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Cryptosporidium and cryptosporidiosis of man and animals in India

M B CHHABRA[1] and K M L PATHAK[2]

National Research Centre on Camel, Bikaner, Rajasthan 334 001 India

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ABSTRACT

The coccidian parasites *Cryptosporidium* spp. have been increasingly reported worldwide from a variety of hosts, causing infection ranging from an asymptomatic carrier state to severe diarrhoea, especially in the young. While prevalence in children with diarrhoea has been found to range from 1.1% to 18.9% at various centres in India. *Cryptosporidium* was also observed as a predominant component of the spectrum of opportunistic enteric pathogens in HIV-infected patients. Neonatal diarrhoea in livestock, particularly in bovines, including outbreaks with mortality, have been recorded. Reported infection in bovines ranged from 11.32% to 69.32% with maximum positive as well as oocyst shedding in diarrhoeic calves upto 30 days of age. Contaminated water, insanitary conditions and contact with animals appear to be major sources of human infection. Zoonotic cryptosporidiosis was more severe and frequently associated with fever. *Cryptosporidium parvum* is the main species in bovines as well as in zoonotic infections, but molecular characterization and genotyping has resulted in identification of at least 6 other species and several more genotypes in India. Diagnosis has mainly relied on coprological techniques based on staining and microscopy, but antigen-detection ELISA and PCR-assays have also been evaluated for greater sensitivity and specificity. Therapeutic management with anticoccidials in calves had limited success, nitozoxanide was reported effective in cryptosporidial diarrhoea in children and azithromycin gave good results in both bovines and humans. This is the first India-specific review of this rapidly emerging disease of zoonotic significance, compiling data and information from both medical and veterinary sources.

Key words: *C*ryptosporidiosis, *Cryptosporidium,* Diarrhoeal disease, India, Zoonosis

The apicomplexan coccidian *Cryptosporidium* (Tyzzer 1907) is an obligate intracellular (but extra-cytoplasmic) parasite with a wide range of vertebrate hosts. First human infection was reported in 1976 but within the next 15 years, its association with diarrhoeal disease worldwide was established, highlighted by a water-borne outbreak involving more than 400,000 persons in Milwaukee, Wisconsin (Fayer *et al.* 2000). The oocyst stage excreted in faeces is important for survival and dispersal primarily as water- and food-borne infection. *Cryptosporidium* is increasingly gaining attention as a human and animal pathogen mainly due to its prime involvement in worldwide waterborne outbreaks (Karanis *et al.* 2007). Up to the year 2007, there were 16 valid species with a further 33+ genotypes described (Smith *et al.* 2007). However by the year 2009, two more species were added making 18 valid species of *Cryptosporidium* from fish, reptiles, birds and mammals reported from different parts of the world, seven species are known to be emerging gastrointestinal parasites of zoonotic importance (Muraleedharan 2009). The first confirmed Indian report of detection of *cryptosporidium* oocysts in faeces of calves came from Uttar Pradesh (Dubey *et al.* 1992).

The major zoonotic species *C.parvum* causes neonatal diarrhoea in livestock with consequent economic loss (Nolan *et al*. 2009). This species is largely responsible for environmental contamination with oocysts. Transmission of zoonotic *Cryptosporidium* occurs through ingestion of oocysts of livestock origin (Hunter and Thompson 2005). Human infection is mainly life-threatening in immuno-compromised cases such as those of HIV-infection. Knowledge on *Cryptosporidium* and cryptosporidiosis has been the subject of several reviews (Fayer and Ungar 1986, Dubey *et al.* 1990, O'Donaghue 1995, Kumar *et al*. 2005, Fayer and Xiao 2008). In India, the reports of human cases of cryptosporidiosis with diarrhoea first appeared in the mid-1980's (Mathan *et al*. 1985, Das *et al.* 1987, Malla *et al.* 1987) while the first report of calf diarrhea associated with *Cryptosporidium* came around the same time (Nooruddin and Sarma 1987). Since then, a fairly large volume of reports and studies particularly on human, dealing with various aspects of the parasite and the disease, have been generated

Present address: [1](chhabra.manmohan@gmail.com) 2518, Sector D-2, Vasant Kunj, New Delhi 110 070.

