Evolving Patterns of Cryptosporidiosis: Issues and Implications in the Context of Public Health in India

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Abstract

Cryptosporidiosis is one of the major causes of diarrhea in immune-compromised individuals and children besides causing sporadic water-borne, food-borne, and zoonotic outbreaks. In 2016, *Cryptosporidium* species infection was the fifth leading cause of diarrhea and acute infection causing more than 4.2 million disability-adjusted life years lost besides a decrease in childhood growth. Human cryptosporidiosis is primarily caused by two species/genotype: *Cryptosporidium hominis* (anthroponotic) and *Cryptosporidium parvum* (zoonotic) besides other six rare species/genotypes. Transmission intensity, genetic diversity, and occurrence of genetic recombination have shaped the genus *Cryptosporidium* population structures into palmitic, clonal, and epidemic. Genetic recombination is more in *C. parvum* compared with *C. hominis*. Furthermore, parasite–host co-evolution, host adaptation, and geographic segregation have led to the formation of "subtype- families." Host-adapted subtype-families have distinct geographical distribution and host preferences. Genetic exchanges between subtypes played an important role throughout the evolution of the genus leading to "adaptation introgression" that led to emergence of virulent and hyper-transmissible subtypes. The population structure of *C. hominis* in India appears to be more complex where both transmission intensity and genetic diversity are much higher. Further, study based on "molecular strain surveillance" has resulted newer insights into the epidemiology and transmission of cryptosporidiosis in India. The identification at the species and genotype levels is essential for the assessment of infection sources in humans and the public health potential of the parasite at large. The results of the study over three decades on cryptosporidiosis in India, in the absence of a national surveillance data, were analyzed highlighting current situation on epidemiology, genetic diversity, and distribution particularly among vulnerable population. Despite creditable efforts, there are still many areas need to be explored; therefore, the intent of this article is to facilitate future research approaches for mitigating the burden associated with this disease.

Keywords
- *Cryptosporidium* species
- subtyping
- epidemiology
- molecular prospecting
- India

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Introduction

Cryptosporidium species, a protozoan parasite, infects the epithelial cells of the intestinal tract of humans and a variety of animals worldwide and one of the major causes of diarrheal disease in humans globally. *Cryptosporidium* species, which belongs to the phylum *Apicomplexa,* was first described as a cell-associated organism in the gastric mucosa of mice in 1907 by E.E. Tyzzer.[1] The genus *Cryptosporidium* was named so because of the absence of sporocysts within the oocysts, a unique difference from other coccidian parasites. This genus did not receive much attention for almost 70 years, till two simultaneous reports of human cryptosporidiosis were published in 1976, one in an apparently healthy individual and other in an immune-compromised person.[2,3] These two reports also set the tune of uniqueness of *Cryptosporidium* species that it can infect both immune-competent and immune-compromised. With the emergence of human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) pandemic, the incidence of cryptosporidiosis was increasingly recognized as the cause of severe life-threatening diarrhea in children as well as in immune-compromised individuals. In humans, immune-competent individuals usually experience self-limiting diarrhea. The self-limiting disease is often manifested by acute profuse watery diarrhea accompanied by abdominal pain and other enteric symptoms including vomiting, low grade fever, general malaise, weakness, fatigue, loss of appetite, nausea, chills, and sweats.[4] In contrast, immune-compromised individuals, particularly those with HIV infection and other immunodeficiency disorders, often suffer from intractable diarrhea. Immune-compromised individuals predisposed to cryptosporidiosis includes HIV seropositive individuals with decreased CD4+ T cell count, patients with malignant disorders undergoing chemotherapy, T cell deficiency disorders like severe combined immunodeficiency, and hyper-immunoglobulin M syndrome, transplant recipients.[5] Diarrhea tends to be much more severe in hosts having defects in either cell-mediated or humoral immunity. In a study of HIV patients, fulminant cryptosporidiosis occurred with CD4+ T cell count less than 50 cells/μL.[6] Extraintestinal cryptosporidiosis has also been reported causing pancreatitis, sclerosing cholangitis, cirrhosis, cholangiocarcinoma, and respiratory involvement.[7] Of late, *Cryptosporidium* species were recognized as one of the important cause of diarrheal illness in children younger than 5 years of age with peak occurrence in children below 2 years of age.[8] Nosocomial infection by direct and indirect person-to-person transmission has also been well documented, causing secondary cases among roommates and family members.[9] Animal-to-person (zoonotic) transmission primarily occurs among veterinarians and veterinary students as well as other people exposed to live stocks. Preweaned calves are considered to be an important source of zoonotic cryptosporidiosis.[10]

