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Clinical features & risk factors associated with cryptosporidiosis in HIV infected adults in India

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Abstract

Background & objectives—Cryptosporidiosis is a leading cause of protracted, life threatening diarrhoea in HIV infected patients. Although data on prevalence are available for Indian patients, no information on risk factors for transmission exists. We therefore undertook this study to identify risk factors for transmission of cryptosporidiosis in HIV infected adults.

Methods—Both symptomatic (diarrhoeal) and asymptomatic HIV infected patients were screened for cryptosporidiosis. All *Cryptosporidium* spp. positive cases were enrolled in the study and interviewed to record socio-demographic information, water supply and animal contact. Data were analysed to study clinical features and potential association with species and genotype.

Results—Of the 28 cryptosporidial infections identified on screening 111 HIV positive patients with diarrhoea, 10 (35.7%) had chronic diarrhoea, 14 (50%) had associated fever and 8 (28.6%) had nausea. Symptomatic patients had a significantly higher number of co-infections with other enteric parasites (P=0.04) than 20 asymptomatics of 423 HIV positive individuals screened. Eleven of 17 (64%) patients with potentially zoonotic infections had diarrhoea. Patients with zoonotic species (64%) also tended to have fever more frequently than those infected with *C. hominis* (58%). Association between area of residence, rural or urban, water source and contact with animals and acquisition of cryptosporidiosis was not statistically significant.

Interpretation & conclusions—Cryptosporidiosis is an important cause of morbidity in HIV infected individuals in India, resulting in chronic diarrhoea. Risk factors for potentially zoonotic transmission of cryptosporidiosis were described in this study, but larger studies need to be done for a clearer understanding of the transmission dynamics of different cryptosporidial species in developing countries.

Keywords

Cryptosporidium; cryptosporidiosis; HIV; India; zoonotic

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Cryptosporidium spp. cause severe, life threatening diarrhoea in untreated HIV infected patients. Until the advent of highly active anti-retroviral therapy (HAART), this was a relatively common opportunistic infection even in developed countries[1,2]. In India, there have been reports from the mid 1990s on the prevalence of cryptosporidiosis from different parts of the country ranging from 8.5[3] to 81 per cent[4] with a high prevalence being reported from the north eastern States[4,5]. A few studies from developed countries have identified social and behavioural risk factors involved in transmission of cryptosporidiosis in HIV infected patients[6-9]. Although we recently published genotyping data from our population showing an association between symptomatic cryptosporidiosis and lower CD4 counts but not with an increased viral load[10], the association between clinical features, risk factors and susceptibility to infection were not examined. Our data[10] showed a strikingly high prevalence (35%) of potentially zoonotic species of *Cryptosporidium*, but potential modes of acquisition were not sufficiently investigated. In this study, we aimed at defining the clinical features in relation to species and identifying the risk factors for transmission of cryptosporidiosis in HIV infected adults in India with the same cohort of patients.

Materials & Methods

Study population and screening

As previously reported[10], 423 asymptomatic (no diarrhoea) and 111 symptomatic (with diarrhoea) HIV-infected adults who presented to the outpatient unit or who were admitted to the Christian Medical Hospital, Vellore, were screened for *Cryptosporidium* oocysts by microscopy using modified acid-fast staining[11,12] between September 2002 and August 2004. Diarrhoea was defined as three or more stools per day for at least 72 h. Acute diarrhoea was defined as diarrhoea of less than 14 days duration. Persistent diarrhoea was defined as diarrhoea for 14 or more days. Samples that were positive for *Cryptosporidium* spp. by microscopy were further followed up for speciation and genotyping by PCR-restriction fragment length polymorphism (RFLP) at multiple loci including SSR rRNA, COWP, TRAP and *Cpgp 40/15*. The genotyping, CD4 count and viral load results have been previously published[10]. All samples were also screened for other stool parasites and all diarrhoeal stool samples (n=111) were screened for enteric bacterial pathogens other than *Campylobacter* spp. and diarrhoeagenic *Escherichia coli.* However, viral agents were not screened in this study.

Data collection and assessment of diarrhoea

Informed consent from *Cryptosporidium* positive cases were obtained. They were then interviewed with a questionnaire which recorded information on the composition of the household in terms of number of residents, family structure, occupation *etc.* The questionnaire used this recorded information from the patients to assess their socio-economic status based on the modified Kuppuswamy scale[13,14]. Questions on housing structure, arrangements for cooking and eating, water supply, toilet facilities and contact with animals were also included. Data on concurrent opportunistic infections were also collected by reviewing case records. For patients with diarrhoea, detailed clinical information on frequency, duration, and associated symptoms such as weight loss was collected. Any history of diarrhoea during the previous 12 months was also recorded. The study protocol, questionnaires and consent forms had been approved by the institutional review board.

