**Research Article** **Microbiology**

**International Journal of Pharma and Bio Sciences** **ISSN 0975-6299**

# CRYPTOSPORIDIOSIS IN INDIA

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## ABSTRACT

Parasites belonging to genus *Cryptosporidium* constitute a large number of animal and human species of intestinal Coccidia. Among several species, *C. parvum* and *C. muris* is well recognized diarrheal agent in immune-competent as well as immunocompromised human host. Cosmopolitan distribution, stability over large variation of temperature, wide host range and zoonotic potential are the unique features which attributed to its increasing prevalence and outbreaks in most parts of the world. Owing to the large population of HIV patients in India, these infections are being increasingly reported. Furthermore, *Cryptosporidium sp*. has been reported as the leading cause of childhood diarrhea in India. Over the last decade, increasing awareness among clinicians and microbiologists along with introduction of simple techniques like modified acid-fast staining and stool concentration has improved the detection of these agents. However, molecular genetics of diverse human and animal species and their epidemiology and zoonotic potential need further evaluation.

**KEYWORDS:** **Cryptosporidiosis; Intestinal Coccidia; Opportunistic parasite; parasitic zoonosi**

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# INTRODUCTION

Cryptosporidium, Isospora and Cyclospora species are commonly termed as intestinal coccidia on account of its habitat in small intestine and association with enteric disease in animals and human.[1] These parasites are unicellular lacking organelle of locomotion (i.e. cilia, flagella, pseudopodia) and require alternation of sexual and asexual reproduction to continue life cycle.[2] Apical microtubule complex is present in these parasites as a common characteristic of phylam Apicomplexa. *Cryptosporidium* is unique from other intestinal Coccida. It produces small acid fast oocyst containing four naked sporozoites without any sporocyst.[3] *Cryptosporidium* develops in an intracellular extracytoplasmic pocket formed by invagination of host cell membrane, known as parasitophorous vacuole.[1, 2] In general, intestinal infection in immune-competent host is self-limiting, uncomplicated and without progress to carrier state.[2] Whereas, immunosuppressed patients, frequently suffers from profuse acute watery diarrhoea progressing to chronic diarrhoea, malnutrition and weight loss.[4] Owing to significant increase in patients with compromised immune status associated with HIV, use of steroids, immunosuppressive and anticancer agents, opportunistic infections by *Cryptosporidium* are increasingly being reported. As per the current estimate, India has a large population of 21 lakh people living with HIV/AIDS.[5] Consequently, opportunistic infection by *Cryptosporidium* in India is on the rise. *Cryptosporidium* has also been reported as a one of the major causes of childhood diarrhea in India.[6] Wide-ranging in distribution, temperature stability, mode of transmission and hosts are the essential characteristics which contribute to the pathogenicity and zoonotic potential of these emerging pathogens.[2, 3] In this review, Indian scenario infection by *Cryptosporidium sp*. and its clinical, microbiological and epidemiological aspects has been addressed.

## *HISTORY OF DISCOVERY*

In 1907, American parasitologist Tyzzer first described a protozoan oocyst containing four naked sporozoites without a sporocyst surrounding it, in gastric glands of common mice.[7] He coined the term *Cryptosporidium* to reflect this unusual morphology i.e. absent or hidden sporocysts. Five years after, he found a smaller parasite of similar morphology in villi of small intestine of mouse.[8] These two species were designated as *C. muris* and *C. parvum* respectively. Subsequently, *Cryptosporidium* infection was reported in fish, reptiles and birds and these species were named accordingly.[1] However, unlike other Coccidia, *Cryptosporidium* lacks host specificity. Thus the traditional taxonomy based on original host remained controversial. Among several species, *C. hominis, C. parvum, C. felis, C. muris, C. meleagridis, C. canis, C. suis* are the common species frequently reported to infect human.[3] Recent molecular research has established two distinct genotypes of *C. parvum*.[2] In contrast to the bovine genotype of *C. parvum* the human genotype is not infectious to animals.