Cryptosporidium species has a complex asexual (merogony) and sexual life cycle and the lifecycle is completed within the epithelial cells of small intestine and colon of the infected hosts with the developing stages present on luminal surface. Asexual and sexual reproduction of the parasite occurs within the extra-cytoplasmic vacuole. This results in production of merozoite that eventually infect the adjacent epithelial cells leading to generation of both thin and thick-walled sporulated oocysts containing sporozoites. Sporozoites released from them infect the epithelial cells. Thick-walled oocysts excreted from infected individual are the infective stage and can survive for many months in temperate and moist conditions, and are resistant to many common disinfectants, particularly chlorine-based disinfectants.[11] Transmission occurs principally through the fecal–oral route, following direct or indirect contact with *Cryptosporidium* oocysts. The oocyst is responsible for the dissemination of the infection. It is of small size (4–8 μm); size may vary with different species and is environmentally robust.[12] A single oocyst is sufficient to produce infection and disease in susceptible hosts.[13] The infective dose is variable for different strains. The infective dose is 9 oocysts for TAMU, 87 for Iowa, and 1,042 for UCP strains.[14] Infected hosts act as reservoirs, allowing the propagation of the infection. It is now recognized that the infectious dose as well as the duration and severity of illness are influenced by both etiological factors that include the species and strain of the parasite, as well as various host factors (age and immune status). Depending on the parasite species and the host's immune competency, the prepatent period (time between infection and active oocyst shedding) is between 1 and 3 weeks, whereas the patent period (duration of oocysts shedding) can range from several days to months or years[15] and such long patency can lead to persistent infection and diarrhea. There are more than 38 known and few unnamed *Cryptosporidium* species, of which *Cryptosporidium hominis* (*C. hominis*) and *Cryptosporidium parvum* (*C. parvum*) are the two most common species that are responsible for nearly 90% of human infections. *C. hominis* is restricted to humans, whereas *C. parvum* can infect other mammals. Other six (6) species that have been associated with human infections include *C. meleagridis*, *C. felis*, *C. canis*, *C. viatorum, C. muris, C. suis, C. andersoni*, and *C. corvine.*[16] *C. hominis* and *C. parvum* differ in their host range, genotype, and pathogenicity. The burden of disease from cryptosporidiosis varies substantially between and within countries/areas. Some of the accumulating data have suggested that there can be variations in the clinical manifestations of cryptosporidiosis according to the infecting *Cryptosporidium* species/genotype or subtypes.[17,18] Three major clinical presentations in immune-competent individuals include asymptomatic carriage, acute diarrhea, and persistent diarrhea. The incubation period may vary from 3 to 14 days and the illness is usually self-limiting.[19] Other symptoms include malaise, fatigue, anorexia, pyrexia, abdominal pain, nausea, vomiting, and weightloss.[20] Oocysts may continue to be shed in the feces following cessation of diarrhea for 7 days (range: 1–15 days). The clinical presentation of cryptosporidiosis leads to severe diarrhea in immune-compromised individuals.

Tinctorial stains such as auramine-phenol, safranin, and modified acid-fast staining methods are commonly used to detect *Cryptosporidium* oocysts in fecal specimens. However,

these staining techniques, although cost-effective and specific, have been reported to be relatively less sensitive compared with immunoassays and polymerase chain reaction based assays.[21] With the limited availability of the treatment options, control of the disease relies mainly on the knowledge of the biology and transmission of the different species of *Cryptosporidium*.[11]

Since the first report of human cryptosporidiosis in India,[22] human cases have continued to increase underpinning many newer aspects and insights with promising progress. The present analyses describe the trend of laboratory confirmed human cryptosporidiosis in India over a period of more than three decades and focuses on source of infection, mode of transmission, risk factors, and distribution of the disease and genetic characteristics of *Cryptosporidium* species in different parts of the country.

Materials and Methods

There has been a significant addition of literature to the incidence and prevalence of human cryptosporidiosis in India. Laboratory confirmation using microscopy or by molecular methods was used to meet the case definition for cryptosporidiosis. A systematic search about all relevant published abstracts and full texts published in English language on human cryptosporidiosis before January 2020 was conducted and all research articles were analyzed. All the selected research articles were classified under following contents such as (a) number of publications per year, (b) state-specific studies, and (c) different aspects of cryptosporidiosis. Various demographics such as age, sex, clinical manifestations (diarrhea, weight loss, etc.), laboratory diagnosis and genotype(s), and subtype(s) association were studied in detail.

Results and Discussion

Based on the search strategy, nearly hundred published reports from different parts of the country addressing relevant information associated with *Cryptosporidium* infections in India were included. Based on number of publications per year, annual publication frequency was determined. From 1990–2001 to 2010, publication was higher mostly reporting about prevalence and thereafter some thematic papers with particular concepts along with study on molecular aspects brought the new knowledge and insight about cryptosporidiosis.

Human Cryptosporidiosis

Cryptosporidium's unusual location within the host cell, sequestered between the cell cytoplasm and cell membrane, its ability to autoinfect through "thin walled" oocysts within the intestine, its innate antimicrobial resistance, and general lack of host specificity (especially in *C. parvum*) are some of the unique features that distinguish it from other enteric protozoa.

Prevalence of Cryptosporidiosis

Prevalence rate of *Cryptosporidium* infection ranges from less than 1% to more than 30% worldwide. The prevalence of *Cryptosporidium* species in humans is reported to be 0.3 to 4.3% in North America, 0.1 to 14.1% in Europe, 1.3 to 13.1% in Asian countries, 2.6 to 21.3% in African countries, 3.2 to 31.5% in central and South American countries.[23] Studies have shown that cryptosporidiosis is more common in developing countries (5% to >10%) than in developed countries(<1–3%).[11,24] According to the US surveillance of cryptosporidiosis (2009–2010), the rate of reported cases was 2.5 and 2.9% per 100,000 populations in 2009 and 2010 respectively.[25] In England and Wales, an average of 4500 laboratory identified cases were reported each year since 1998 to 2008.[26] The reported prevalence of *C. hominis* is more than *C. parvum* in the developing world including India,[27,28] whereas *C. parvum* has been reported to be the prevalent species in western countries and also in many of the middle eastern countries including Kuwait (94%), Iran (74 and 75%), and Jordan (50%).[29] In India, variable prevalence was observed in different studies and in different populations, such as HIV-positive and negative adult patients with diarrhea, children under 5 years of age with acute and persistent diarrhea, and other groups such as in cancer patients and transplant recipients.

