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 over-view 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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WEB TABLE I EPIDEMIOLOGY OF CRYPTOSPORIDIUM IN ACUTE DIARRHEA FROM STUDIES WITH NON-DIARRHEAL CONTROLS

Author, year [Ref]	*Place of study*	*Study population*	*Detection methods*	*Positivity rates*	*Associated risk*	*Clinical associates/ Remarks*
Hospital-based studies						
Breurec, *et al*. 2015 [10]	Bangui, Central African republic	Age: < 5yr. Cases- 333 Controls 333	Microscopy followed by multiplex PCR	Cases- 42/333 (12.6) Controls- 9/333 (2.7) Attributable fraction= 10.5 (7.3–11.7)	Young age: Infants adjusted OR= 4.6 (1.8–11.7), toddlers OR= 2.9 (0.6–14.8) , Rainy season	
Nhampossa, *et al*. 2015 [11]	Rural Southern Mozambique	Age: <5 yr Cases- 784 Controls 1545	Immunoassays	a) 0-11 m- Case- 84/431 (20%), controls 86/861 (10%). b) 12-23 m- Case 44/233 (19%), control 46/502 (9%). c) 24-59 m- Case 11/120 (9%) controls 18/232 (8%)	Infancy: adjusted OR 15.26 (11.96–18.56),	Incidence rate was 2.10 (1.45–2.76) per 100 child years at risk
Tellivik, *et al*. 2015 [12]	Dar es Salaam, Tanzania	Age <2 yr Case- 701 Controls-588	Multiplex real-time PCR	Cases-16.3%, Controls-3.1% OR = 6.2; 95% CI: 3.7-10.4; P < 0.001;	Immunodeficiency- HIV positive 24.2, HIV negative 3.9%; OR = 7.9; 95% CI: 3.1–20.5: P < 0.001. Stunting- OR = 2.12; 95% CI: 1.2–3.8, P = 0.011;Rainy season: OR = 2.41; 95% CI: 1.5–3.8, P <0.001	*C.hominis* in 84.7% and C. parvum in 7.6%
Vadlamudi*, et al.* 2013 [13]	Birmingham, Alabama Retrospective 7 year cohort	Age: 3-17 year with inflammatory bowel disease- 7 positive cryptosporidium, 21 negative for infection	Stool rapid immunoassay test with immunocard STAT!®	7/170 (4.1%)	No differences in baseline characteristics	5/7 had severe dehydration 3/5 treated with NTZ recovered within 3 days
Wang*, et al.* 2013 [14]	China	Age- 1m-18yr Cases- 78 Controls- 499	Not mentioned	Cases-51.4% Controls- 2 %	Not evaluated	Positive had more co-infection than controls with Giardia, E.bieneusi and C. difficile

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Amatya, *et al.* 2011 [15]	Nepal	Age: <15 yr Cases-863 Controls-100	Modified Ziehl Neelsen method	Cases-4.1% Controls- 0%	Age- 6-10 yr	
Opintan, *et al.* 2010 [6]	Accra, Ghana. Cross sectional <5 years	Age: 0-60 m Cases-170 (145 AD and 13 PD), Controls-104	Single locus Quantitative qPCR on fecal DNA	Cases- 14/170 (8.2%), Controls- 1/104 (0.9%), OR = 9.244 [95% CI 1.197–71.4], *P* = 0.011)	Malnutrition WAZ<-1 OR-9.244 (1.197–71.371); P=0.011	NA NA
Haque, *et al.* 2009 [16]	Dhaka hospital Bangladesh	Age: ≤14 yr cases-1088 ; Controls-1623	Antigen detection kits, real-time PCR assay,	Cases- 94/2039 (4.6%);controls- 41/1623 (2.5%)	Young age <12m age (57/1088 cases vs 14/485 controls; p=0.037).	Cases less likely to have abdominal pain, than control subjects (15% *vs*. 37%; *P*<.001) *C.hominis* most frequent (61%), followed by *C.parvum*,
Abu-Alrub, *et al.* 2007 [17]	West bank, Palestine Prospective, hospital based	Age: 1m-15 yr 760 cases, 62 ND controls	Modified acid fast staining	Cases- 11.6% Controls- 3.2%	Young age: Incidence 14.4% <5 yr, 7.7% 5 to 10 yr, 5.9% 11 to 15 yr. Rural area or refugee camps than urban area	NA
Mirzaei M, 2007 [18]	City of Shiraz, Fars Province, Iran	Children <15 yr-Cases-51; controls-38	Ziehl-Neelsen acid-fast staining.	Cases- 18/51 (35.3%) Controls (2.6%)	NA	
Mukhopadhyaya, *et al.* 2007 [19]	Western Nepal. Prospective (6 years).	Age: Below 5 years AD-155, PD-204, ND control-100	Modified acid fast staining	AD- nil, PD- 2/204 (0.1%), controls- nil	Lack of breastfeeding for PD. No relation with water source/ sanitary practices	
Sanchez-Vega, *et al.* 2006 [20]	Mexico	Age < 1 year Cases-100 Controls-100	Modified Kinyoun acid-fast technique, and observed by light microscopy at 100×, 400×,1,000×.	Cases- 18% Controls-0%	Incidence higher in males 77.78%. Sanitation and hygiene was adequate	All cases had fever and abdominal distension, none had vomiting and dehydration
Olesen, *et al.* 2005 [21]	Stool collection centre, Denmark. Prospective	Age: 0-5 years. Cases-424 Controls-870	Ziehl-Neelsen acid fast staining	Cases- 1.7% (6/351), Controls-0%	NA	NA