[2]Deputy Director General (Animal Sciences), Indian Council of Agricultural Research, Room No.113, Krishi Bhavan, Dr. Rajendra Prasad Road, New Delhi 110 114.

to justify a comprehensive review from an Indian perspective.

Epidemiology

Based on microscopic examination of faecal samples with Ziehl Neelsen staining, an incidence of 13.1% in children with diarrhoea was recorded in Vellore, Tamil Nadu (Mathan *et al.* 1985), 1.3% in Chandigarh (Malla *et al.* 1987) while Das *et al.* (1987) in West Bengal found *Cryptosporidium* as the sole agent for acute diarrhoea in 4.45% children and Pherwani *et al.* (1989) reported 4.4% positivity in diarrhoeic children in Mumbai. Cryptosporidiosis as an opportunistic pathogen among cancer patients had been reported with incidence ranging from 1.3% to 2.4% (Sreedharan *et al.* 1996). In a survey of intestinal parasites among the HIV-positive asymptomatic drug users in north-east India, *Cryptosporidium* sp. (94.4%) was most commonly seen (Anand *et al.* 1998). In a hospital based study in north-eastern India, 7.2% (151/2095) of stool samples were positive for *Cryptosporidium* (Nath *et al.* 1999). Cryptosporidiosis was commonly found in hospital cases of diarrhoea, specially in children (Sethi *et al.* 1999) in north India while in HIV-seropositive individuals with diarrhoea, *C. parvum* was the most common (10.8%) in the same area (Mohandas *et al.* 2002). It was regarded as the third commonest microbial infection in AIDS patients. C*ryptosporidium* infection was recorded in 3% of animal handlers in Calcutta (Chattopadhyay *et al.* 2000) which is not insignificant considering that infection is uncommon in normal adults. In diarrhoea patients at Nehru Hospital, Chandigarh (Sehgal *et al.* 2001). C*ryptosporidium* at 4.4% was the second most common parasite detected after *Giardia.* In Puducherry, Parija *et al.* (2001) found the parasite in 5% patients with diarrhoea, age ranging 1–45 years. Prevalence of cryptosporidiosis among children with diarrhoea ranged from 1.1 to 18.9% in different parts of the country (Rao *et al.* 2008). The pathogen was also found significantly associated with infectious diarrhoea in elderly (60 years or older) patients with isolations from 18.3% (Gambhir *et al.* 2003), most infections occurring during the rainy season. A study in Chennai (Kumar *et al.* 2002) found coccidian parasites in etiologic agents of diarrhoea among HIV-positive patients with 13.7% detected positive for cryptosporidiosis. Among HIV-seropositive patients in and around Amritsar (Bajaj *et al.* 2003), diarrhoeal illness was present in 35% and C*ryptosporidium* was detected (18%) as the commonest protozoan pathogen. Faecal examination of HIV-infected patients having diarrhoea in Manipur (Singh *et al.* 2004), revealed 57.6% positive for parasitic infections, of which *C. parvum* was identified as predominant. According to Sadraei *et al.* (2005), opportunistic protozoa should be expected in HIV-infected patients with low CD4+ and diarrhoea. Cryptosporidial infections were observed in 20% of renal transplant recipients (Udgiri *et al.* 2004) but only a few were symptomatic. *Cryptosporidium* was found to be a predominant component of the spectrum of opportunistic parasitic infections, accounting for 23.37% in Mumbai (Lanjewar *et al.* 1996), 17.5 in Mangalore, South India (Shenoy *et al.* 2003), 43.0% in Manipal, Karnataka (Singh *et al.* 2003), 25.0% in Vellore, Tamil Nadu (Rao Ajjampur *et al.* 2007), 28.7% in Madurai (Ramakrishnan *et al.* 2007), 33% in Delhi (Dwivedi *et al.* 2007), 18.66% in Kashmir (Hajam *et al*. 2008), 43% in Kolkata (Chakraborty *et al.* 2008), 39.8% in Varanasi (Tuli *et al.* 2008) and 31.3% in a rural community in Maharashtra (Gupta 2011). The last named (Tuli *et al.* 2008) also reported an inverse correlation between CD4 counts of HIV-patients and enteric protozoans of which C*ryptosporidium* spp. was the one most isolated. *Cryptosporidium parvum* was the dominant entity of enteric protozoan parasites identified in HIV-seropositive individuals with diarrhoea in Gulbarga, Karnataka state (Kanta and Gangane 2008). Early detection of opportunistic protozoan infections and prompt treatment can delay the progression of HIV-infections (Uppal *et al*. 2009). In animals, cryptosporidial oocysts in zebu calves (17.7%) and buffalo calves (16.6%) were reported from Bareilly, Uttar Pradesh (Dubey *et al.* 1992). Prevalence in and around Calcutta was recorded in 11.32% of cattle, 12.97% of buffaloes and nil in of dogs, the positivity being higher in diarrhoeic animals (Chattopadhyay *et al.* 2000). A later report (Das *et al.* 2003) from the same area, using a different staining technique, found 20.8% samples positive, with higher % positivity in diarrhoeic cattle. Further, the study found highest infection during the cooler months whereas as per an earlier report (Prasad *et al.* 1989), the infection was highest in the warm and humid months. Cryptosporidiosis in buffalo calves matches that of bovines in prevalence, but appears less severe clinically (Gopalnath 1997). Nighot *et al.* (2002) reported first record of natural cryptosporidiosis in chicken in India. In Puducherry, Kumar *et al.* (2004) recorded the highest infection (25%) in calves below 1 year of age, 2.5% in adult cattle, 7.69% in goats and 4.35% in poultry. In Andhra Pradesh, Shobhamani *et al.* (2005) found prevalence of cryptosporidiosis in 19.52% of 374 non-diarrhoeic and 48.38% of 277 diarrhoeic calves. Oocysts of *Cryptosporidium* were detected in faecal samples of both diarrhoeic and non-diarrhoeic cattle and buffalo calves at Izatnagar, Uttar Pradesh (Jeyabal and Ray 2005). Another study (Shobhamani and Singari 2006) reported the overall prevalence in calves with diarrhoea in and around Tirupati, Andhra Pradesh at 31.8% of 651 faecal samples from dairy herds, with highest positivity in young calves upto 60 days of age, and slightly higher infection in summer than in monsoon and winter months. Interestingly, farm calves, mainly Holstein Friesian (HF) and Jersey crossbreds were found to have a much higher prevalence (32.89%) than rural calves (17.39%). Das and Rajan (2006) reported C*ryptosporidium* infection in equines. In dairy farms in Punjab (Singh *et al.* 2006), oocysts were detected in 50% of diarrhoeic and 25.68% non-diarrhoeic