Geographical and Seasonal Distribution

Epidemiologic surveys indicate that human cryptosporidiosis is distributed worldwide both in urban and rural populations.[30] Geographic differences that exist in the disease burden are attributable to distribution of *C. hominis* and *C. parvum*. *C. hominis* has been shown to be more prevalent in North and South America, Australia, Japan, Africa, and other developing countries,[27] suggesting that the anthroponotic transmission is of greatest importance in these regions. However, *C. parvum* is the predominant species in United Kingdom, Northern Ireland, France, Switzerland, Portugal, Slovenia, Czech Republic, and New Zealand.[10] The seasonal differences in the distribution of cryptosporidiosis have been described from various regions of the world that also vary with geographical locations. It has been observed that *C. hominis* is highly prevalent in autumn in the United Kingdom and New Zealand, whereas *C. parvum* is more prevalent during spring in Canada, Ireland, and the Netherlands. This is believed to be related to increased exposure to animal oocysts following the calving and lambing season for *C. parvum*, as well as to increased travel, exposure to water, and attendance at day care centers for *C. hominis*.[10] In China, it has been observed that increase in temperature and precipitation was considered to be associated with an increase in the incidence of cryptosporidiosis. It was also observed that in moist tropical climates, precipitation was a strong seasonal driver for cryptosporidiosis; in contrast, in temperate climates, the incidence of cryptosporidiosis peaked with the increase in temperature.[31] In India, the incidence of cryptosporidiosis among children residing in the more temperate northern parts of India correlated positively with temperature and negatively with humidity, but correlations were not observed for children residing in the more tropical southern region.[18] Effect of climate and meteorology on cryptosporidiosis was studied by Jagai et al,[31] who developed

a meta-analysis framework to assess the link between environmental exposure to protozoa via drinking and recreational water and cryptosporidiosis infection. The study observed a marked increase in cryptosporidiosis infection during warm and rainy seasons. Regardless of above report, the seasonal patterns and distributions are incomplete and remain to be studied extensively to explore transmission dynamics of this disease.

Cryptosporidiosis in Vulnerable Groups

1. Cryptosporidiosis in HIV-infected adults

Cryptosporidiosis is one of common opportunistic infections among immune-compromised individuals. The prevalence of cryptosporidiosis in HIV-infected patients with diarrhea has been reported to differ in range depending on the population studied, degree of immune-suppression, and use of antiretroviral therapy (ART). Reports on the prevalence of cryptosporidial diarrhea in HIV infected adults from different parts of India from the mid-1990s have ranged from 0.7 to 83% in symptomatic and from 1.4 to 57% in asymptomatic individuals.[32,33] In a study conducted between March 2002 and March 2007 at National AIDS Research Institute, Pune, India, by Kulkarni et al,[34] intestinal parasites were detected in 35% of the HIV infected patients, of which 12% (16 of 137) were infected with *Cryptosporidium* species. Vignesh et al[35] reported an overall prevalence of 2.9% of *Cryptosporidium* in HIV-infected patients between 2003 and 2006 from Southern India. The prevalence percentage in HIV infected patients from other parts of India is 20% (9/45) from Kashmir,[36] 19% (19/100) from Ahmedabad,[37] 17.2 (7/43) and 20.6% (7/34) from New Delhi,[38,39] 25% (28/111) from Vellore,[28] 28.7% (23/80) from Madurai,[40] 9.64% (32/332) from Maharashtra,[41] and 39.8% (146/366) from Varanasi.[42] In some of North Eastern states, the prevalence of *Cryptosporidium* in HIV-infected people was reported to be higher than 70.0%,[43] though the study had included very small number of patients. The results of many of these studies have indicated that HIV-infected individuals had a low CD4+ cell count in almost 50% of cases. The species *C. hominis* and *C. parvum* were primarily responsible for cryptosporidiosis in HIV-infected patients in our country. Others species that have been confirmed as human pathogens in HIV patients in India are *C. meleagridis*[28,44] and *C. viatorum*.[45] A comparatively large proportion of individuals infected with *C. meleagridis* have been observed in Haiti[46] and Peru.[47] Some of the relatively recent studies on HIV infected patients also showed similar trend with reference to cryptosporidiosis.[48,49] Asymptomatic cryptosporidial infection in HIV population is under-appreciated and in few HIV-infected cases extraintestinal manifestations like biliary, pulmonary, and middle ear involvement have been described in AIDS patients.[50] Introduction of the highly active antiretroviral therapy (HAART) has had a remarkable impact both on reduction in occurrence and clinical course of cryptosporidiosis. In a multinational cohort study of HIV-positive individuals from Australia and 10 European countries, conducted during 1997 to 2001 and 1994 to 1996, showed significant HAART-induced decrease in progression to cryptosporidiosis (3.1 to 0.2%).[51] To some extent, similar observations have also been noted in our country.

2. Cryptosporidiosis in Children

Cryptosporidium species have been recognized as one of the leading causes of moderate-to-severe diarrhea particularly in children younger than 5 years of age.[52] Initial studies before 1990 from the country reported increased detection of *cryptosporidium* from Children.[22,53-56] These studies were based on staining techniques results. The very first study from Vellore[22] showed parasite *Cryptosporidium* in 13.1% children under 6 months of age with acute diarrhea and in 9.8% age-matched controls. The study is of significance for the fact that symptomless individuals may get colonized by *Cryptosporidium* species. Around the same period, study from Calcutta (Present Kolkata), *Cryptosporidium* was detected as the only pathogen in 18 (4.47%, 18/402) children below 9 years of age and diseases were self-limiting.[53] Subsequently many case reports and series were reported from nearly all parts of the country.[55,57,58] Stratifying the children as HIV seropositive with and without diarrhea, the reported prevalence is 20 and 14%, respectively.[38] Hospital and community-based studies have reported *Cryptosporidium* as the cause of pediatric diarrhea, in 1.3 to 18.9%. A prevalence rate of 67% has been reported by Sarkar et al in children belonging to a semiurban community in southern India[59] and 27% by enzyme-linked immunosorbent assay in a study conducted in Delhi.[60] In developing countries, prevalence of *Cryptosporidium* in diarrheal children under 5 years reflect both exposure and immunity. Mortality in children under 5 years of age in developing countries due to cryptosporidiosis is 30 to 50%.[61]