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Roy, *et al.* 2004 [22]	Foodborne Diseases Active Surveillance Network (foodnet) across seven states in US	Age <6m->65 yr. *Cases (positive crytosporidiosis) - 282 ND controls-490	Acid-fast staining, direct fluorescent antibody staining, or commercial ELISA	NA	Risk- international travel OR-7.7 (2.7 to 22.0), contact with cattle (OR = 3.5; 95% CI = 1.8 to 6.8), contact with persons >2 to 11 years of age with diarrhea (OR = 3.0; 95% CI = 1.5 to 6.2), and freshwater swimming (OR = 1.9; 95% CI = 1.049 to 3.5). Eating raw vegetables was protective (OR = 0.5; 95% CI = 0.3 to 0.7).	
Adjei, *et al.* 2004 [23]	Accra, Ghana	Age <5yr 277 with AD and 77 ND controls	The modified Ziehl Neelson staining procedure	Cases-- 27.8% Controls-15.6%	Age- 12-24m. Weight for age deficit >25%. No role of gender, water supply, history of antibiotic therapy or contact with animal	Most common symptom-fever (71-75%), nausea (47% cases)
Khan, *et al.* 2004 [24]	Dhaka, Bangladesh	Age: <5 yr *Cases-46 positive for cryptosporidium, 46 controls with negative growth	Modified Acid-fast staining	Prevalence- 47/1672 (2.8%) in stool samples.	No risk with age, gender, breastfeeding, malnutrition (WAZ, HAZ or WHZ), pulse rate, history of contact with animals, water supply	Watery diarrhea (96%) and vomiting (57%) were most common features. Only 11% dehydrated and 13% had fever. PD occurrence was higher in Cases than controls
Al Braiken, *et al.* 2003 [25]	Jeddah, Saudi Arabia	Age: <5yr Cases-63 Controls-190	Modified Acid-fast staining	Cases-32%, controls-4.7%; P<0.001	No risk with age or gender.	
Nunez, *et al.* 2003 [26]	Havana City, Cuba	Age: Pediatric age Cases-113 Controls- 288	Modified Ziehl-Neelsen techniques	Cases- 13/113 (11.5%) nil in controls	No difference with age or gender.	Those with cryptosporidial diarrhea had longer diarrhea duration & higher risk for PD than those with isolated cyclopspora (p<0.01)

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Tumwine, *et al.* 2003 [27]	Uganda	Age: 0-60m Cases (AD/ PD)- 1779 Controls-667	modified acid fast staining and PCR-RFLP for *C.parvum* only.	Cases- 444 (25.0%) Controls- 57 (8.5%); (r2=80.2, *P* = 0.0001)	Higher if low maternal education. Risk if , wasting (OR 1.59,1.27–1.99), underweight (OR 1.42 1,1.14–1.78), stunting (OR 1.31 1.04–1.64) No difference with gender, or if breastfed
Kirkpatrick, *et al.* 2002 [28]	Port-au-Prince, Haiti. Prospective	Age: <18 months in 3 groups. 17 with cryptosporidial diarrhea, 17 with non-cryptosporidial diarrhea, 15 as healthy controls	Ziehl-Nielsen modified acid-fast Stain and Gram stain,	Cases- 28/60 (46%)	Lack of breast feeding, low HAZ (p=0.03) low WAZ (p=0.01), vitamin A deficiency (p=0.04). No association with low WHZ
Bern, *et al.* 2000 [29]	Guatemela Stool surveillance	Age: 3 months-19 years Cases- 697 Controls- 2709	Modified AFB staining, ultraviolet epifluorescence. Direct IFA with *Cryptosporidium*-specific OW50 monoclonal antibody.	Cases- 7.1% Controls- 5.3% (p-NS)	Young age <2yr; p=0.03). Seasonality-rainy season.
Albert, *et al.* 1999 [30]	Dhaka, Bangladesh	Age <5 yr Cases- 814 with age matched ND controls.	Modified acid fast staining	Cases- 11(1.4%) Controls-3(0.4%) ; p=0.03	Anthropometry comparable between two groups
Cegielski, *et al.* 1999 [31]	Tanzania.	Age: 15-60 months Cases- 55 with AD, 59 with chronic diarrhea, 20 controls	Kinyoun and auramine-rhodamine stains and direct fluorescent monoclonal antibodies	Cases:AD- 7/55 (13%), Chronic diarrhea 5/59 (8%) Controls- Nil	Young age (p0.4 Malnutrition Seropositivity- No relation Environmental hygiene- no relation
Iqbal, *et al.* 1999 [32]	Rawalpindi, Pakistan	Aged <5yr Cases-475 Controls-150	For *C.parvum* only-modified Ziehl-Neelsen stain	Cases- 10.3%; controls-3.3%	Young age: 19–24 months of age (21.8% cases and 10% controls). No association with gender, Sociodemographic Information, drinking water supply, and contact with domestic animals

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Katsumata, *et al.* 1998 [33]	Surbaya, Indonesia- hospital and community based	Age: 0 to ≤60 mo *Hospital based*- Cases- 917 AD Controls-1043 *Community*- Cases- 257 AD Control- 4111	Phase-contrast Microscopy at 6003 magnification followed by Kinyoun staining	*Hospital based*- cases- 2.8% Controls- 1.4% *Community:* Cases-8.2% Controls- 0.7%	Young Age <2 years (OR-0.95, 0.89-0.99), Rainy season (OR-10.65, 1.38-82.17), contact with cats (7.05, 3.61-13.76), overcrowding (OR-1.46,1.19-1.79). No relation with gender, drinking water supply, bathing in public bath	NA
Chacin Bonilla, *et al.* 1997 [34]	Maracaibo, Zulia State, Venezuela.	Age:0-60 mo Cases- 310 Controls-150	Modified Ziehl-Neelsen stain	Cases-11.2% Controls- 6%	No age differences or difference in malnutrition prevalence. No effect of breastfeeding	Duration of AD 5-16 days. Dehydration seen in 91.2% positive cases. Mostly stools were watery
(b) Community-based studies						
Mills, *et al*. 2015 [35]	Multicentric- 8 sites - Dhaka, Bangladesh; Fortaleza, Brazil; Vellore,India; Bhaktapur, Nepal; Loreto, Peru; Naushero Feroze, Pakistan; Venda, South Africa; and Haydom, Tanzania	Age: 17 day till 24 months Prospective 2145 children: 7318 diarrhoeal and 24 310 non-diarrhoeal stools	Enzyme immunoaassay	Attributable fraction - 2.0 (1.3-2.6) in <12 months; highest; and 3.8% (2.8-4.7)in 12-24 months	Maximum isolation of cryptosporidium coincided with peak diarrheal season at each site	
Bodhidatta , *et al.* 2010 [36]	Western Thailand. Prospective	Age-3m-5 year old Cases-236, Controls-236	ELISA based kits	Cases- 4/207 (2%) Controls-12/227 (5%); p-NS	NA	NA

