-up and children with persistent diarrhea had a decrease in anti IgA and IgM titers. There was a good correlation between anti *C. parvum* and *C. hominis* gp15 and cp23 antibodies, but in the case of gp40, some evidence of subtype-specific immunity was seen.[76,77,79] Longitudinal studies have shown high rates of seroconversion in children in Vellore but also suggest a rapid waning in antibody levels.[80,81] Presence of preexisting anti-gp15 IgG in maternal sera and in preweaning sera was associated with a slight protection from cryptosporidial diarrhea.[81] Presence of IgA to oocyst lysates in breast milk resulted in significant protection from both all cryptosporidial infections (38%) and cryptosporidial diarrhea (64%) and was found to bind to the surface of oocysts.[82] These studies suggest that preexisting but short-lived antibodies may prevent infection and limit duration of infection.

CONCLUSIONS

In this review, we have described the burden and epidemiology of cryptosporidiosis in children in the Indian subcontinent. Estimates of cryptosporidial diarrhea are limited to mostly India and Bangladesh where numerous hospital- and community-based studies have been carried out.[27,29,35,40,46,47] There is a lack of data from other sites including Pakistan, Nepal, Bhutan, Sri Lanka, and Maldives. Hospital- or community-based studies to determine the burden of disease in early childhood using molecular tools in these countries are necessary. This will also determine the circulating species and genotype in the region and help in unraveling transmission patterns and risk factors. Among the countries for which limited data are available, two have achieved MDG4 goals (Nepal and Bhutan) and make the case for an important study on whether this has impacted rates of cryptosporidial morbidity and mortality in the community. Detailed studies on immune response to identify correlates of protection from cryptosporidial

diarrhea especially cell-mediated immunity need to be carried out. Extremely high rates of asymptomatic cryptosporidiosis in the community were also seen in these studies and had been suggested to be the true burden of infection.[53,83] Asymptomatic or non diarrheal infections along with cryptosporidial diarrhea have been found to be linked to malnutrition (stunting and wasting with failure to catch up), decreased physical fitness, and poor cognition and potentially enteropathy.[10,11] The mechanisms by which episodes of asymptomatic and symptomatic cryptosporidiosis cause longer sequelae need to be explored and could include but is not limited to studies on changes in microbiome in children, studies on biomarkers of intestinal inflammation and integrity and more in-depth analysis of genetic susceptibility. More detailed studies exploring interventions for prevention are also required. Whether currently underway studies on water, sanitation and hygiene interventions will play a role in preventing both symptomatic and asymptomatic infections needs to be seen. In summary, cryptosporidiosis causes significant mortality and morbidity in vast numbers of children in the Indian subcontinent and urgently warrants increased research and resources for prevention.

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Conflicts of interest

There are no conflicts of interest.

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