Besides *C. hominis, C. felis* and *C. parvum* (mouse genotype) have been reported from India.[28] *C. meleagridis*1/35 (2.9%) and *C. muris*1/35 (2.9%) have also been detected in Saudi Arabia.[62] Differences in the distribution of *Cryptosporidium* genotypes in humans are considered as an indication of differences in infection sources. Detection of *C. parvum* in some populations has been considered to be the result of zoonotic transmission but recent subtyping studies have shown that not all *C. parvum* infections in humans are the result of zoonotic transmission. Among the *C. parvum* GP60 subtype families identified, alleles IIa and IIc are the two most common types. The former has been identified in both humans and ruminants, whereas the latter has been seen only inhumans.[63] Asymptomatic cryptosporidiosis in children in India has been reported within a range of 0 and 9.8%. These findings emphasize the lack of correlation between disease severity or symptoms and *Cryptosporidium* infection that may persist beyond the clinical illness. Community-based studies in India are lacking, and differences between rural and urban population have not been studied intensively and extensively except two studies.[57,59] Generally, people living in rural areas are expected to have higher prevalence of *Cryptosporidium* related to poor sanitation conditions in most of rural areas, a lack of necessary general health knowledge, and health habits of people.

In contrast to many studies related to childhood cryptosporidiosis, persistent diarrhea due to cryptosporidiosis and malnutrition have been increasingly reported from Africa.[64] The exact degree to which malnutrition and diarrhea are associated with cryptosporidiosis is not known;

however, they are assumed to have a complex bidirectionalrelationship.[65] Notably, a correlation between early childhood cryptosporidiosis and growth reduction or even failure has been identified particularly in children younger than 12 months, in addition to long-term cognitive deficits and impaired physical fitness later in life.[66,67] Only study from India has shown association between malabsorption and coccidian parasites.[68]

Cryptosporidiosis in Transplant Recipients

Reports of cryptosporidiosis prevalence varied widely depending on the non-HIV population examined. Enteric infections in 65 patients undergoing bone marrow transplantations (BMT) with diarrhea were studied.[69] Enteric pathogens were found in 60% of patients before or after transplantations. *Cryptosporidium* species did contribute significantly to morbidity besides many bacterial and rotavirus infections in this study. Cryptosporidial infection was first reported in a renal transplant patient by Weisburger et al[70] in 1979. In India, 60 living renal transplant recipients were examined, of which 12 patients (20%) had *cryptosporidium* oocysts including 16.6% who had symptomatic diarrhea. Patients responded well to spiramycin (2 g/day for 10 days).[71] In a retrospective analysis, it was observed that 9% (119/1235) renal transplant recipients developed diarrhea, and *Cryptosporidium* was found in 34/119 (28.5%). Nine of 680 (1.3%) patients were on a cyclosporine (CSA)-based regimen, and 25/643 (3.8%) patients were on a tacrolimus (Tac)-based regimen. It was observed that relative risk of developing *Cryptosporidium* infection was lower on the CSA-based regimen, compared with the Tac-based regimen (odds ratio: 0.35, 95% confidence interval: 0.17–0.72, $p = 0.003$). Patients with Tac and mycophenolate mofetil combination therapy had a significantly high risk of *Cryptosporidium* infection.[72] We conducted a study from 38 transplant recipients comprising 29 postrenal, two liver, and seven BMT recipients presenting with diarrhea and 50 transplant recipients (42 postrenal transplant, eight BMT) without diarrhea. Presence of intestinal parasites was examined by light microscopy and multilocus genotyping of *Cryptosporidium* species was analyzed. Twenty-one percent (8/38) patients were positive for *Cryptosporidium* species. Multilocus genotyping of *Cryptosporidium* species detected four isolates of *C. hominis;* two of *C. parvum,* one of mixed genotype, and genotype of one could not be ascertained. All the *C. hominis* isolates were detected in adult postrenal transplant (PRT) recipients, whereas the *C. parvum* isolates included a child with BMT and an adult with PRT.[73] In an another study from Lucknow, 358 renal transplant recipients and 200 healthy controls were examined that showed positivity of 8.4% (30/358) in former group and none from controls ($p < 0.001$). *C. parvum* was identified in (33.3%) and *C. hominis* in 20/30 (66.7%).[74]

Cryptosporidiosis in Cancer Patients

Cancer patients often experience transient or constant impairments in immunity due to administration of chemotherapeutic drugs. Prevalence of cryptosporidiosis in cases of malignant disorders in India has been reported to be 0.3[75] and 1.3% (7/560).[76] A recently published case–control study from China has shown occurrences of *Cryptosporidium* species in patients with digestive malignancies before chemotherapy. The infection rates of *Cryptosporidium* spp. in colorectal, esophageal, liver, and small intestine cancers were 17.24% (20/116, $p < 0.01$), 6.25% (⅛, $p < 0.029$), 14.29% (1/7, $p < 0.001$), and 40% (⅔, $p < 0.001$). All the *Cryptosporidium* spp. obtained were *C. parvum*, suggesting potential zoonotic transmission. The other notable findings observed were the plausible associations of subtypes IIaA15G2R1 and IIaA15G2R2 in patients with colorectal cancer and IIaA13G2R2 subtype with both colorectal and liver cancers.[77]

Food- and Water-Borne and Respiratory Cryptosporidiosis

Cryptosporidium has been incriminated in many water-borne outbreaks. There were reports of 19 outbreaks in United Kingdom and United States between 1984 and 1996, affecting an estimated number of 427,100 individuals. By the end of 2016, at least 524 water-borne outbreaks of cryptosporidiosis have been reported globally, including drinking and recreational water.[78] *Cryptosporidium* oocysts have been detected in surface water. The commonness of fecal wastes from human and nonhuman hosts suggests that many environments, particularly water and soil, act as vehicles for the spread of the disease. The oocysts have also been identified as contaminations in different types of food, mainly on numerous fresh vegetables and fruits, and at least 26 food-borne outbreaks have been reported worldwide.[79] In India, no study has been performed till date to ascertain, if at all there are any food and water-borne outbreaks.