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Samie , *et al.* 2009 [37]	Vhembe district, South Africa Cross sectional	Age 5-15 years. Case- 39 Controls- 256	The modified Ziehl Neelson staining procedure	Cases- 17.9% Controls- 14.4% ; P=0.56	NA	NA
Wongstitwilairoong , *et al.* 2007 [38]	Sangkhlaburi, Thailand. Prospective	Age:3m- 5year 236 cases of diarrhea and 236 ND controls	DMSO-MAFB staining and prospect *Giardia*/*Cryptosporidium* Microplate Assay, an enzyme immunoassay	Detection- 0.8% cases vs 2.5% controls	No gender differences	Only 4 detected by smear and additional 12 by EIA assay
Robertson , *et al.* 2002 [39]	Melbourne and Adelaide, Australia.	Aged 0-18 years *Cases (positive crytosporidiosis) -335 Control (healthy)- 1331	Detection confirmed by accredited Pathology laboratory	NA	Swimming in public pool OR-2.7 (1.9-3.8), contact with <6 yr old with diarrhea OR-7.4 (4.0-13.8), drinking unboiled water OR-3.1 (1.5-6.5), calf contact OR- 5.1 (1.5-17.3)	NA
Peuch , *et al.* 2001 [40]	New South Wales, Australia. Telephonic interview	Age: < 15 years. Cases- 100 Controls-200	NA	NA	Risk if swimming in untreated water 4.8 (1.1-20.3) or public pool 2.7 (1.4-5.1). Protective-bottled water 0.4 (0.2-0.9) No difference with gender, childcare contact, contact with pets.	Abdominal cramps frequent in cases.
Newman , *et al.* 1999 [41]	Fortaleza, Northeastern Brazil Prospective 4 year cohort	Age: Birth till 4 years. Total 1054 stool samples from 189 children	Modified Acid-fast and auramine stains	Cases: AD- 8.4%, PD-16.5%, Control- 4%	Low B.wt, overcrowding. Malnutrition, age or gender- No increased risk	Vomiting, mild fever common. Dehydration rare 15 cases of recurrent cryptosporidium infection.
Nath , *et al.* 1999 [42]	Sunderpur, Varanasi	Age: < 5yr Cases- 607 Controls- 529	Safranine-methylene blue stain.	Cases- 23/607 (3.8%), Controls- 9/529 (1.7%)	Age: 49-60m and 13-24m. No gender risk.	Majority (63.3%) were mucoid stools. None positive for RBC or WBC
Agnew , *et al.* 1998 [43]	Fortaleza, Brazil.	Age: 3-27 months. Total 154: 43 Cases: positive cryptosporidium,	Modified acid-fast and auramine stains	Cases: 43/154 (27.9%) Controls: NA	Young age <1 yr ($p<0.05$). Lower HAZ ($p<0.01$)	Mean duration of cryptosporidial diarrhea 12.3± 2.0 days. Significant lower

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Checkley , *et al.* 1995 [44]	Lima, Peru. Prospective cohort over 2 years.	207 children aged 0-3 months	Light microscopy followed by both acid-fast and monoclonal Antibody fluorescent-labeled stains	Detection rate 45% (94/207): 63% in asymptomatic infection.	Higher risk of asymptomatic infection in 0-5 months old	Weight gain in symptomatic infection - 338 gm less; asymptomatic infection- 154gm less than those without diarrhea and not infected.

IFA- Immunofluorescence Assay, qPCR- real time polymerase chain reaction, PCR-RFLP−Polymerase chain reaction- restriction fragment length polymorphism, ELISA- enzyme-linked immunosorbent assay, HAZ- Height for Age Z score, WAZ- weight for age Z score, AD- Acute diarrhea, ND- Non-diarrhea, PD- Persistent diarrhea.

WEB TABLE II EPIDEMIOLOGY OF CRYPTOSPORIDIUM IN ACUTE DIARRHEA FROM STUDIES WITHOUT NON-DIARRHEAL CONTROLS

Author, year	*Place of study*	*Study population*	*Detection methods*	*Positivity rates*	*Associated risk*	*Clinical associates/ Remarks*
Hospital-based studies						
Mengist, *et al.* 2015 [46]	Ababa, Ethiopia	Age: 1-18 yr. 180 HIV positive- 79 on HAART, 101 non-HAART	Modified Ziehl Neelsen staining	HAART- None Non-HAARt-4/101 (3.9%)	Low CD4 counts <350/mm3; adjusted OR, 95%CI: 13(10.5, 97.6), P<0.01] than in non-HAART group	No opportunistic parasite in those on HAART. *Entamoeba histolytica* was commonest; seen in 10%
Bera , *et al*. 2014 [47]	Delhi, India	Age: < 5 yr. 168 children with AD	Kinyoun method, followed by ELISA	Kinyoun method- 7/168 (4.1%), ELISA 48/168 (28.6%)	No association with breastfeeding, drinking water supply, contact with animals, malnutrition	Dehydration seen only in 4 patients.
Eraky, *et al.* 2014 [48]	Benha, Egypt	Age: 1-14 yr 430 samples	Microscopy followed by PCR	50/430 (11.6%)	Not mentioned	Increased detection of *C. parvum* (82%) than *C. hominis* (12%), mixed (6%)
Charles , *et al.* 2014 [49]	Haiti	Age: <15 yr 3602 children with AD	Luminexxtag GPP polymerase chain reaction in 210 samples for parasites	24/ 210 (11.4%)	No increased risk with seropositivity	HIV positive were at risk for invasive bacterial pathogens
Feng , *et al.* 2012 [50]	Shanghai, China	Age: 1 month–19 yr. 6284 Children	PCR and restriction fragment length Polymorphism analysis	102/6284 (1.6%)	Young age: <6m (8.4%, 95% CI 5.6–11.2) than older (1.9%, 95% CI 1.4–2.4)	
Haider , *et al.*, 2012 [51]	Karachi, Pakistan	Age: 0-19 yrs 339 stool Samples with abdominal pain, Vomiting, constipation, diarrhea and fever.	Kinyoun method	37/339 (10.9%)	More common in summers, no correlation with rainfall.	Cryptosporidium was mono-infection in 26/37. Diarrhea in 54%, abdominal pain in 48%, constipation in 38%, fever 30%, vomiting 28%
Elgun , *et al.*, 2011 [52]	Adana, Turkey	Age: <12 yr HIV positive children	Modified Ziehl–Neelsen staining, ELISA	5.2% by staining, 67.6% with ELISA		ELISA sensitivity-100%, specificity-80.1%