Statistical analysis

Data were entered into Microsoft Excel 2002 and analyzed using STATA version 8.0 (Stata Corp., College Station, TX, USA). Statistical comparisons were made using Fisher's exact and Chi-square tests.

Results

The most common parasites seen in patients with diarrhoea were *Cryptosporidium* (25%, 28/111) and *Isospora* (20%, 22/111). Data on genotyping of cryptosporidial infections in the 48 HIV infected individuals with cryptosporidiosis enrolled in the study have been previously published[10]. This data set included 28 patients with diarrhoea and 20 patients with no diarrhoea. The species identified were Cryptosporidium hominis, C. parvum, C. felis, C. muris, and *C. meleagridis*. Cryptosporidial diarrhoea was associated with decreased CD4 counts, below 200 ($P< 0.009$), but not high viral loads[10].

Clinical features in HIV infected individuals with symptomatic cryptosporidiosis

Of the 28 cases with cryptosporidial diarrhoea, 17 patients had acute diarrhoea and 11 patients had persistent diarrhoea. Among these patients, 10 patients reported diarrhoea for more than 3 months, 8 patients had three or more episodes of diarrhoea in the previous 12 months, while for 10 patients the current diarrhoeal episode was the first episode in a 12 month period. Diarrhoea was associated with fever in 14 (50%) patients, with nausea alone in 4 (14.3%), and with nausea and vomiting in 4 (14.3%) patients. Watery diarrhoea was reported in 19 (68%) cases with only 1 case of bloody diarrhoea. 11 of the 17 (64%) patients with potentially zoonotic infections had diarrhoea. Patients with zoonotic isolates tended to have fever more frequently (64%) than those infected with *C. hominis* or the human genotype (18/31, 58%) but the difference was not statistically significant. Weight loss was reported by 9 of 11 patients with persistent diarrhoea and 5 of 17 patients with acute diarrhoea and there was no difference between patients infected with zoonotic isolates (6/11, 54%) and those with a *C. hominis* infection (15/26, 58%) Association of diarrhoea with CD4 count has been mentioned previously[10].

On a review of the case records, both symptomatic and asymptomatic patients were found to have concurrent opportunistic infections which included candidiasis, cryptococcal meningitis, tuberculosis and toxoplasmosis (Table). However, the numbers, in each category and cumulatively, were too small to carry out any meaningful statistical analysis. When analysed for other stool parasites, among the symptomatic cases, 3 were co-infected with *Giardia*, 2 with *Isospora* and one each with *Hymenolepis nana*, *Cyclospora* and *Strongyloides* respectively while only one asymptomatic patient had a hookworm co-infection. There was a significantly higher number of co-infections with other enteric parasites among the symptomatic (8/28) than asymptomatic (1/20, P=0.04) cases. Bacterial pathogens (*Salmonella*, *Shigella*, *Aeromonas* and *Vibrio cholerae*) were detected in only 13 of the 111 patients screened and only one patient co-infected with *Aeromonas* had a concurrent cryptosporidial diarrhoea.

Demographic data and socio-economic risk factors

Demographic and risk factor data were collected for 39 of 48 of these individuals infected with *Cryptosporidium* spp. Information was not available for 9 patients either because they did not return for follow up or when a confirmatory house visit was conducted, they were discovered to have provided incorrect addresses.

The study included 27 males (mean age 38.6 yr) and 21 females (mean age 33 yr). Of the 39 patients for whom demographic data were collected, 14 lived in rural areas and 25 in urban areas. There was no statistically significant association between place of residence and diarrhoea in the cases, as 64.3 per cent lived in rural areas and 56 per cent in urban areas. Socio-economic status based on income, possessions and education showed that only 3 were from a high socio-economic group, 5 from a middle income group and the majority of cases (31) were from the low socio-economic status groups. Two patients from the high socio-economic groups went on to receive anti-retroviral therapy.

The risk factors for acquiring cryptosporidiosis in HIV that were assessed in the 39 patients included exposure to children less than 2 yr of age, occurrence of diarrhoea in other members of the household, defecation sites, drinking water supply and contact with animals. In 7 households, there were children aged <2yr of which in 2 households, these children belonged to the nuclear family of the patient and in remaining five households, they were part of the extended family that shared the home. None of the patients reported diarrhoea in other members of their households during the two weeks prior to their hospital visit. There was also no significant association between presence of young children in the household, diarrhoea in other household members or defecation sites with diarrhoea in these patients. Piped water supply was used for drinking by 26 patients, bore well water by 9 patients and open well water by 3 patients. Three of 14 rural households had piped water in their homes. The 3 upper income households used bottled water or filters. There was no significant association of type of water supply with symptoms or infecting genotype.