Respiratory cryptosporidiosis has been reported in immunocompromised patients as well as in children and was supported by experimental intranasal infection of piglets. The symptoms associated with this route are respiratory (laryngo-tracheitis) and could be accompanied by mild diarrhea. Reports of cryptosporidial infections of the respiratory tract[80] as well as gastric cryptosporidiosis[81] among immune-deficient and immune-competent individuals have indicated that this protozoan is not restricted only to the gastrointestinal tract rather exhibits extraintestinal tropism. Two reports of respiratory cryptosporidiosis, one in an adult[82] and the other in a 10-year-old child, have been reported in India.[83]

Molecular Epidemiology of Cryptosporidiosis

Despite the high prevalence of cryptosporidiosis in developing countries, genetic characterization of *Cryptosporidium* species has not been studied extensively in India except few.[18,27,84,85]

Cryptosporidium Species and Their Genotypes in Humans

Genotyping tools based on DNA sequences of different antigens and housekeeping genes have identified *C. hominis* and *C. parvum* species primarily responsible for infections in both immune-competent and immune-compromised individuals.[86] Studies using small subunit ribosomal RNA have revealed presence of *C. canis, C. felis,* and *C. meleagridis* in

AIDS patients in the United States, Kenya, Thailand, Peru, and many European countries.[87,88] Likewise, some putative human *C. muris* infections have been reported from immune-compromised patients from different parts of the world including India.[27] Similarly, *C. cervine* and *C. suis* have been reported from Canada and United States, respectively.[89,90] Above-mentioned reports signify presence of wide spectrum of zoonotic infections in different geographic regions. Recently *C. andersoni* was identified in a few sporadic cases of cryptosporidiosis and was the predominant species (79.59%,78/98) in diarrheal patients from Assam in India.[91]

Subtyping (subgenotyping)

Thus, molecular methods, besides diagnosis, have also been applied for differentiation of *Cryptosporidium* at subtype level.[86] Identification of the isolates at the subtype/subgenotype level is useful for understanding of the population structure of *Cryptosporidium* species.[92] One of the popular subtyping tool is the DNA sequence analysis of the variable fragment of the gene encoding a sporozoite surface glycoprotein gp60 (also called cpgp40/15 or gp40). The gp60 gene is similar to microsatellite sequence by having tandem repeats of the serine-coding trinucleotide TCA, TCG, or TCT at the 50 (gp40) end of the gene. However, in addition to variations in the number of trinucleotide repeats, there are extensive sequence differences in the nonrepeat regions, which categorize *C. parvum* and *C. hominis* each to several subtype families. Sequence analysis of gp60 gene is widely used in *Cryptosporidium* subtyping because of its sequence heterogeneity and relevance to parasite biology. It is the single most polymorphic marker identified so far in the *Cryptosporidium* genome. gp60 is located on the surface of apical region of invasive stages of the parasite, and is one of the dominant targets for neutralizing antibody responses in humans.[93] Thus, it is possible to link biologic characteristics of the parasites and clinical presentations with the subtype family identity. Nomenclature of gp60 subtypes starts with the subtype family designation Ia, Ib, Id, Ie, If, etc. for *C. hominis,* and IIa, IIb, IIc, IId, etc. for *C. parvum,* respectively. Some of the *C. parvum* subtype families, such as IIa and IId, are found in both humans and ruminants. *C. parvum* subtype type families, especially IIc, have been reported in humans.[10,94] In an Indian study,[95] conducted at three different centers (Vellore, Trichy, and Delhi), subtypes Ia and Ie were the most commonly identified subtypes among the *C. hominis*-infected children, followed by subtypes Ib and Id. "If" subtype, however, was reported mainly in Delhi, with only one instance from Vellore. Restriction fragment length polymorphism patterns suggestive of mixed infection with Ia and If were also reported in one child from the Delhi. Among the seven children with *C. parvum* infection, one each with subtypes IIc and IId was identified in the Delhi. Sequencing and phylogenetic analysis showed the presence of a newly identified subtype, "IIm" in the southern centers, that is, in Vellore and Trichy (numbers T415 and V740), and another previously unreported subtype, named IIn in accordance with current nomenclature conventions,[96] in the Vellore center (numbers V416 and V640). However, no significant difference in severity of diarrhea among children infected with *C. hominis* subtypes Ia, Ib, Id, and Ie was noted. A trend toward association of shorter duration of diarrhea with subtype Id than with the other subtypes was observed. Similarly, there was a trend toward association of older age with subtype Ia than with other genotypes.

Differences in molecular epidemiology of *C. hominis* between the developing and developed countries have been noticeable. In developing countries, the complexity of *C. hominis* infections in humans is reflected by the occurrence of multiple subtype families and multiple subtypes within families Ia and Id. Thus, three to four different *C. hominis* subtype families have been reported in humans in India.[27,28] Extensive *C. hominis* heterogeneity in developing countries is an indicator of intensive and stable cryptosporidiosis transmission in these areas. Four common *C. hominis* subtype families, Ia, Ib, Id, and Ie, are usually seen in humans in developing countries. However, there are geographic differences in the distribution. For example, all the four common subtype families were seen in children and HIV seropositive adults in India. Within each subtype family, one subtype is frequently seen in certain areas but not in others. For example, there are only two common subtypes within the *C. hominis* subtype family i.e., IbA9G3 and IbA10G2. The former is commonly seen in Malawi, Kenya, and India, whereas the latter is commonly seen in South Africa, Botswana, Jamaica, and Peru.[47,97] The high virulence of the Ib subtype family in immune-competent persons is also reflected in the number of outbreaks caused by the parasite. Almost half of the *C. hominis* outbreaks in the United States, especially the major ones, were caused by the Ib subtype family, particularly the IbA10G2 subtype. IbA10G2 is apparently a major *C. hominis* subtype responsible for cryptosporidiosis outbreaks in Europe.[98]

Genotypes and subtypes along with clinical association have been shown in ►**Tables 1**, **2**, and **3**.