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Moyo , *et al.*, 2011 [53]	Dar es Salaam, Tanzania	Age:<5 years 280 with diarrhea (AD-84%, PD-9.6%, dysentery- 6%)	Immunocard STAT! Rapid Assay- (distinguishes Giardia from C. Parvum)	18.9% (51/280).		
Yu , *et al.* 2011 [54]	Hunan Province, China.	Age: <2yr 140 with AD	Auramine–phenol staining, modified acid-fast staining, and the polymerase chain reaction.	1.4%, (2/140)		PCR detected one additional case than rest two
Ajjampur , *et al.* 2010 [55]	Delhi, Trichy and Vellore, India	Age:<5 yr 2579 with AD	Microscopy, PCR-RFLP, and/or sequencing at the Small-subunit (SSU) rrna and Cpgp40/15 loci for species determination and subgenotyping, respectively	70/ 2,579 (2.7%)	Young Age: 75% children <2yr. Season: Hotter summers and dried winters in Delhi; negative correlation with humidity	*C.hominis* most type with subgenotypes- Ie, Ia, Ib, and Id
Idris , *et al.* 2010 [56]	Jakarta, Indonesia	Age: 6m- 18yr. 42 with HIV/ malignancy/ WHA <70%/ immuno-suppressive drug and PD or recurrent diarrhea	Modified Ziehl–Neelsen staining.	2/42 (4.7%)	Both were HIV positive (low CD4). Higher risk for any protozoal infection in toddler age <3 yr, WHA<70%, unhygienic water supply	Treatment: paromomycin syrup at 10 mg/kg three times a day for ten days.
Areeshi , *et al.*, 2008 [57]	Antananarivo, Madagascar.	Age: 1day-16 yr 215 with AD	Modified Ziehl–Neelsen Method and commercial enzyme immuno-assay (prospect). Confirmed samples were genotyped	12/215 (5.6%)	Young age: <2yr- 10/12 (83%) were in second year of life	*C. hominis* (Gp60 Type I) most frequent. Additional 4 children were false positive with EIA, later found negative with microscopy.
Natividad , *et al.* 2008 [58]	Philippines	Age: <18 yr 2160 stool samples	Merifluor Giardia-Cryptosporidium direct fluorescence kit	Solitary infection- 2.9%, Mixed infection with Giardia- 4.8%	Highest isolation 0-4 yr. No difference with gender. Higher risk in rainy season	
Pelayo , *et al.*, 2008 [59]	Havana, Cuba	Age: 2-8 yr Children with diarrhea(denominator NA)	Modified acid-fast staining, fluorescein-Labelled mixture of Giardia- and Cryptosporidium-specific monoclonal antibody and nuclear stain DAPI followed by PCR	30 children positive.	Higher risk if not BF in 2-5 yr group. Most infected children washed hands and fruits	Most common symptom- anorexia (68%), abdominal pain- 57%, Vomiting was rare (7%). All C. Hominis positive, none for C.parvum.

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Morse , *et al.*, 2007 [60]	Malawi.	Age: < 5 yr 848 stool samples over 22 months	Modified Ziehl–Neelsen(mzn), auramine phenol (AP), immunofluorescence (IF) followed by (PCR–RFLP) of oocyst-extracted DNA using 18S rrna and COWP gene loci.	5·9% (50/848)	Young Age- 85% were <2 yr. Higher rate in rainy season. No role of gender.	*C. hominis* in 48% and *C. parvum* in 18%. N18S rrna confirmed 100% cases.
Gatei , *et al.* 2006 [61]	Microbiological lab data, Kenya	Age: 0m- 5yr 4899 stool samples collected over 2 years	Modified Ziehl–Neelsen staining. Further Genotyping using nested PCR for 18S rrna	4%.	Young age: 13-24 months age (5.2%). PD- High OR (2.19; CI 1.463–3.29). Season- Driest seasons that follow slight rains. No role of gender	66.4% AD, 21% recurrent diarrhea, 9% PD. 51% had abdominal swelling and vomiting. 87% isolates were *C.hominis*
Hamedi , *et al.* 2005 [62]	Bandar Abbas Iran,	Age: 6m-7yr 245 with diarrhea	Modified Ziehl–Neelsen staining.	17/245 (7%) in AD. 6/14 (5.7%) in PD	Young age (<24m), underweight (P=0.01), those with siblings <10 yr age. Breastfeeding protective. No association with source of drinking water, parents' occupation,	Positive isolate- had longer duration of diarrhea (10 vs 3 days)
Dlamini , *et al.* 2005 [63]	Swaziland, Southern Africa	Age:<5 yr 48 with diarrhea	Anti-Cryptosporidium monoclonal antibody (TCS Water Sciences, UK) and stained with 4'6 diamidino-2-phenylindole (DAPI)	2/48 (4.2%)	NA	NA
Lee , *et al.* 2005 [64]	Chungju, Korea	Age: 0-10 yr 286 with diarrhea	Modified Acid-fast staining- positive considered as *C.parvum*	1/286 (0.3%)	NA	NA