animals. The prevalence of infection peaked in young calves between 0 and 30 days in both diarrhoeic and non-diarrhoeic groups (86.4 and 66.6%, respectively). High mortality and case fatality rates were observed in young calves. In West Bengal (Gunjan Das *et al.* 2006), 32.9% of calves were found affected with Cryptosporidiosis. The observations of another study from the same state (Roy *et al.* 2006c), were essentially corroborative of the same trend with prevalence of C*ryptosporidium* infection at two different farms being significantly higher than at the local Khattal. In a study to assess the significance of *cryptosporidium* as the causative agent (Roy *et al*. 2006a), overall detection was almost identical at 17.46% and 18.04% (470 samples/year) in the two years. Detection in diarrhoeic cases was 26.79 and 27.49% and in non-diarrhoeic cases, 8.13 and 8.59% in related period. Examination of faecal samples of mithun (*Bos frontalis)* in Nagaland (Rajkhowa *et al.* 2006), revealed overall prevalence of *C.parvum* at a high 56%, highest (81%) prevalence in young mithuns of 1–6 months of age, and in diarrhoeic animals (94%) in comparison to non-diarrhoeic group (51%). Prevalence in mithuns kept in semi-intensive system was higher (64%) than those in free-range conditions (40%). Sheikh *et al.* (2007) reported the overall prevalence of 29.37% after testing 395 faecal samples of dairy calves of upto 6 months in Kashmir valley. Higher prevalence in calves < 1 month of age (33.79%) and higher shedding of oocysts were observed. Prevalence was higher in winter (43.97%) and low in autumn (16.48%). In a subsequent report (Sheikh *et al.* 2009) on cryptosporidial infection in lambs in Kashmir valley, overall prevalence of 38.18%, higher in diarrhoeic (43.72%) than non-diarrhoeic (29.87%) lambs were recorded. Diarrhoea as well as oocyst excretion rate was more frequent in lambs < 1 month of age (66.67%). Prevalence during winter/spring was significantly higher (46.70%) as compared to summer/autumn (27.75%). Infection in dairy calves at Hisar, Haryana (John and Kapoor 2009) at faecal examination was 45% overall, with higher prevalence rate found in diarrhoeic calves (64.86%), < 1 month age calves (69.23%) and crossbred calves (50%). The pattern of prevalence and intensity studied by Mallinath *et al.* (2009) in bovine calves in and around Bengaluru was essentially similar to the earlier studies, viz. higher in calves < 1 month-old than adult animals, and in diarrhoeic as against non-diarrhoeic samples. Interestingly, while prevalence was highest in HF crossbreds, followed by Jersey cross, no C*ryptosporidium* oocyst were found in non-descript breeds or in Deoni and Surti buffalo calves.