Multilocus Sequence Typing

This technique further allows the detection of length polymorphisms and nucleotide substitutions due to use of microsatellite and minisatellite markers and the single nucleotide polymorphisms (SNP), respectively.

Various markers that have been used over the years for multilocus sequence typing (MLST) include 70 kDa heat-shock protein (HSP70) in chromosome 2, T-rich fragment (designated Chrom3T) coding for a hypothetical protein-cgd630 in chromosome 3, and the following genes in chromosome 6 that are 60 kDa glycoprotein (GP60), 47 kDa protein (CP47), serine repeat antigen (MSC6–7), mucin-like protein (Mucin1), 56 kDa trans-membrane protein (CP56), 47-kDa protein (CP47 microsatellite), a serine repeat antigen (MSC6–7 minisatellite), a hypothetical retinitis pigmentosa GTPase regulator (RPGR minisatellite), and a hydroxyproline-rich glycoprotein (DZHRGP minisatellite and microsatellite).[84] Of these, the most polymorphic is the glycoprotein gene GP60. At the GP60 locus, subtype nomenclature is based on the combination of the number of serine coding trinucleotide repeats TCA, TCG, or TCT present in the microsatellite region and the SNP in the rest of the sequence.[99] The

Table 1 Worldwide distribution of Cryptosporidium genotypes and subtypes

Country	*Cryptosporidium* spp.	Subgenotype	Subtypes		Reference
Ontario	*C. hominis* (40%) *C. parvum* (50%) *C. cervine* Genotype (10%)	Ia, Id, Ie, IIa,	IaA19R3, IaA23R4, IdA19, IeA11G3T3	IIaA17G2R1 (2), IIaA16G3R1 (1), IIaA15G2R2 (1)	Trotz-Williams, 2006[100]
Jamaica	*C. hominis* (73.5%) *C. parvum* (20%) *C. canis* (2.9%) *C. felis* (2.9%)	Ib, Ie, IIc	IbA10G2 (22) IeA12G3T3 (3)	IIcA5G3d (7)	Gatei et al, 2008[97]
Mexico	*C. hominis* (83.3%) *C. parvum* (16.7%)	Ia, Ib, Id, Ie, IIa	IaA14R3(2), IaA15R3 (3), IbA10G2 (1), IdA17 (1), IeA11G3T3 (3)	IIaA15G2R1(1) IIaA16G1R1(1)	Valenzuela et al, 2014[101]
Portugal	*C. hominis* (35.7%) *C. parvum* (64.3%)	Ia, Ib Id, Ie, If IIa, IIb, IIc, IId	IaA19R3(1), IbA10G2(10) IdA15(1), IeA11G3T3(2) IfA14G1(1)	IIaA15G2R1 (9), IIbA14(1) IIcA5G3a (1), IIcA5G3b(6) IIdA17G1(2), IIdA19G1 (3) IIdA21G1(2), IIdA22G1(1)	Alves et al, 2006[102]
Brazil	*C. hominis* (63%) *C. parvum* (14.8%) *C. felis* (18.5%) *C. canis* (3.7%)				Lucca et al, 2009[103]
Brazil	*C. hominis* (57.1%) *C. parvum* (14.2%) *C. meleagridis* (14.2%)				Bushen et al, 2007[104]
Peru	*C. hominis* (73%) *C. parvum* (11.3%) *C. meleagridis* (8.8%) *C. canis*/*C. felis* (6.2%) *C. suis* (0.5%)	Ia, Ib, Id, IIc	IaA12R3 (1), IaA12R4 (5) IaA12R5 (3), IaA13R2 (1) IaA13R6 (1), IaA13R7 (9) IaA13R8 (12), IaA14R7 (2) IaA17R6 (1), IbA10G2 (35) IbA13G3 (4), IdA10 (25) IdA12 (2), IdA15G1 (1) IdA20 (12), IeA11G3T3 (13)	IIcA5G3a (16), IIcA5G3b (4) IIcA5G3c (2)	Cama et al, 2007[17]
Peru	*C. hominis* (70%) *C. parvum* (13.3%) *C. meleagridis* (7.8%) *C. canis* (1.5%) *C. felis* (4.7%) *C. hominis* & *C. parvum* (1.5%) *C. canis* & *C. meleagridis* (0.7%)		IaA11R4 (3), IaA12R4 (7) IaA13R4 (1), IaA13R7 (1) IaA14R6 (5), IaA15R3 (3) IbA10G2 (23), IdA10 (9) IdA15 (1), IdA20 (2) IeA11G3T3 (19)	IIcA5G3a (12), IIcA5G3b (1) IIcA5G3c (1)	Cama et al, 2008[47]
Australia	*C. hominis* (53.6%) *C. parvum* (46.3%)	Ib, Id, IIa	IbA10G2, IbA9G2 IdA26, IdA15	IIaA18G3R1, IIaA17G3R1 IIaA20G5R1, IIaA20G3R1 IIaA17G4R1, IIa16G3R1	Waldron 2009[105]
Australia		Ib, IIa	IbA10G2R2, IbA15G1R2 Ib2A18G1R4	IIaA18G3R1, IIaA20G3R1 IIaA23G3R1, IIaA23G3R1 IIaA5G3R2	Jex et al, 2007[106]
Australia	*C. hominis* (42.8%) *C. parvum* (57.2%)	Ib, IIa	IbA10G2, IbA15G1	IIaA17G2R1, IIaA18G3R1 IIaA19G3R1, IIaA20G3R1	Ng et al, 2008[107]
Australia	*C. hominis* (78.6%) *C. parvum* (19.8%) *C. meleagridis* (1.6%)	Ib, Id, Ie, If, Ig IIa, IId			Ng et al, 2010[108]
Australia	*C. hominis* (82%) *C. parvum* (18%)	Ia, Ib, Id, If, IIa, IIc	IaA23 (1), IbA5G2T3 (1) IbA9G2 (1), IbA9G2T1 (1) IbA10G2 (21), IdA15G1 (9) IdA16 (1), IdA25 (3) IfA11G1T1 (1), IfA12G1 (2)	IIaA15G2R1 (2),IIaA17G2R1 (1) IIaA18G3R1 (3), IIaA19G2R1(1) IIaA19G3R1 (1), IIcA5G3a (1)	O Brien et al, 2008[109]
UK	*C. hominis*	Ia, Ib	IbA10G2(10), IaA12R3(1) IaA22R2(1) IaA30R3(1) IaA25R3 (1), IgA24 (2)		Chalmers et al, 2008[98]

