REVIEW ARTICLE

Epidemiology of Cryptosporidium in Pediatric Diarrheal Illnesses

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Context: *Cryptosporidium spp*. is a zoonotic infection, now being recognized as a significant cause of diarrhea in both immunocompetent and immunocompromised hosts. However, there still exist significant knowledge gaps in its estimated global burden, epidemiology, diagnosis and management.

Evidence acquisition: A semi-systematic search was performed across PubMed to select studies on epidemiological burden of cryptosporidium diarrhea using the following keywords- ['crytosporidiosis' OR 'cryptosporidium'] AND ['diarrhea' OR 'diarrhoea']. Articles were included if participants were 'Humans', belonged to pediatric (0-18 y) age group, and were published after 1990. The results were compiled separately for acute and persistent diarrhea.

Results: *Cryptosporidium spp* is commonly detected in stools of both cases (acute/ persistent diarrhea) and asymptomatic controls. The prevalence is higher in children with diarrhea than non-diarrheal controls (1.7-35% *vs* 0.3-15%); varying widely across different studies. The positivity rate is higher in younger children (<2 years) suffering from diarrhea. The main symptoms associated with cryptosporidiosis include fever, vomiting and abdominal pain with propensity for prolonged duration of diarrhea. It predisposes to malnutrition, which is also a risk factor for cryptosporidiosis. The prevalence is higher in HIV positive patients; certain socio-demographic factors play a more important role than mere geographical distribution for infection.

Conclusions: The high positivity rates during both acute and persistent diarrhea highlights the need to suspect this infection even in immunocompetent children.

Keywords: *Acute diarrhea, Etiology, Malnutrition, Persistent diarrhea, Systematic review.*

Diarrhea is a leading cause of morbidity and mortality in under-five children [1]. Though bacteria and viruses are the predominant agents for pediatric diarrhea, intestinal parasites (*Entamoeba histolytica Giardia lamblia, and Cryptosporidium spp.*) are also well-known etiological agents. Cryptosporidium is a ubiquitous enteric protozoan with 11 species, of which *C. hominis* and *C. parvum* commonly affect humans. The former is isolated frequently from developed countries, while the latter is commoner in developing countries or as zoonotic infection in developed nations. The burden of cryptosporidiosis is thus largely incurred by developing countries due to sub-optimal sanitation practices [2,3]. In children, cryptosporidiosis is associated with both acute and persistent diarrhea. It affects both immune-competent and immunocompromised individuals, with a more chronic illness seen in the latter [2].

Earlier prevalence of cryptosporidiosis varied from 1% in high-income countries to 5-10% in low- and middle-income countries (LMIC). Recently, the prevalence of cryptosporidium in childhood diarrhea has shown an upward trend, possibly due to use of newer detection methods [4]. Detection rates have increased even in immune-competent healthy children. The recent Global Enteric Multicentric Study (GEMS) on 9,439 children with moderate-to-severe diarrhea and 13,129 control children from seven countries of Asia and Africa, attributed four major pathogens as cause of moderate to severe diarrhea – Rotavirus, Cryptosporidium, Enterotoxigenic *Escherichia coli* and *Shigella* [5]. India reported the highest estimated incidence of moderate-to-severe diarrhea among seven countries evaluated. This study highlighted the pathogenic role of cryptosporidium, which was associated with increased risk of death (hazard ratio 2·3; 95% CI 1·3,4·3) in children aged 12-23 months. Cryptosporidium was also identified as a leading parasitic cause of diarrhea (both acute and persistent) detected among 8.2% of symptomatic children (OR 9.24; 95% CI 1.20,71.37) [6].

At most centers, there is still a lack of lucid understanding of the clinical presentation and risk factors for cryptosporidium infection. There is a need to provide an updated review on epidemiology and clinical

manifestations of pediatric cryptosporidiosis, as existing reviews mainly focus on newer diagnostic assays and biotechnological advances in this field [7,8]. There is lack of a systematically conducted consolidated review on its epidemiology, required to plan control and treatment strategies. We planned this review to provide insight into epidemiological burden, including risk factors, of cryptosporidiosis in acute and persistent childhood diarrhea. The review further aimed at understanding clinical correlates and presentation of cryptosporidiosis in children.