Transmission of cryptosporidiosis is frequent from person to person, animal to animal, animal to man, or through indirect routes like contaminated water and food. Outbreaks of chlorine-resistant C*ryptosporidium* have motivated water authorities to reassess the adequacy of current water quality regulations (Sharma *et al.* 2003). *Cryptosporidium* oocysts had been isolated from the water supply of Chennai (Muthukumar *et al.* 2007), presenting a public health hazard necessitating surveillance of city water quality. A study on detection of enteric parasites in unprocessed food from northern India (Rai *et al.* 2008) indicates that unhygienic practices of food handlers may be a major contributor in the transmission of *Entamoeba, Giardia* and C*ryptosporidium* especially in raw meat and milk. Populations at greater risk include animal handlers (Chattopadhyay *et al.* 2000), others with close contact with animals (Gambhir *et al.* 2003) and slum dwellers due to poor sanitary practices (Palit *et al.* 2005).

Species identification

Initial reports in India, human as well as animal, were based mainly on oocyst morphology and staining characteristics. As such identification was made upto the genus level or as *C. parvum,* being the predominant cause of diarrhoeal disease in bovines as well as human infections. However, subsequently two separate definitive genotypes were recognized, one strictly human and the other, primarily zoonotic. The former had then been proposed as a separate species called *C. hominis* (Morgan-Ryan *et al.* 2002). Nighot *et al*. (2002) observed a case of cryptosporidiosis in chicken which they regarded as the first record in India. They however did not identify the species which from the description of lesions appeared to be *C. baileyi.* The occurrence of *C. andersoni* in cattle was reported by Kumar *et al.* (2004). In Punjab (Singh *et al.* 2006), *C. parvum* was reported as the prevalent infection in cattle associated with calf diarrhoea, on oocyst identification. Roy *et al.* (2006) carried out molecular characterization of the causative agent and concluded that *C. parvum* was mainly responsible for bovine disease in India. Likewise, another study based on molecular characterization of bovine *Cryptosporidium* isolates in India (Paul *et al.* 2008) indicated that *C. parvum* was the only species prevalent in neonatal bovine calves of 3 different geographical regions of the country. According to an international group (Feng *et al.* 2007), genotyping studies on specimens from geographically diverse sources, which included India, indicated that 85% of pre-weaned calves were infected with zoonotic *C. parvum* while in the post-weaned animals, *C. bovis* and a C*ryptosporidium* deer-like genotype were much more prevalent. In Bengaluru, Mallinath *et al.* (2009) identified two species, viz. *C. parvum* and *C.andersoni* in bovines based on oocyst morphology and micrometry while from Triupati (A.P.), Shobhamani *et al.* (2009) identified *C. parvum* and *C.muris* from diarrhoeic calves. A molecular epidemiological survey among cattle (Paul *et al.* 2009a) demonstrated *C.andersoni* as the major *Cryptosporidium* species affecting juvenile and adult cattle in 3 different agro-climatic geographic regions of India. PCR-RFLP analysis and nucleotide sequencing of bovine and human isolated species revealed that *C. parvum* and *C. hominis* were the species mainly responsible for diarrhoea