(*Continued*)

Table 1 (*Continued*)

Country	*Cryptosporidium* spp.	Subgenotype	Subtypes		Reference
Europe	*C. hominis* (75%) *C. parvum* (25%)		IbA10G2 (5), IdA15G1 (1)	IIaA18G3R1 (1), IIdA22G1 (1)	O Brien et al, 2008[109]
France	*C. hominis* (31.6%) *C. parvum* (42%) *C. meleagridis* (5.2%) *C. felis* (10.5%) *C. muris* (1.7%)				Guyot et al, 2001[110]
Ireland	*C. hominis* (24%) *C. parvum*		IbA10G2(25)	IIaA18G3R1(48), IIaA18G3R1(9) IIaA20G3R1(7), IIaA15G2R1(6) IIaA19G3R1(2), IIaA17G1R1(1) IIaA10G2R1(1), IIaA14G2R1(1) IIaA16G3R1(1), IIaA17G2R1(1) IIaA20G5R1(1), IIaA21G3R1(1) IIdA26G1 (1)	Zintl et al, 2008[111]
South Africa	*C. parvum* (22)	Ic, Id, Ib, Ie, Ia			Leav et al, 2002[112]
Ethiopia	*C. hominis* (18%) *C. parvum* (65.7%) *C. viatorum* (7.1%) *C. canis* (1.4%) *C. felis* (3.5%) *C. meleagridis* (2%) *C. xioai* (1.4%)	Ib, Id, Ie, IIa, IIb, IIc, IId, IIe, If like	IbA10G2(1), IdA20(10), IdA24(1), IdA26(2), IeA11G3T3(5)	IIaA13G2R1 (1), IIaA14G2R1 (1), IIaA15G2R1(60), IIaA16G2R1 (1), IIaA16G2R1(4), IIaA17G2R1(2), IIaA18G2R1 (1), IIaA19G1R1 (1), IIbA12(1), IIcA5G3(2), IIdA17G1(1), IIdA19G1(1), IIdA22G1(2), IIdA24G1(1)	Adamu et al, 2014[113]
Kenya	*C. hominis* (68.2%) *C. parvum* (18.2%) *C. meleagridis* (13.6%)				Nyamwange et al, 2012[116]
Nigeria	*C. hominis* (44.2%) *C. parvum* (32.5%) *C. hominis & C. parvum* (5.2%) *C. meleagridis* (6.5%) *C.* rabbit genotype (6.5%) *C.* cervine genotype (3.9%) *C. canis* (1.3%)	Ia, Ib, Id, Ie, Ih, IIa, IIc, IIi, IIm	IaA18R2 (3),IaA22R2 (1), IaA24R2 (2), IaA25R2 (2), IaA28R2(1), IaA21R1(1) IbA10G2(3),IbA13G3(7) IdA11(2), IdA17(2) IeA11G3T3(3) IhA14G1(1)	IIaA15G2R1(1), IIaA16G1R1(1) IIcA5G3a (9), IIcA5G3b(8) IIiA11(2), IImA14G1(2)	Molloy et al, 2011[114]
Jordan	*C. parvum* (22) *C. hominis* (20) *C. meleagridis* (1) C. *canis* (1)	IbId,IIa IIb, IIc	IbA6G3(3), IbA9G3(3) IbA10G2 (1), IbA20G2(1) Id IdA21 (2), IdA24 (5)	IIaA15G1R1(2), IIaA20G3R1(1) IIcA5G3a (2), IIdA14G1 (1) IIdA20G1 (5), IIdA24G1 (1) IIdA29G1 (1)	Hijjawi et al, 2010[29]
Yemen	*C. hominis* (3%) *C. parvum* (97%)	Ie, IIa	IeA11G3T3 (1)	IIaA15G2R1(7)	Alyousefi et al, 2013[115]
Japan	*C. hominis* (75%) *C. parvum* (25%)	IeIa, IIc, Ib			Abe et al, 2006[92]
Kuwait	*C. hominis* (26.5%) *C. parvum* (73.5%)	Ia, Id, Ie, IIa, IIc, IId			Iqbal et al, 2011[117]
India	*C. hominis* (49) *C. meleagridis* (2) *C. felis* (1)	Ia,Ib,Ie,Id	IbA9G3 (17), IeA11G3T3 (5), IaA12G1R3 (1), IaA19R3 (4), IaA21R3 (1), IaA22R3 (1), IdA15G1 (13), IdA16 (4)		Gatei et al, 2007[84]
India	*C. hominis* (81%) *C. parvum* (12%) *C. felis (5.2%)* *C. parvum* (mouse genotype	Ia, Ib, Ic, Id, Ie			Rao Ajjampur et al, 2007[118]
India	*C. hominis* (88.1%) *C. parvum* (10.5%) *C. meleagridis* (1%)	Ia, Ib, Id, Ie, If, IIc, IId, IIm, IIn	IaA18, IaA19, IeA11G3T3, IfA13G1	IIcA5G3, IIdA15G1, IImA7G1, IInA8	Ajjampuret al, 2010[95]

(*Continued*)

Table 1 (*Continued*)