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Pereira , *et al.* 2002 [65]	Goiânia, capital of Goiás State in Brazil.	Age: 2wk-10 yr 445 Children with diarrhea	Direct immunofluorescent assay f/b immunomagnetic separation,	64/445 (14.4%) with DFA, 83/445(18.7%) with immunomagnetic separation	Risk- Infancy OR-0.5, 90%CI 0.36–0.68, P<0.0001, day care attendance (OR 2.1; 1.1-3.8), childhood contact with diarrhea (OR 1.9; 1.4-2.7), late rainfall (OR-3.7;1.7-8.3). No risk with gender (OR-2.2, CI=0.13-3.8). No relation with breast-feeding, diet and type of food hygiene, source and type of treatment of drinking water, presence of sewage, and animal exposure	
Torres , *et al.* 2001 [66]	Uruguay	Age: 1 to 20 m. 224with PD (135) or AD (89).	Modified Ziehl-Neelsen (Kinyoun) procedure	19/224 (8.4%) in diarrheal group.	NA	NA
Essers , *et al.* 2000 [67]	Bern, Switzerland	Age:5 weeks- 15 yr. 312 with AD	Use of auramine-carbolfuchsin And visualized by fluorescent microscopy on concentrated stool sample	15/312 (4.8%).	No etiology specific seasonality seen.	Children with cryptosporidial diarrhea were younger than those with bacterial infection
Burgner , *et al.* 1999 [68]	New South Wales, Australia	60 oncology patients with diarrhea (mean age 5.5 yr). 172 non-oncology patients with diarrhea (mean age 4 yr)	Modified Ziehl–Neelsen staining.	Nil in 149 samples of oncology patients vs 23/173 (13.3%) samples from non-oncology patients	NA	No seasonal variation seen. Only *C. Pavum* investigated for
Nath , *et al.* 1999 [69]	Banaras Hindu University, Varanasi	Age: 0-15 yr. Children with AD	M/E- counterstaining with methylene blue	99/1337(7.4%)	Detection higher in rainy season	Absence of fecal leucocytes and erythrocytes in all

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Enriquez , *et al.* 1997 [70]	Mexico city, Mexico	Age: <5yr 403 cases with AD	Modified Acid fast stain, Indirect IFA (OW50)	26/403 (6.4%)	Age below 1 yr. Weight for age deficit >25%-OR-2.9; Ci 1.1-7.5. Breastfeeding protective (9% vs 37%;P<0.01) No relation with gender, antibiotic use, dwelling characteristics, water supply	Majority cases had fever and vomiting No increased detection of seropositivity IFA detected 26; Staining only 20/26 (sensitivity 76.9%, specificity 98.9%)
Brannan , *et al.* 1996 [71]	Romania	Age: 12-52 m 60 HIV infected children and 32 malnourished (WAZ<65%) children	Immunofluorescent assay and trichrome-stained fecal smear. ELISA based IgA & IgG tested in positive subjects	11/60 (18%)in sero-positive vs nil among malnourished	Seropositivity and increasing severity of malnutrition	No increased detection of Giardia in positive cases
Gennari-Cordoso , *et al.* 1996 [72]	Uberlândia, State of Minas Gerais, Brazil	Age 0-12 yr 94 with AD	Safranin/Methylene Blue and the Kinyoun (modified) staining method	4.26%	Older age: 0-2yr=5.08%, 8-10yr =33.3%, Rainy season	Mixed infection in 20%
(b) Community–based studies						
Daniels, *et al*. 2015 [73]	Odisha, India	Age 6m-79 yr 85 stool samples	Immunomagnetic separation and direct immunofluorescence antibody tests	12%	Increased propensity for <2 yr (OR 0.8-17; p=0.09) No relation with gender, rural/ urban.	Geometric mean concentration of oocysts maximum in dog stool samples, followed by sheep, goat, cattle and buffalo. Cattle contributed to 61% of animal population and greatest environmental parasitic load. Water contamination higher in ponds than tube wells (95% CI: 2.9–10.1)
Helmy , *et al.* 2014 [74]	Ismailia province, Egypt	Age: <10 yr 165 stool samples with AD	Enzyme immunoassay (EIA), ICT, PCR	1.8%		Detection – 2.4% with EIA, 6.7% ICT, 49% PCR

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Sarkar, *et al.* 2013 [75]	Vellore, India. Prospective 2 yr	Age: 0-6m. 176: Protected (80)- family consuming bottled drinking water, Unprotected (80)- municipal drinking water supply	Stool microscopy, anti-Gp 15 antibody by ELISA and PCR 13/76 of asymptomatic children positive only by serology	186 episodes in 118 (67%) children.	No difference with type of drinking water. Risk with HAZ<-2 at 6 m age for diarrhea, not sp. Cryptosp. (OR-1.4, 1.03–1.91)	Diarrhea with cryptosporidium lasted longer (4d) & was more severe than non-cryptosp diarrhea.No associated with fever, vomiting 76 (64.4%) had only asymptomatic infection, 12 (10.2%) only diarrhea and 30 (25.4%) had both. *C. hominis* isolated in 80%
Wegayehu, *et al.* 2013 [76]	North Shewa Zone, Oromia Region, Ethiopia	aged 1-14 yr. 384 apparently healthy children	Modified Ziehl-Neelsen staining method	28/384 (7.3%)	Higher in those with close contact with cattle (8.5% vs 6.2; *P*=0.39). No difference with age or gender	
Mondal, *et al.* 2012 [77]	Mirpur, Dhaka, Bangladesh	Age 0-7 days 147 babies till 12m age	Real time PCR	18/420 (4.28%)	Children stunted at birth (r=0.25, *P*=0.057)	
Abu Samra , *et al.* 2012 [78]	Four sites in South Africa (part of rotavirus surveillance)	Age: 0-5 years Total 442 stool samples	Modified Ziehl–Neelsen staining. 80% subjected to molecular analysis (18S PCR and gp60 PCR)	12.2% (54/442);	Young age: 40/54 in infants	*C. hominis* more frequent (76%) than C. Parvum (20%)
Vahedi, *et al.* 2012 [79]	Mazandaran province, Iran	Age: child-adult. 475/962 with AD were <20 yr	Ziehl-Neelsen acid fast stain and Auramin Phenol fluorescence	0.1%	No difference with gender or age	
Vandenberg, *et al.* 2012 [80]	Brussels	Age: not specified 130 children	Microscopy, antigen tests, and real-time PCR	38/122 (31%)		29/38 (76%) symptomatic, 9/38 (24%) asymptomatic 27 symptomatic treated with Paromomycin for 2 weeks. 9 /27 also with nitazoxanide.
Sejdidni, *et al.* 2011 [81]	Albania	6m-16 yr. 321 healthy children	Formalin–ether concentration and Ziehl–Neelsen staining	0.3%	No relation with gender or age	
Siwila, *et al.* 2011 [82]	Kafue district, Zambia Prospective over 12 months	Age:36-72m 100 healthy children- 786 stool samples	Immunofluorescence microscopy	241/786 (30.7%). 86/100 had ≥1 positive episode	No relation with gender or season	Diarrhea frequent- 69/100. 63/69 were positive