in cattle and human, respectively at Kolkata in India (Roy *et al.* 2010)

Characterization of Cryptosporidium *sp.*

Isolates from HIV-infected individuals by multi-locus genotyping (Muthusamy *et al.* 2006) identified *C. hominis, C. parvum, C. felis, C. muris and C. meleagridis.* C_{PgP} 40/15 PCR-restriction fragment length polymorphism identified six subgenotypes. Cryptosporidial diarrhoea was associated with decreased CD4 counts below 200 (P, 0.009). In Spain *Cryptosporidium felis* was reported (Llorente *et al.* 2006) in a diarrhoeic 4-year old boy who had come via adoption from an orphanage in Kolkata. After oocyst findings in faecal samples, species was determined by RFLP analysis and sequencing of the PCR product. Molecular characterization of C*ryptosporidium* by PCR restriction length polymorphism analysis from children in Kolkata (Das *et al.* 2006) identified at least 3 genotypes. Out of 40 positive samples, 35 were positive for *C.hominis,* 4 for *C. parvum* and 1 was positive for *C.felis.* In a similar study in children in an urban slum in South India (Rao Ajjampur *et al.* 2007), the two most common species were *C.hominis* (81%) and *C. parvum* (12%). Other species identified were *C. felis* and *C. parvum* (mouse genotype). Among 5 subgenotypes identified, one revealed similarities to *C. meleagridis.* Fifty C*ryptosporidium* positive specimens collected from pediatric patients in Kolkata during 2001–04 (Gatei *et al.* 2007), were analysed for parasite genetic structure using multilocus sequence typing (MLST). Genotype analysis showed the presence of *C. hominis, C. meleagridis* and *C. felis* in 49, 2 and 1 patient, respectively (two patients had mixed infection of *C. hominis* and *C. meleagridis).* A study from Andhra Pradesh (Nagamani *et al.* 2008) using DNA extraction and PCR-RFLP for species identification of *Cryptosporidium* in young children, HIV-seropositive adults and HIV-seronegative adults, found the parasite in all 3 groups, with *C.hominis* (71.7%) and *C.parvum* (18.9%) as the only 2 species detected. The high occurrence of *C.parvum* in this series was considered as significant indicator of zoonotic transmission.

Clinical manifestations

Cryptosporidium infects primarily the intestinal epithelium but may be disseminated to epithelial cells of other organs e.g. bile duct or the respiratory tract (Krauss 2003). In clinically overt disease, the main symptoms are profuse water diarrhoea, abdominal pain (Sehgal *et al.* 2001). Children in the age group of six months to one year were found to be the most vulnerable (Nagamani *et al.* 2007). In immuno-compromised individuals such as HIV-positive patients, the diarrhoea is more persistent and often associated with fever, weight loss and anorexia (Rao Ajjampur *et al.* 2007, Kanta and Gangane 2008). Symptomatic patients had a significantly higher rate of co-infections with other enteric parasites. Intestinal parasites including C*ryptosporidium* were found associated with malabsorption syndrome, particularly in malnourished children (Behera *et al.* 2008). A case of respiratory infection caused by *C.parvum* in a 10-year-old child who had been on corticosteroids for 6 months (Shrikhande *et al.* 2009). The child had history of low-grade fever, breathlessness and cough with expectoration of 3 months' duration, but was non-reactive for HIV. Simultaneous gastrointestinal involvement with *C.parvum* was observed.