## *GLOBAL SCENARIO*

Since the discovery, *Cryptosporidium* was not appreciated as pathogenic protozoa until 1955, when an outbreak in turkey flock was reported.[9] Subsequently, it was found to cause overwhelming wasting diarrhea in immunocompromised human host.[10] *Cryptosporidium* is highly infectious with infective dose as low as 132 oocysts.[1] Transmission is largely faeco-oral and clinical illness essentially varies according to host's immune status and infective dose. Its pathogenic potential in immunocompetent persons was uncovered when several water borne outbreaks were reported. The largest of all outbreaks occurred in Milwaukee, Wisconsin, USA in 1994 affecting around 4,00,000 people through the public water supply.[11] In developing countries, cryptosporidiosis is endemic and mostly affects adults with HIV infection and children. The prevalence of *Cryptosporidium species* among children with diarrhea varies from 3 to 13% in underdeveloped countries like Brazil, Venezuela, Indonesia, Thailand, South Africa, Ghana, India and Bangladesh.[2] In contrast, in developed parts of world like Britain, United States, Canada, Australia and Denmark it accounts for only 1 to 4% of childhood diarrhoea.[2] Higher incidence is associated with travel to an endemic area and conditions

of poor sanitation, overcrowding as in daycare centers.[3]

## *INDIAN SCENARIO*

### *Opportunistic infection in HIV patients*

Although India has achieved an overall decline in annual new HIV infections during the last decade, about 21 lakhs people are living with HIV/AIDS.[5] The prevalence of HIV is considerably high in states like Manipur, Mizoram, Nagaland, Andhra Pradesh, Karnataka and Goa. Pediatric population contributes to approximately 7% of HIV burden in India.[5] HIV patients not on anti-retroviral drugs, with low CD4+ count, children with AIDS are particularly at risk of developing opportunistic infections. Several studies have reported correlation between CD4+ count and cryptosporidiosis in HIV patients.[12-16] Gautam *et al.* reported 17.2% prevalence of *Cryptosporidium* infection among drug-naive HIV patients in North India.[16] CD4 counts ≤100 cells/cmm and plasma viral load >4.0 log(10) copies/mL had positive correlation with *Cryptosporidium* infection. In contrast, a study conducted in South India had correlation of Cryptosporidial diarrhea only with CD4 counts ≤200 cells/cmm, but not with high viral loads.[14] The rate of isolation of enteric pathogen is higher in HIV patients with diarrhoea compared to asymptomatic patients and it ranges from 26% to 62.7%.[13, 17-20] Intestinal protozoa especially Coccidian parasites were the predominant isolate in most studies (table 1).[4, 13, 15, 17-19, 21-23] The prevalence of *Cryptosporidium* infection in HIV patient varies with respect to geographical area. While *Cryptosporidium* infection is more common than other coccidia in North India, studies conducted in South and Western parts of India show *Isospora beli* accounting for a majority of enteric pathogen (table 1).[4, 15, 19]

**Table 1**
***Intestinal coccidia in HIV patients from different regions of India.***

| Region | Subjects | Result | Reference |
|---|---|---|---|
| North India | 154 HIV patients | *Cryptosporidium sp.* 60.4%<br>*Isospora belli* 9.03% | Jha *et al.* [21] |
| North India | 120 HIV patients | *Cryptosporidium sp.* 10.8%<br>*Cyclospora cayetanensis* 3.3%<br>*Isospora belli* 2.5% | Mohandas *et al.* [18] |
| North India | 26 HIV patients with diarrhoea | *Cryptosporidium sp*. 11%<br>*Isospora belli* 31% | Prasad *et al.*[17] |
| North India | 75 HIV patients | *Cryptosporidium sp*. 33%<br>*Isospora belli* 2.7% | Dwivedi *et al.*[13] |
| South India | 100 HIV patients | *Cryptosporidium sp*. 2%<br>*Isospora belli* 21% | Janagond *et al.*[19] |
| South India | 534 HIV patients (111 with diarrhoea & 423 without diarrhoea) | *Cryptosporidium sp*. 25%<br>*Isospora belli* 20% | Rao Ajjampur *et al.* [4] |
| Western India | 544 HIV patients | *Cryptosporidium sp.* 24.8%<br>*Cyclospora cayetanensis* 7.7%<br>*Isospora belli* 2.5% | Mathur *et al.*[22] |
| Western India (Gujarat) | 100 HIV patients | *Cryptosporidium sp.* 2%<br>*Isospora belli* 18% | Mehta *et al.*[15] |
| North East India (Manipur) | HIV positive asymptomatic injecting drug users | *Cryptosporidium sp*. 94.4%<br>*Isospora belli* 10.7% | Anand *et al.*[23] |