METHODS

Study design and Sources of literature: A semi-structured systematic search strategy was used. The primary database used to search information was Medline through PubMed. The search was performed between 5 August 2014 and 06 June 2016. Both MeSH-based and keyword-based searches were done, and information from studies was synthesized in a narrative manner.

Search strategy: In order to capture the most relevant data from the vast data source, and retain the methodological quality, we decided *a priori* to use a systematic search process. We searched the major heading of 'cryptosporidium' under medical subject headings (MeSH), and combined it with the MeSH term 'diarrhea'. In order to find more related articles pertinent to the research question, we also performed a keyword-based search using keywords ['crytosporidiosis' OR 'cryptosporidium'] AND ['diarrhea' OR 'diarrhoea']. As per a pilot test search done on 2 August 2014, we found that this search strategy shortlisted many articles related to zoonotic burden of cryptosporidiosis. To retain the focus of review on epidemiology and clinical data, we decided to add additional filters of 'Humans' to exclude animal based studies, and 'Age- birth till 18 years' to restrict the review to this age group. To maintain the relevance of epidemiological data in current scenario, we selected only articles published in last twenty years. Only one article duplicated from both search designs, MeSH and keyword-based, and was retained.

Inclusion/exclusion and Outcome variables: We recorded the search date, search terms, search string and search output; and checked each searched item for eligibility. We restricted to include only articles that contained clinical information on prevalence/ incidence or clinical features or management. We considered the following parameters for eligibility of abstracts by the authors: (*i*) Title, (*ii*) Examination of Abstract or Introduction (where abstract was not published), (*iii*) Examination of full-text. The titles/abstracts mentioning animal studies or genetic studies on cryptosporidiosis were dropped. The article which contained subjects from both pediatric and adult age was included only for their pediatric data. Certain abstracts whose full text could not be retrieved were included with the available information only.

Data collection and analysis: The main outcome parameters to be addressed were 'prevalence of crytosporidiosis and its risk factors in Pediatric diarrhea'. In view of anticipated heterogeneity in the study settings, patient profile and microbiological methods of detection, we decided *a priori* that no meta-analysis of data would be performed. The shortlisted articles included both hospital- and community-based studies on pediatric diarrhea. The data collected was stratified depending on clinical presentation of acute or persistent diarrhea. Standard case definitions for acute diarrhea (lasting <7 days) and persistent diarrhea (lasting ≥14 days) were used [9]. The abstracts on acute diarrhea were further stratified into those with controls and without controls. Control population was defined separately in different studies as 'Non-diarrheal' or 'healthy controls'. Control population in studies with persistent diarrhea included healthy children or those with acute diarrhea. Narrative reviews and isolated case reports were dropped from analysis but information was considered for discussion. Few studies were retrieved which analyzed microbiological flora from stool samples. These studies were included if samples were obtained from pediatric patients. Few studies had included only children with cryptosporidiosis to analyze risk factors associated with transmission. These were also retained for analysis.

The data collected was stratified and tabulated.

RESULTS

Acute Diarrhea

Epidemiological features in studies with non-diarrheal controls

The prevalence of cryptosporidiosis was reported over a broad range across different studies at both hospital and community level. We identified a total of 33 studies (***Web Table* I)** [6,10-44], which had enrolled both diarrheal (cases) and non-diarrheal (control) children (23-hospital based, 9 community-based). The prevalence was higher and varied from 1.7-35% among those with diarrhea than 0.4-15.6% in children without diarrhea, from LMIC Asia and Africa. The prevalence among diarrheal cases from certain high-income and high- to middle-income countries was found comparable (11.2% from Venezuela [34], 18% from Mexico [20] and 27% from Brazil [43]) to that in developing countries. This wide range of prevalence could result from different time frames of

each study – prospective or cross-sectional, different methods used for diagnosis, and special efforts made to detect the organism in studies reporting high prevalence. Detection improved with additional methods like auramine staining [31,43], and direct or indirect fluorescence using monoclonal antibodies [44]. Among case-control studies, a higher prevalence was seen among hospitalized children than in community. However, Katsumata, *et al.* [33] from Indonesia detected higher prevalence in community than hospital diarrheal samples (8.2% and 2.8%, respectively).