Twenty one patients reported contact with animals. As mentioned previously, zoonotic species identified were 9 *C. parvum* (previously called bovine genotype), 5 *C. felis*, 1 *C.parvum* (mouse), 1 *C. meleagridis* and 1 *C. muris*. Of these 17 patients that have potentially acquired infection by zoonotic transmission, information was available for 15 and of these 9 (60%) reported contact with animals, and all but one of the patients with *C. felis* infection reported animal contact. The animals reported were mainly cows in both urban and rural households, with two rural households with goats and one high socio-economic status urban household reporting the presence of dogs. The association of contact with animals with particular species of *Cryptosporidium* was not statistically significant, but the numbers in each category are small and the association between animal exposure and cryptosporidiosis could not be assessed as data on animal exposure for patients without *Cryptosporidium* were not obtained.

Discussion

Cryptosporidiosis is a substantial threat to HIV infected individuals, who have a lifetime risk of infection of around 10 per cent[15]. Cryptosporidiosis remains an important cause of diarrhoea in the immunocompromised due to the lack of effective therapy. Although cryptosporidiosis in HIV infected patients has been widely reported in India including three reports from our centre[4,5,10,16-21], little is known about circulation and transmission patterns of the infection in this part of the world. Studies from developed countries have found sexual behaviour patterns, immigrant status, pet ownership especially dogs and farm animals, travel outside the country, toileting children and some ethnic populations to be some of the risk factors associated with cryptosporidiosis[6-9,22]. In developing countries, one case-control study from Guinea-Bissau[23] found that significant risk factors were keeping pigs and dogs and storage of cooked food for later consumption. Breastfeeding was negatively associated with risk. However, animal-human mixing patterns, access to potable drinking water and antiretroviral therapy are all factors that play a major role in the risk of acquiring cryptosporidiosis and are different from region to region. Molecular typing data from our centre[10] indicated that though *C. hominis* was the most common species identified, other zoonotic species were also relatively common in HIV infected individuals and so raised questions about the sources of infection. A previous report analyzing isolates from Kenya, Switzerland and the United States identified animal contact in patients (using a questionnaire) infected with zoonotic isolates[24], but this association was not clearly seen in our study. However, the information on animal contact was based on a response to the questionnaire from the patient so casual animal contact not reported by the study participant cannot be ruled out in all cases. While the most common species associated with human cryptosporidiosis is *C. hominis*, several studies indicate that immunocompromised individuals are susceptible to a wider range of species and genotypes and that host factors may play a role in controlling susceptibility to these divergent parasites[25,26]. Immunodeficiency therefore, alters host susceptibility to *Cryptosporidium* spp.

that are not normally infectious in humans[1]. Additionally, variation in virulence among different species has also been demonstrated in volunteer studies with respect to attack rate and duration of diarrhoea[27]. Considerable circumstantial evidence from previous studies have shown a zoonotic exposure in these patients[28], so the poor association with animal exposure in our study indicates that these potentially zoonotic species circulate in the human population but will require further studies involving characterization of isolates from infected animals. Although no statistically significant association was found between most of the risk factors studied and acquisition of cryptosporidiosis in these patients, further studies with a larger number of HIV infected adults for whom more extensive follow up data are available, will be required. This will help in understanding the transmission and natural history of cryptosporidiosis in India. It is necessary to document the public health significance of this infection in immunocompromised hosts in order to formulate more rational approaches to the control of this disease, especially in the developing countries.

It must be noted that these studies can be difficult to conduct because of the stigmatizing nature of the illness. During the course of this study, many problems were encountered because patients did not return for scheduled visits to hospital and when home visits were made, it was discovered that patients had moved or provided incorrect addresses. Such problems have also been encountered by other investigators, who have shown that approximately 70 per cent of individuals return for the results of HIV related tests and less than 50 per cent participate in further studies[29,30].

In conclusion, cryptosporidiosis is an important cause of diarrhoea and associated morbidity in HIV infected individuals. Further studies need to be done with a larger sample to understand risk factors associated with zoonotic transmission of *Cryptosporidium* spp. in the population.

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Table

Socio-demographic characteristics and concurrent opportunistic infections in patients with symptomatic and asymptomatic cryptosporidial infection

Patient characteristics	Symptomatic (n=28)	Asymptomatic (n=20)
Age (yr) (Mean) (SD)	35.5 (6.9)	37.2 (8.7)
Male : female ratio	1.15:1	1.5:1
Piped water supply (%)*	16 (69.6)	10 (62.5)
Area of residence Urban: rural*	1.5:1	2.2:1
Animal contact* (%)	14 (60.9)	7 (43.8)
Concurrent opportunistic infections		
Candidiasis	5	3
Tuberculosis	6	6
Toxoplasmosis	1	0
Cryptococcal meningitis	1	1

* Data available for 23 symptomatics and 16 asymptomatics individuals