Country	*Cryptosporidium* spp.	Subgenotype	Subtypes		Reference
India	*C. hominis* (67%) *C. parvum* (22.4%) *C. meleagridis* (1.7%)	Ia,Ib, Id, Ie, IIc, IId, IIe	IaA18R3, IaA19R3, IaA21R3, IaA26R3, IaA27R3, IaA29G1T3R3b IbA9G3 IdA14G1, IdA15G11, IdA16G1, IeA11G3T21, IeA11G3T3, IfA13G1	IIcA5G3a IIdA14G1, IIdA15G1 IIeA7G1b	Sharma et al, 2013[44]
India	*C. hominis* **64%** (54/84) *C. parvum* **27%** (23/84)	Ie, Ia, Id Ib If IIc II d IIa IId IIb	IdA15G1 (41.7%, 10/24), IbA9G3 (16.7%, 4/24), IeA11G3T3 (4.2%, 1/24), IfA14G1 (8.3%, 2/24), IbA10G2 (4.2%, 1/24), IdA17G1 (4.2%, 1/24) and IaA19R3 (4.2%, 1/24)		Yadav et al, 2016[119]

Table 2 Subtype families of Cryptosporidium spp. reported from Indian subcontinent

C. hominis (98%) *C. felis* (2%)	Ia, Ib, Ie, Id	Gatei et al, 2007[84]
C. hominis (64%) *C. parvum* (18%) *C. parvum (mouse)* (2%) *C. melegridis* (2%) *C. muris* (2%)	Ia, Ib, Ic, Id, Ig, If, IIa, IIb, IIc	Muthusamy et al, 2006[27]
C. hominis (87.5%) *C. parvum* (10%) *C. felis* (2.5%)		Das et al, 2006[120]
C. hominis (81%) *C. parvum* (12%) *C. felis* (5.2%) *C. parvum* (mouse genotype)	Ia, Ib, Ic, Id, Ie	Rao Ajjampur et al, 2007[118]
C. hominis (88.1%) *C. parvum* (10.5%) *C. meleagridis* (1%)	Ia, Ib, Id, Ie, If, IIc, IId, IIm, IIn	Ajjampur et al, 2010[95]
C. hominis (67%) *C. parvum* (22.4%) *C. meleagridis* (1.7%)	Ia,Ib, Id, Ie, IIc, IId, IIe	Sharma et al, 2013[44]

Table 3 Subtype family association with clinical outcome of Cryptosporidiosis

	Cama et al, 2007[17] (HIV adults)	Cama et al, 2008[47] (children)	Ajjampur et al, 2010[95] (children)	Ng et al, 2010[107]	Chierico et al, 2011[121] (HIV adults)	Iqbal et al, 2011[117] (children)	Adamu et al, 2014[113] (HIV adults)
Ia	No association	Diarrhea	No association		No association with wasting		
Ib	Marginally with diarrhea, vomiting	Nausea, vomiting malaise, diarrhea	No association				
Id	Chronic diarrhea	Diarrhea	Shorter duration of diarrhea	Abdominal pain		Fever, dehydration, severe diarrhea	
IIc		Vomiting			Wasting syndrome		
IIa							Diarrhea

Abbreviation: HIV, human immunodeficiency virus.

diversity at MLST is more pronounced at *C. parvum* loci compared with *C. hominis*. The high genetic diversity of CP47 and MSC6–7 has also been reported and CP47 locus has been suggested as an alternative for the typing *C. hominis* and *C. parvum*. CP47 has a high resolution for both length polymorphism and MLST-based typing that distinguishes *C. hominis* and *C. parvum* by size.[84]

Clinical Association of *Cryptosporidium* Subtypes

Number of studies in last over a half decade emphasized on the significant role of subtypes in the clinical manifestation of the cryptosporidiosis (►**Table 3**). A recent study reported that the Ib subtype family appeared to be much more virulent than other subtype families and was significantly associated with diarrhea, nausea, vomiting, and

general malaise.[96] Worldwide, IbA9G3 and IbA10G2 are the two common subtypes reported within the Ib subtype family. Subtype IbA10G2 has a global distribution. IbA9G3 has been commonly reported in humans in Malawi, Kenya, India, and Australia, whereas IbA10G2 in South Africa, Botswana, Jamaica, Peru, United States, Canada, Australia, and European countries.[97,98] It has been reported that IbA10G2 is responsible for more than half of the water-borne outbreaks of gastroenteritis in the United States, United Kingdom, Canada, and France.[96] Variations in the pattern of clinical manifestations were observed among *C. hominis* subtype families. Infections with subtype family Ib were associated with nausea, vomiting, general malaise, and diarrhea. Infection with other subtype families (Ia, Id, and Ie) were generally associated with diarrhea symptom only.

In a study on pediatric cryptosporidiosis from Kolkata, IbA9G3, IeA11G3T3, IaA12G1R3, IaA19R3, IaA21R3, IaA22R3, IdA15G1, and IdA16 subtypes were identified at gp60 locus.[84] However, association of these subtypes with the clinical manifestations has not been reported in this study. The subtypes identified from Delhi were IdA15G1 (41.7%, 10/24), IbA9G3 (16.7%, 4/24), IeA11G3T3 (4.2%, 1/24), IfA14G1 (8.3%, 2/24), IbA10G2 (4.2%, 1/24), IdA17G1 (4.2%, 1/24), and IaA19R3 (4.2%, 1/24). Four (16.7%) patients, however, had mixed infections with IdA15G1 and IeA11G3T3 subtypes.[85]

Conclusion and Future Perspective

The occurrence and distribution of *Cryptosporidium* species among human hosts are similar to that described elsewhere including report of unique variants. Conditions contributing to observed cryptosporidiosis prevalence indicate contact and exposure variability of both human and animal populations. Molecular epidemiological studies on cryptosporidiosis have significantly broadened the understanding of transmission dynamics and information about distribution of circulating genotype(s) and subtype(s). Distribution of different subtypes and association of particular subtypes with the some of the outbreaks and diseased conditions clearly indicate emergence of virulent ones.

Conflict of Interest
None declared.

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