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Adamu, *et al.* 2010 [83]	Nine regions of Ethiopia	Age 1-45 years 1034 diarrheal stool samples.	Modified Ziehl-Neelson staining. Gene analysis from positive samples for COWP, SSU-rrna and GP60 gene fragments	79/1034 (7.6%)	No relation with seropositivity. Direct physical contact with calves-35.4% (28/79)	*C. parvum* in 39/41, rest were *C. hominis*
Ajjampur, *et al.* 2010 [84]	Vellore, India. 2 year longitudinal follow up	Age: ≤2 yr. twenty subjects 1036 samples- 196 diarrheal and 840 surveillance samples	SSU RNA PCR based detection	35 episodes of cryptosporidiosis among 20 subjects.	Single vs multiple episodes- EBF as risk (P=0.001) No risk with gender, SES, maternal education, child's age, presence of animal in house	71% (25/35) had diarrhea, 28.6% (10/35) were asymptomatic. Relapses usually asymptomatic 31/35 episodes were due to *C. hominis*. Multiple episodes in 8/20 children (40%).
Mondal, *et al.* 2009 [85]	Mirpur, Urban slum, Dhaka, Bangladesh	Age: 2-5 years. 289 children	Modified Ziehl–Neelsen staining	Incidence episode /100 child year- 7.27 with WAZ >-2 and 12.19 with WAZ<-2	Low WAZ<-2 RR= 1.7 (1.1, 2.6); attributable proportion as 40%. No risk with age, stunting, drinking water source	
Cama, *et al.* 2008 [86]	Pampas de San Juan de Mirafl ores, Lima. 4-year longitudinal	Age: birth cohort. 368/533 children included	Modified Ziehl–Neelsen staining. Followed by genotyping with Gp 60	109/533(20.4%) children		Duration longer with *C. hominis* (10.3d) vs rest (5.8d)*. Diarrhea (in all) Rest- malaise, abdominal pain, vomiting with *C.parvum* only. None had fever or dysentery *C. hominis* in 70% f/b *C. Parvum* in 31%.
Chacín-Bonilla, *et al.* 2008 [87]	San Carlos island, Venezuela	Age: 1month-15 yr). 248 subjects *Also included adults till 86 yr*	Modified Ziehl-Neelsencarbolfuchsin staining	60/248 (24.2%).	Risk factors- extreme poverty, living in a hut or small residence, lack of sanitary latrine, contact with contaminated soil, overcrowding	
Kirkpatrick, *et al.* 2008 [88]	Dhaka, Bangladesh Prospective over 3 years	Age: 2-5 years. 226 Children-	Cryptosporidium antigen-detection kit (Techlab)	96/226 (42.4%)	No relation with age	Predisposition with HLA B*15 (OR 2.16; *P*=.04), DQB1*0301 allele (OR, 2.75; *P* =.005

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Abreu-Acosta, *et al.* 2007 [89]	Santa Cruz de Tenerife, Canary islands, Spain	Stool surveillance for 0-60 year. Only 9 patients had age record as <12 yr	Modified Ziehl-Neelsen stain and PCR	Cases: 4/9 (44.4%)	Isolation higher in immunocompromised (18.2%) than immunocompetent (4.6%); P<0.05. No relation of age or gender.	Only 9 subjects had recorded age <12 yr
Carvalho-Almeida, *et al.* 2006 [90]	São Paulo City, Brazil	Age: 4-72 m. 64 children- Stool samples after 10-35 days of occurrence of index case	Kinyoun Method, a modified Ziehl-Neelsen technique	13/64 (20.3%).	Young Age<1yr-53.8% (7/13). No association with drinking water source or contact with pets	Symptoms in those tested positive-diarrhea, low fever, vomiting.
Bentley, *et al.* 2004 [91]	Highlands of Central Guatemala,	Age: 2-13 yr with diarrhea or abdominal pain	Kinyoun's modified acid-fast stain2	14% with diarrhea and abdominal pain, 21% with diarrhea& 29% with abdominal pain	Infection not affected by age	
Laubach, *et al.* 2004 [92]	Lake Atitlan, Guatemala	Age: 2-13 yr. 100 children with AD and abdominal pain	Kinyoun's modified Acid-fast stain	32%	No risk with seasonality. Males aged 2-5 yr lower prevalence.	Postulated-Higher prevalence due to contact with lake
Valentiner-Branth, *et al.* 2003 [93]	Bandim II and Belem of Bissau, the capital of Guinea-Bissau.	Age: 0-3 weeks. 200 children- Prospective till 2 yr.	Modified Ziehl-Neelsen technique	Incidence- 0.33%.	Higher in boys (OR, 5.35; 95% CI, 2.15 to 13.3, P=0.01). No relation with breastfeeding	Pathogenecity of *C. parvum* high (OR, 4.53; 95% CI, 2.75 to 7.46)
Miller, *et al.* 2003 [94]	Trujillo, Venezuela	Age: 4m-5 yr. 45 children	Kinyoun acid-fast stain	33/45 (73%)		Possible that their investigation coincided with an outbreak in this community
Mederios , *et al.* 2001 [95]	RibeirãoPreto, Brazil	Age: <10 yr. 1836 children with AD, Prospective 4 yr study	Modified Kinyoun method	34/1836 (1.8%)	Young age <2yr-88.2%	
Perch, *et al.* 2001 [96]	Guinea-Bissau, West Africa	Age: <5yr. 2681 children over 7 yr period	Modified Ziehl–Neelsen technique.	351/2681 (13.1%).	Young age: 6-11m. No relation with gender (RR-1.02 (0.84–1.25). risk in rainy season	Abstract only