Since speciation of *Cryptosporidium* is challenging and requires molecular methods, the information about distribution of different *Cryptosporidium sp*. in India is inadequate. While *C. hominis* and *C. parvum* are the common species causing diarrhoea in HIV patients, other species like *C. felis, C. muris* and *C. meleagridis* have also been reported.[4]

### *Childhood diarrhea*

Cell mediated immunity plays an important role in defense against *Cryptosporidium*.[1, 2] Owing to their underdeveloped immune system, children are particularly susceptible to cryptosporidiosis. In developing countries, malnutrition and common childhood infections like measles perpetuate immunocompromised state and have been implicated as critical predisposing factors.[23] Breastfeeding has been reported to have protective role against cryptosporidiosis in infants.[1] It helps in enhancing the development of immunity and also it precludes the use of water contaminated with oocysts of

*Cryptosporidium*. In India, intestinal cryptosporidiosis has been reported in both symptomatic and asymptomatic (non-diarrhoeatic) children irrespective of their HIV status. A study conducted in semiurban slum area in southern India, detected 67% prevalence of *Cryptosporidium* among <2 year children.[24] However, most of the children with intestinal cryptosporidiosis were asymptomatic and type of drinking water (bottled water or municipal drinking water) had no association with these infections. In a multicentric study involving 2,579 under-five children with diarrhoea admitted in hospitals of Delhi, Trichy, Vellore, 2.7% children had cryptosporidial diarrhea.[6] *C. hominis* with its subgenotype Ie, Ia, Ib, and Id was the most common species accounting for 88% of all *Cryptosporidium* isolates in all centers. The authors also reported the identification of a novel *C. parvum* subgenotype (IIn) in Vellore. Das *et al.* reported *C. hominis* (87.5*%), C. parvum* (10%), and *C. felis* (2.5%) were the commonest species infecting children in Kolkata.[25] A similar observation was reported from semiurban community in South India.[26] Intestinal *Cryptosporidium* infection has been found to affect child health in long term. Inability to eliminate the parasite has been manifested as multiple episodes of diarrhoea, persistent diarrhoea, malabsorption, stunting of growth and longer shedding of *Cryptosporidium* oocysts.[24] The high frequency of asymptomatic cyst-passers reported in India may be a consequence of repeated or persistent exposure to *Cryptosporidium* from early age and is significant from epidemiological point of view.[24, 27]

### *Infections in immunocompetent adults*

Although cryptosporidiosis is rare among immunocompetent adults, it has been reported from various parts of the country. Especially, rural area, urban slums, low socio economic status, unsafe water supply, living conditions with poor hygiene, contact with animals/pets and homosexuality have been implicated as essential risk factors in adults.[3, 28-30] The prevalence of *Cryptosporidium* among adults varies from 1.36% to 21%.[29, 31, 32] As in children and HIV patients, *C.hominis* and *C. parvum* are also the major species of *Cryptosporidium* associated with infection in adults. In a study from North India, Sharma *et al.* reported *C. hominis* and *C. parvum* accounted for 73% and 24% *Cryptosporidium* isolates in adults.[33]