As per hospital data, the prevalence was higher among cases in studies which enrolled children below five years (1.4-46%) of age than those with age range till adolescence (4.1-17%). However, two studies reported higher prevalence in the older age group than the younger. Wang, *et al*. [14] evaluated concurrent infections (*Giardia, E. bieneusi* and *C. difficile*) in children during an outbreak of cryptosporidium in China with a point prevalence of 51%. This exaggerated prevalence was related to outbreak, and was not indicative of true overall prevalence [14]. Mirzaei, *et al.* [18] also reported 35% prevalence in children below 15 years over a 3-month period, which was higher than that seen in adults in his study. The age distribution of subjects below 15 years was unavailable [18].

Cryptosporidium was also detected in asymptomatic controls at both hospital and community setting, though at a significantly lower prevalence (0-6%; ***Web Table* I**) than cases. Two studies from Africa detected higher prevalence of infection in controls than that reported by other studies, (8.5% [23] and 15.6% [27]); however, the positivity rate was less than that in cases.

Among the community-based studies, a higher prevalence was seen in cases (3.8-45%) than controls (1.7-4%). A higher prevalence (two-fold) of infection in controls than cases was reported from Thailand [36,38], which was probably an incidental occurrence that signified the burden of latent infection among asymptomatic children below five years of age. Both studies had used enzyme immunoassays for detection, with sensitivity of more than 95% in the latter [38].

The median prevalence in community-based studies was almost similar to hospital-based data. However, two studies which had evaluated younger children (below 2 years) reported high prevalence in community (45% and 27.8%, respectively) [43,44]. The only case-control study from India was from Varanasi, which had recruited total 1136 children aged below 5 years. The detection of cryptosporidium was 3.8% in cases and 1.7% in controls (OR 2.94; P <0.01) [42].

Epidemiological features in studies without controls

A relatively greater number of studies (49) were found which described epidemiological patterns in diarrheal children without simultaneous enrolment of controls – 24 hospital-based and 25 community-based (***Web Table* II**) [46-97]. The detection rate was generally higher in community-based studies (0.1%-45%) than hospital-based studies (1.4%-18.9%). The detection rate was greater in studies which used additional diagnostic methods over acid-fast staining, varying from 18.7% with direct fluorescence [65], 18.9% with Immunocard [53], 42.4% with antigen detection kit [88], and 45% with direct fluorescence using monoclonal antibody [44]. The detection rate improved from 4% with routine microscopy to 28% with immunoassay in an Indian study [47]. Prevalence was higher if study had enrolled immunocompromised seropositive children [71], or those attending day-care center [65,80]. Detection was also greater if stool samples were analyzed within few days of occurrence of index case (20%) [90].

Clinical features of infection

Cryptosporidiosis occured frequently in younger than older children in most of studies. On further age-stratification, children aged below 2 years of age were more predisposed to infection (***Web Tables* I** and **II**). The vulnerability in this age group may be explained by diminished maternal antibody protection and increased exposure to pathogens by virtue of their feeding practices. The Indian data in GEMS study identified attributable-fraction of cryptosporidiosis in moderate-to-severe diarrhea as being second highest after rotavirus, in children aged 0-11 months and 12-23 months (Rotavirus 27 and 25.4, and cryptosporidium 11.7 and 8.4 weighted percent of total diarrheal episodes, respectively) [5]. The annual burden of cryptosporidiosis in Indian children aged below 2 years was estimated to 3.9–7.1 million diarrheal episodes, 66.4–249.0 thousand hospitalizations, and 5.8–14.6 thousand deaths [5]. Few studies did not find any significant association with age [24-26]. Almost all studies precluded the role of gender as a predisposing factor (***Web Tables* I** and **II**).

Among infants who presented with acute diarrhea due to cryptosporidium, fever, nausea and abdominal distension were commonly seen, but not dehydration [20]. Similarly, in children younger than 5 years, fever and vomiting were commoner findings unlike dehydration [23,24,41,45,70]. The diarrheal pattern in cryptosporidiosis was mainly watery diarrhea (***Web Tables* I** and **II**). Few studies also reported mucoid stools in children with cryptosporidiosis [32,69]. A study from slums in Southern India reported prolonged oocyst shedding in 40% of

children affected with repeated cryptosporidial infections, which may adversely impact growth during childhood [84]. Diarrhea due to cryptosporidium had a propensity for prolonged course [26,31,34,62,86], and its detection rate in stool samples was higher in children with persistent diarrhea than in acute diarrhea [27] (***Web Table* III**). The subtype *C. hominis* was associated with longer duration of diarrhea while *C. parvum* resulted in more systemic features [86].