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MØlbak, *et al.* 1997 [97]	Bandim II, Guinea-Bissau, West Africa.	Age: <5yr. 1064 children from 301 households. 3y prospective diarrhea surveillance.	Modified Ziehl–Neelsen staining.	236/1064 (22%)	Young age <1yr-108/236. No association with baseline weight, height.	Those with infection had subsequent poor weight gain
Checkley, *et al.* 1995 [44]	Lima, Peru.	Aged 0-3 months. 207 children. Prospective cohort over 2 years.	Light microscopy followed by both acid-fast and monoclonal Antibody fluorescent-labeled stains	94/207 (45%).	Age: 18-23 months old (RR-5.71; 1.43-22.81); HAZ<-2SD higher OR 1.52 (0.82-2.82). No relation to weight	Symptomatic cryptosporidiosis had less weight gain- 0-5months= 427gm (34-888), 6-12 months=208 gm (134-549)

HAART- Highly active antiretroviral therapy, RFLP- restriction fragment length polymorphism, PCR- Polymerase chain reaction, ICT- Immunochromatographic test. : IFA- ImmunofluorescenceAssay, HAZ- Height for Age Z score, WAZ- weight for age Z score, AD- Acute diarrhea, ND- Non-Diarrhea, PD- Persistent diarrhea.

WEBTABLE III STUDIES REPORTING ON ISOLATION OF CRYPTOSPORIDIUM IN CHILDREN (<18 YEAR) WITH PERSISTENT DIARRHEA

Study, year [Ref]	*Objective*	*Study population*	*Place of study*	*Detection method*	*Results*	*Comments*
Saneian, *et al*. 2010 [104]	To estimate the prevalence of enteric cryptosporidiosis in children 1m-10 years presenting with diarrhoea	606 children (mean age 42.4±30.0 months, 58.1% females)	Three university hospitals of Iran	Modified acid-fast method	PD in 184 (30.4%) of children. Prevalence of *Cryptosporidium* infection higher in PD, than AD (12.5% *vs* 1.2%; *P*<0.001). no difference in age/ gender of infected and non-infected children	Hospital-based study. May reflect referral bias as very high proportion had PD. Only AFB staining used; immunological and molecular methods not used.
Moore, *et al.* 2010 [105]	10-year cohort study related to prolonged episodes of acute diarrhoea (ProD; duration 7-13 days) PD	414 (193 males, 47%) children were followed from birth	In a 5-block area of a shantytown, Brazil	Microscopy	*Cryptosporidium* isolated more frequently from ProD and PD than control specimens (*P*=0.017 and *P*=0.022, respectively). *Cryptosporidium* was seen more frequently in ProD than AD specimens (*P*=0.023).	Cryptosporidium associated with ProD and PD
Mukhopadhyay, *et al*. 2007 [19]	Cross sectional study from 1998-2004	253 PD cases, 155 AD and 100 healthy controls	Hospital based study in Western Nepal	Microscopy	Giardia most common- (61/90-67%), *Cryptosporidium* in (2/90-0.2%)-one had HIV other had malnutrition.	Infection higher during monsoons. Breastfeeding protective against protozoal infections
Tumwine, *et al.* 2005 [102]	A cross-sectional study on the clinical epidemiology of *E. bieneusi* and *Cryptosporidium* in children with PD, with and without HIV/AIDS	Two hundred forty-three children,145 (59%) males, age range of 1–60months with PD	Uganda's Mulago National Referral Hospital	Immunofluorescence microscopy & confirmation with PCR	*Cryptosporidium* detected in 76/243 (31.3%) with PD. Risk higher with HIV- Positive infection in 67/91 (73.6%) with HIV compared with only 9/152 (5.9%) without HIV (OR : 44.36; 95% CI 18.39 to 110.40; *P* < 0.0001).	*Cryptosporidium* more common in HIV-associated PD.
Abdel-Messih, *et al.* 2005 [106]	The prevalence and clinical characteristics of Cryptosporidium-associated diarrhoea in the Nile River Delta of Egypt was	1275 children evaluated	Benha Fever HOSPITAL and Abu Homan District Hospital, Egypt	Commercial ELISA	Risk for *cryptosporidium*: Age <12 months of age- 2.4 times more likely(p<0.01), age 12 to 23 months 1.9 (p<0.05) times more likely, lack of breastfeeding [Breastfeeding protective (p=0.07)]. *Cryptosporidium* diarrhea associated with vomiting, severe PD and the need for hospitalization.	No other organism studied. No controls. The proportionate contribution of *Cryptosporidium* can not be determined.

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Khan, *et al.* 2004 [24]	To investigate the epidemiology, clinical features, and systemic antibody responses of cryptosporidiosis in Bangladeshi children	92 under-five children (46 *Cryptosporidium*-infected patients as cases while 46 age-matched controls with diarrhoea, but without Cryptosporidial infection)	ICDDR, Dhaka, Bangladesh	Modified acid-fast method	*Cryptosporidium* diarrhea commoner in less than two years of age, was accompanied by watery diarrhea, vomiting, and associated with PD. Other than duration of diarrhoea, there were no significant differences in clinical or epidemiologic features between cases and controls.	No other organism studied. The proportionate contribution of Cryptosporidium can not be determined
Shoaib, *et al*. 2003 [107]	To show the importance of modified acid fast stain in the diagnosis of *Cryptosporidium* in childhood diarrhoea.	300 children presenting with ProD/ PD. Stool samples from children aged 1-14 years with chronic diarrhoea in cross sectional manner.	Karachi, Pakistan	Modified acid-fast method	Five (1.7%) were found to be positive for Cryptosporidiosis. Only one case positive for *Cryptosporidium* was also positive for Giardia cyst showing mixed infection.	No controls. Only AFB staining used. Analyses of stool samples sent for analysis; true prevalence can not be commented on.
Amadi, *et al*. 2001 [108]	To analyze impact of HIV infection on infectious disease, clinical presentation, and mortality in Zambian children with PD and malnutrition.	Two hundred children (94 boys and 106 girls, 6-24 months old) with PD ($\geq$ 2 weeks) and malnutrition (<80% WAZ)	Nutrition ward of the Departmentof Paediatrics at University Teaching Hospital in Lusaka, Zambia	Modified Ziehl-Neelsen staining	*Cryptosporidium parvum* seen in 28% children who were HIV-seropositive vs 18% in HIV-seronegative children (P-NS). Cryptosporidiosis was significantly associated with mortality.	Study suggests that etiological profile not different in HIV-positive and HIV-negative children with PD
Alam, *et al.* 2001 [109]	To differentiate (a) non-severe PD (with no or mild dehydration) and (b) severe PD (with moderate or severe dehydration), and to identify individual characteristics associated with severe PD.	295 under-five children with PD(duration >14 days).	ICDDR, Bangladesh	Microscopy	*Cryptosporidium was* significantly associated with severe PD (with moderate or severe dehydration) ($p<0.05$).	
Lima, *et al.* 2000 [110]	To elucidate the epidemiology, nutritional impact, and causes of PD over 45 month period	189 children aged 0-3 years	Shantytown Northeastern Brazil	Modified acid fast staining	0.42 episodes of PD/child/year. *Cryptosporidium* in PD: cases- 23.9% (13/71), controls- 5.2% (15/289);*P*=0.002. PD resulted in decrease in WAZ and WHZ	Risk factors for PD-age:13-24 m, no toilet at home.