### *Extra-intestinal infections*

*Cryptosporidium* mainly spreads by faeco-oral route. However, it may be transmitted by inhalation of aerosols. Although the habitat is small intestine, *Cryptosporidium* may involve stomach, oesophagus and other organs. Extra-intestinal infections are frequently reported among immunocompromised host. Biliary and respiratory systems are commonly involved.[2, 3, 34] There are inadequate published data available on Extra-intestinal cryptosporidiosis in India. AIDS cholangiopathy is characterized by raised liver enzymes, papillary stenosis, sclerosing cholangitis with attenuation of intrahepatic biliary radicals and thickening of common hepatic duct. These pathological changes often associated with biliary cryptosporidiosis. Devarbhavi *et al.* reported one case of biliary cryptosporidiosis among 30 patients with AIDS cholangiopathy.[35] Another case was detected by Sharma *et al.* in a 35-years-old male who was diagnosed by colonic biopsy.[36] *Cryptosporidium* oocysts were found at mucosal surface in this patient. Shrikhande *et al.* has described a case of pulmonary cryptosporidiosis in a non HIV patient on corticosteroids therapy, presenting with low-grade fever, difficulty in breathing and chronic cough with expectoration.[37]

### *Parasitic zoonosis*

Owing to wide host range, *Cryptosporidium* has emerged as an important zoonotic infection. Contact with the livestock, cattle or pet animal like cat, dog predispose human to cryptosporidiosis. In a study conducted in rural areas of Ahmednagar, Maharashtra detected 1.36% and 10.89% prevalence of cryptosporidiosis in non-diarrheatic human and cattle respectively, suggesting cattle could be the source of human infection.[29] The infection is more frequent in young animals and animals with diarrhoea. Singh *et al.* reported 25.7% and 50% occurrence of *Cryptosporidium sp*. in non-diarrheatic and diarrheatic neonatal dairy calves respectively in Punjab.[38] Whereas, in Kolkata the prevalence was low (7.1% and 32.9% in non-

diarrheatic and diarrheatic cattle respectively).[39] The prevalence of cryptosporidiosis in livestock shows nationwide variation from 16.2 % to 39.65 %.[40-42] In a study from West Bengal Khan *et al.* has documented the evidence of *Cryptosporidium* zoonotic transmission from cattle to farm workers.[43] *C. parvum* has been found to be the predominant isolate in livestock, followed by *C. bovis, C. ryanae and C. andersoni and C. hominis, C. parvum* and *C. bovis* was detected in farm workers. The human isolates of *C. parvum* and *C. bovis* were identical to animal isolates in 18S rRNA sequence and RFLP analysis.

### *SEASONAL VARIATION OF IN CRYPTOSPORIDIOSIS IN INDIA*

Environtal ecology has major effect on transmission of cryptosporidiosis.[44] Most studies in Indian subcontinent has reported considerably high incidence of cryptosporidiosis during monsoon in both animal & human hosts.[12, 31, 45, 46] Due to the longer viability of oocysts in water and chlorine resistance, sporadic cases as well as water borne outbreaks of *Cryptosporidium* are common in monsoon. Tuli *et al*. have reported direct correlation between isolation of *Cryptosporidium* oocysts from patients and increased rain fall.[12] Saha Roy *et al*. detected highest prevalence of bovine cryptosporidiosis was during the rainy season followed by summer and winter.[45] In contrast, a higher rate of cryptosporidial iolation from children with diarrhea in Delhi was detected during hotter and drier season.[6]

## CONCLUSION

Cryptosporidiosis is an emerging parasitic infection worldwide. In India, it is highly endemic among livestock which could be the likely source of zoonotic transmission to human. Its ability to cause infection in a wide range of animal hosts as well as in human, stability to thermal stress and adverse environmental condition, resistance to water chlorination and low infective dose add to its outbreak potential. A large population of HIV patients, children in urban slums and adults with close contact with animals in rural areas and farms are at risk of cryptosporidiosis in India. Owing to lack of awareness and knowledge about the extent of this infection, it is often neglected and under reported. The information about several *Cryptosporidium species* infecting man and animals and their genotypes is inadequate and needs further research.

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