Risk factors

Malnutrition: The relation between malnutrition and cryptosporidiosis is bi-directional. Cryptosporidium impairs nutrient absorption and results in growth failure and stunting [2], as has also been documented in prospective studies [44,97]. In addition, higher isolation rate of cryptosporidium is seen among malnourished children, defined as low weight-for-age, height-for-age or weight-for-height, in different studies [6,23,27,31, 43,56,62,70]. Kirkpatrick, *et al.* [28] concluded both underweight and stunting as stronger risk factors for infection than wasting, and also found vitamin A deficiency as a risk factor [28]. Mondal, *et al.* [85] found underweight as a more significant risk factor than stunting among 289 slum children from Bangladesh [85]. Even stunting at birth was a significant risk factor among slum children at Bangladesh [77]. Two studies from Bangladesh [24,30] and one from Brazil [41] did not conclude any significant relation with anthropometric variables. One of these studies [24] measured growth cross-sectionally, while another [30] had a short follow-up period of three months. Two separate studies did not find any association with baseline weight or height, but documented a significant detrimental effect on weight and height on follow-up (P<0.02), notably in infants [43,97].

Immunodeficiency: It is a predisposing factor for various opportunistic infections, including cryptosporidium [98]. The prevalence of cryptosporidiosis among children seropositive for HIV from India was reported as 29% in those with diarrhea, 14% in those without diarrhea and nil in seronegative subjects [99]. The prevalence varied from 5.2% [52] to 18% [71,89] as per different studies. However, cryptosporidium detection had no relation to HIV-positivity in some studies [31,49,83]. The GEMS study also detected cryptosporidium as a significant diarrheal pathogen regardless of HIV status [5]. A case-control study from Italy did not find any child with cryptosporidiosis to be immune-deficient [45]. The literature suggests that though most cryptosporidial infections occur in children who are not immunodeficient, seropositive children have a higher predisposition to the infection [12]. The risk of infection reduces in seropositive children with administration of Highly active antiretroviral therapy (HAART) [positivity HAART-0%, Non-HAART 3.9%]. Low CD 4 counts (<350 cells/ mm3) increased the risk of infection in the latter group [OR 13 (95% CI 10.5 to 97.6), P<0.01] [46].

Environment and sociodemographic factors: The geographical distribution has not been conclusively established as a risk factor for cryptosporidiosis. Rural environment is considered favourable for transmission of intestinal infections due to suboptimal sanitary facilities, frequent animal exposure, and limited access to safe water [2,73]. However, urban areas are also at-risk because of possibility of contamination of water supply systems. Abu-Alrub, *et al*. [17] found higher prevalence of cryptosporidium among children dwelling in rural/ refugee area in Palestine, but data from Malawi, Africa [2], did not report any difference in prevalence of cryptosporidiosis in rural or urban area.

Socio-demographic factors are likely to play a more important role than mere geographical distribution, as cryptosporidiosis is a zoonotic infection. Contact with cattle and cats is a significant risk factor as reported in both hospital-based [21,33], and community-based studies [39,73,76,83]. A village-based study from Odisha, India reported cattle to contribute maximum to environmental load of oocysts than dogs and cats [73]. However, few studies did not find any significant association with animal exposure [23,24,32,40,55,90]. In addition, contact with contaminated water in public swimming places was reported as a risk factor as per adult surveillance data across US and Australia [22,39,40]. Asymptomatic infection was detected in a significant proportion of children residing in slum area of Vellore, Southern India (28.4%) [84], postulated to result due to compromised hygiene and sanitation services. However, contrary to the belief of protection against infection with use of packaged water, studies from Vellore, India [75] and the West [39,40] have reported lack of association between the two (adjusted RR = 0.86; 95% CI, .60-1.23) [75]. They postulated multiple transmission pathways from asymptomatic infected controls than drinking water source. The environmental factors reported as risk factors for infection include swimming in public pools and contact with cattle [73] or with another person with diarrhea [39,40]. Both hospital- and community-based surveys did not find other environmental factors like food hygiene, presence of sewage [65] and socio-demographic factors like maternal education [27,84] and socio-economic status [84] as risk factors for cryptosporidium.