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Bhandari, *et al.* 1999 [101]	To determine whether there is an association between *Giardia lamblia, Entamoe bahistolytica* or *Cryptosporidium* infection and PD.	175 children aged 0-36 months suffering from PD	Slums of Northern India	Modified acid fast staining-Kinyoun method	The study didn't find any association of Entamoeba histolytica or *Cryptosporidium* with PD	
Sodemann, *et al.* 1999 [111]	To identify episode-specific risk factors for PD	319 episodes of childhood diarrhea	Suburban area of Guinnea-Bissau, West Africa		Cryptosporidiosis was an independent risk factor for the development of PD- (OR = 5.53 (2.10-14.6)	
Newman, *et al.* 1999 [41]	To elucidate the epidemiology of *Cryptosporidium* infection in an endemic setting,	1476 episodes of diarrhea, accounting for 7581 days of illness (5.25 episodes/child-year), were recorded	Urban slum community of Ceara´ in Fortaleza, Brazil.	Acid fast and auramine staining	*Cryptosporidium* prevalence- Total- 7.4% , PD-16.5%, AD-8.4%-, no diarrhea- 4.0% (P<.001). Disease course was highly variable and was not associated with the presence of co-pathogens.	
Cantalice, *et al.* 1998 [112]	To verify the incidence of *Cryptosporidium* in children with PD	70 hospitalized children HIV-negative, younger than 2 years, with PD	Data not found	Not available	*Cryptosporidium* prevalence-20%. More common in younger than 3 months (13/57, 23%). Malnutrition was present in 91.5% of patients, irrespective of cryptococcal status	Article in Portuguese Details of diagnostic methods not available
Fraser, *et al.* 1998 [113]	To examine the role of enteric pathogens and household characteristics in PD	251 infants	In an urban settlement of Bedouin tribes in Israel	Modified acid-fast staining & confirmed by immunofluorescent assays	None of the enteric pathogens examined, including *Cryptosporidium* parvum and enteroaggregative *Escherichia coli*, were associated with PD	
Mølbak K, 1997 [97]	To determine the interaction between nutritional status and cryptosporidiosis	an open cohort of 1064 children younger than 3 y of age	Guinea-Bissau, west Africa	Modified acid-fast staining	Cryptosporidiosis in infancy was accompanied by an estimated weight loss of 392 g (95% CI: 247, 538 g) in boys and 294 g (95% CI: 109, 479 g) in girls, corresponding to 3.7% and 2.9% of mean weight, respectively, at 2 y of age. No significant catch-up growth in weight or height (P=0.02). No tendencies of low weight (P=0.38) or height (P = 0.16) in children who acquired cryptosporidiosis.	The present study suggests that cryptosporidiosis in infancy has a permanent effect on growth.
Koopmans, *et al.* 1997 [114]	To study the etiologic role of toroviruses as a cause of gastroenteritis	Children <18 mo of age; 33 with AD, 41 with PD and 17 controls	Urban slum, Brazil		Presence of *Cryptosporidium* oocysts was commoner in PD although not significant (*P* = 0.08).	

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Newman RD, 1994 [103]	To examine the transmission of *Cryptosporidium* infection in households with an identified person with cryptosporidiosis.	Thirty-one households with a child <3 years age (index case) who was positive for *Cryptosporidium* parvum using acid-fast and auramine-stained stool smears.	An urban slum in Fortaleza, Brazil.	Acid fast and auramine staining	Forty-five percent of index cases of Cryptosporidium infection were associated with PD. Secondary cases seen in 18 (58%) of 31 households involving 30 persons, yielding an overall transmission rate of 19%.	*Cryptosporidium* parvum is highly transmissible and infective in the family setting
Jirapinyo P,1993 [115]	A prospective study was done to identify *Cryptosporidium* in the stools of young children admitted to hospital	387 stool samples from 387 individuals---young children, aged 2 months to 3 years,	Data not found		Prevalence of *Cryptosporidium* significantly higher with prolonged diarrhea than acute diarrhea or No diarrhea ($p < 0.05$).	
Mølbak K, 1993 [116]	To investigate the epidemiology of and mortality from cryptosporidiosis in young children in Guinea Bissau, West Africa.	1315 children aged less than 4 years	301 randomly selected houses in a semi-urban area in the capital, Bissau Guinea Bissau, West Africa.	Modified Acid fast staining	Prevalence of *Cryptosporidium*: overall- 7.4% (239/3215), in PD- 15% (77/513) and in AD-6.1% (148/2428);($p < 0.0001$). Risks- younger children (median age 12 months) and beginning of the rainy seasons. Mortality due to Cryptosporidiosis more in infancy (relative mortality 2.9 (95% CI 1.7 to 4.9)). The excess mortality could not be explained by malnutrition, or by socioeconomic factors, hygienic conditions, or breast feeding.	
Lima AA,1992 [117]	To report results of stool analyses and duodenal aspirates in persistent diarrhea	37 children presenting with persistent diarrhea	Northeast Brazil	Modified acid fast staining	*Cryptosporidium* seen in 13% PD.	

AD- Acute diarrhea, ND- Non-diarrhea, PD- Persistent diarrhea, ProD- prolonged diarrhea, AFB- Acid fast staining, HIV- Human immunodeficiency virus.