Rainy humid environment has been found more

conducive for parasitic growth, survival and transmission [33,58,60]. However, hospital-based studies from India and Pakistan reported higher occurrence of infection in hot summer months with no relation to humidity [51,55]. As per a multi-site study across India, prevalence of cryptosporidiosis had positive association with minimum and maximum temperature, but negative with relative humidity. These differences were appreciable in areas with seasonal temperature fluctuations only [55]. Jagai, *et al.* [100] concluded presence of both high ambient temperature (seen in temperate countries) and high rainfall (seen in the tropics) as contributory seasonal factors for infection. The MAL-ED study from 8 sites in World reported peak incidence of cryptosporidium coincident with peak diarrheal season at respective sites. Thus, it may not be season alone but unhygienic practices also which are responsible for propagation of infection [35].

Persistent Diarrhea

Cryptosporidiosis has a propensity for prolongation of the diarrheal episode [24]. Initial studies from India in 1990s did not report increased isolation of cryptosporidium in children with persistent diarrhea, unlike Giardia [101]. However, these studies used modified acid-fast staining for documenting cryptosporidium in stool samples. Recent studies from other parts of world have used better detection methods than simple microscopy and found higher prevalence of cryptosporidium in persistent diarrhea (16-31%) [102,103], with prevalence being higher than that in acute diarrhea (***Web Table*** **III)** [101-117].

The risk factors identified for development of persistent diarrhea in cryptosporidiosis include young age (<2 years) [21,100] and lack of breastfeeding [106]; Vomiting and dehydration were other clinical features that were seen in a significant proportion of these children [24,106,109].

Persistent diarrhea negatively impacts nutritional status in children in terms of weight, height and weight for height [110]; few studies have reported an association between this condition and cryptosporidiosis [108]. The pre-infection weight and height of children presenting with diarrhea was found comparable in different studies, irrespective of positivity of cryptosporidiosis [24,97]. Both Mølbak, *et al.* [97] and Lima, *et al.* [110] documented significant growth faltering in children with cryptosporidium infections, suggesting a two-way association of malnutrition with cryptospori-diosis. Among infants, there was a greater faltering in height on follow-up till 180 days (though not statistically significant) unlike weight loss which remained similar on follow-up till 180 days [97].

Immunodeficiency is reported as an important risk factor for cryptosporidiosis in persistent diarrhea [108]. A hospital-based study at Uganda found significantly higher odds of cryptosporidium in HIV-positive than HIV-negative children with persistent diarrhea (OR 44.36; 95% CI 18.39 to 110.40). The risk of infection was also higher in those with low CD4 cell count (<25%) than those with higher CD4 counts (OR 6.45; 95% CI 3.28 to 12.76). The authors also commented on higher isolation of *C. parvum* species in children with HIV than *C. hominis* (OR 0.167; 95% CI 0.036 to 0.771) [102].

DISCUSSION

The present review compiles available evidence on epidemiology of cryptosporidium diarrhea in the pediatric age group. The prevalence of cryptosporidium in pediatric diarrhea is high in both acute and persistent diarrhea, being higher in the latter group. The available evidence concluded young age, malnutrition and certain socio-demographic factors as associated risk factors, with inconclusive association with exposure to animals and sanitation. HIV-positivity has a definite association with cryptosporidium in persistent, but not necessarily in acute diarrhea.

The UNICEF fact sheet 2014 mentions diarrhea among top four causes of under-five mortality in children in world, contributing to 9% of total deaths. India alone contributes to 21% of all under-five deaths globally [1]. As per GEMS study, *Cryptosporidium spp.*, which were initially thought to be only opportunistic protozoal infection, have now been identified as the third leading cause of moderate to severe diarrhea, ranking after rotavirus and Shigella, and are associated with an increased risk of death in children aged 12-23 months [5]. Recent secondary analysis of data from the GEMS study, which analyzed over 15,000 stool samples showed annual incidence (per 100 child years) of cryptosporidiosis varying from 2.52-4.88% to 3.18-3.48% in less-severe and moderate to severe diarrhea, respectively in infants. The incidence was lesser (1.36-1.41%) among toddlers with moderate to severe diarrhea but similar (4.04-4.71%) in those with less severe diarrhea [118]. The attributable incidence (per 100 child years) in less severe diarrhea from India was reported as 4.73 (0.61–8.86) in those <11 months and as 3.43 (-0.78-7.64) in children aged 12-23 months. The odds of risk of cryptosporidiosis in moderate diarrhea varied with age as 2.44 (1.34-4.44), 3.22 (1.90-5.47) and 2.19 (1.23-3.87) in children aged < 6months, 6-11 months and 12-17 months, respectively [118]. Further, the authors estimated around 202,000 cryptosporidium-attributable deaths, with around 59,000 excess deaths occuring among cryptosporidium-attributable diarrhea

cases over expected if cases had been cryptosporidium-negative.

In most recent studies, a significant proportion of healthy children with diarrhea were detected positive for cryptosporidium. The protozoan was also detected in mixed infections. Thus, screening for cryptosporidium should be contemplated in settings of prolonged or persistent diarrhea. Lack of specific clinical signs or pattern of illness also justifies its screening. In addition to diarrhea, cryptosporidium had significant impact on childhood growth in both symptomatic and asymptomatic infections with greater severity in symptomatic infection than asymptomatic infection [44]. This devastating effect on growth after cryptosporidial infection is attributed to impaired intestinal absorption due to mucosal inflammation, which gets worsened in malnourished children [4].

We have not systematically addressed certain key areas like diagnosis and treatment in this review. Microscopy using modified acid-fast staining is a cheap and readily available method, though its sensitivity gets compromised with lack of good staining, visual expertise and parasitic load. Fluorescent staining with auramine stains improve detection but may affect specificity, which can be overcome with immunoflourescent stains [4]. The GEMS study – the largest ever study of etiology of acute diarrhea, which documented a high prevalence of cryptosporidium – also used immunoassays for detection of *Cryptosporidium spp.* and *Giardia* [119]. The literature demonstrates better sensitivity and specificity of serological and molecular methods over conventional microscopic examination of oocysts [6,38,47,49,54]. Martin-Ampudia, *et al.* [120] detected cryptosporidium in 62 (15.5%) stool samples with additional parasitological testing. This indicated under-notification of cryptosporidiosis and highlighted the need for its routine testing in children. Only one study reported false positive results with EIA over microscopy [57]. Molecular analysis, mostly based on 18S rRNA, can differentiate different species. The high cost and need for technical expertise limits its use to research settings [4].

The limitations of the present review are include search strategy limited to PubMed; lack of quality assessment of studies; and absence of a meta-analysis. Moreover, some more studies might be available since the last date of search for this review. There is clinical heterogeneity among included studies in terms of study population, geographical areas, time frames and microbiological methods used for diagnosis of cryptosporidium infection. We identified the following research areas: prevalence of cryptosporidium infection in diarrhea from community-based studies in low- to middle-income countries; longitudinal studies documenting short-term and long-term outcomes of children suffering from cryptosporidium infection; and development of rapid, sensitive and cost-effective kits for detection of cryptosporidium. Moreover, specific treatment options for cryptosporidiosis are still limited. The difficulty in *in vitro* propagation of cryptosporidium is a major obstacle in developing specific therapeutic agents, in addition to lack of standardized animal models [4]. An exaggerated pro-inflammatory cell-mediated immune response with elevated levels of interleukin 8,10,13 and tumor necrosis factor-α was reported in malnourished children with cryptosporidiosis [28], which suggests for development of newer treatment strategies, including immunotherapy. Vaccine therapy is under consideration but lacks direction due to incompletely understood human immune responses in cryptosporidiosis. The vaccines being investigated are based on Cp15, Cp40 and Cp23 antigens [4,121]. Further elaboration of the protozoan's genome will assist us in developing newer immunotherapy agents.

CONCLUSION

Cryptosporidium is an important human pathogen with manifestations varying from asymptomatic colonization to acute and persistent diarrhea. The infections, though more common and prolonged in immunodeficient children, are well documented in immunocompetent children, up to the extent of most common parasitic cause in almost all studies, and most common cause of acute diarrhea and persistent diarrhea in some of the studies. The present review establishes a comprehensive overview of the epidemiological attributes of cryptosporidium diarrhea in childhood. A better awareness and under-standing of this pathogen will improve the epidemiological and etiological diagnosis of pediatric diarrheal illnesses, and will emphasize the need to develop improved diagnostic and therapeutic agents.

Contributors: DS, SB and RL: conceptualized the review;. AD and DS: collected the initial data; AD, DS, SB, RL: pooled and interpreted the results; AD: prepared the first draft of manuscript which was revised by all others. All authors read and approved the final manuscript.

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