Among animals, *Cryptosporidium* is an important cause of calfhood morbidity. In India, association of cryptosporidiosis with diarrhoea in calves is known since Nooruddin and Sarma (1987) and was subsequently substantiated by several reports (Shobhamani *et al.* 2005, 2006; Singh *et al.* 2006). Although concurrent infections with other enteric pathogens were detected (Shobhamani and Singari 2006), C*ryptosporidium* could cause significant pathogenic effects as the single infectious agent. Majority (68.87%) of the calves with cryptosporidial infections had watery diarrhoea indicating this feature as characteristic of clinical cryptosporidiosis. Further, clinical manifestations observed (Shobhamani *et al.* 2006) were duration of diarrhoea from 2–23 days, dehydration, mild fever, depression and varying degree of anorexia. In calves above one month of age the diarrhoea was self-limiting. Mortality is generally low but in severe fulminating infections, it may be as high as 35% in young calves (Singh *et al.* 2006). In this outbreak, no mortality was recorded in calves older than 30 days. Haemato-biochemical changes included significantly decreased Hb, PCV, TLC, mild leucopenia, neutropenia, lymphopenia, eosinophilia, hypoglycemia, hypoproteinemia, reduced serum sodium and potassium values (Shobhamani *et al.* 2007a). Association between cryptosporidial infection and diarrhoea in lambs has also been reported (Sheikh *et al.* 2009) with high (66.67%) frequency in lambs below one month age. Gross and histopathological lesions observed in naturally infected chicken were documented involving bursa and intestines (Nighot *et al.* 2002).

Immunity

Cryptosporidiosis is self-limiting in immunocompetent humans but can be life-threatening in immuocompromised hosts such as HIV patients. Both duration and outcome of cryptosporidial diarrhoea depend on the immune status of the host. Severe chronic infections may develop in immunocompromised hosts with lymphocyte or gammaglobulin deficiencies which suggest that both cell-mediated and humoral immune responses are involved in resolution of infections and development of protection. Older animals are less severely affected and age resistance has been demonstrated in some host species (Singh *et al.* 2006). *Cryptosporidium* induces significant lymphoproliferative response in immuno-competent individuals while cytokine responses were identical in infected immunocompetent and

immunocompromised persons (Kaushik *et al*. 2009a). However, protective immune responses in cryptosporidiosis appear to involve multiple aspects of the immune system which may not be fully described by proliferative and cytokine responses. A later study (Kaushik *et al.* 2009b) showed specific serum IgG and IgA production in patients infected with C*ryptosporidium,* both HIV-seropositive and sero-negative as compared to uninfected subjects suggesting induction of C*ryptosporidium* specific humoral immune response, with no role of HIV status. However, according to these authors, such antibody responses may not necessarily be protective from symptoms.

Diagnosis

Round, sporulated oocysts (4 to 5 ìm) may be detected microscopically in faecal sample. Concentration by floatation is usually done with Sheather's sugar solution and staining by modified Ziehl Neelsen (ZN) technique. In India, this technique has been widely in use for detection in humans (Sehgal *et al.* 1989, 2001; Mohandas *et al.* 2002; Nagamani *et al.* 2008) as well as in animals (Chattopadhyay *et al.* 2000, Jeyabal and Ray 2005, Singh *et al.* 2006, Shobhamani *et al.* 2009). As alternative, kinyoun acid fast staining (Kumar *et al.* 2002, Das *et al.* 2003, Roy *et al.* 2006a) or Safranin methylene blue (Sethi *et al.* 1999, Bajaj *et al.* 2003) had been used by some investigators. According to Kumar *et al.* (2004) mucus portion of the faecal sample was found ideal for diagnosis. Detection of C*ryptosporidium* oocysts in bovines using indirect immunofluorescence assay with *C.parvum* oocyst monoclonal antibody was reported by Roy *et al.* (2006b) who found this method more sensitive than the conventional staining techniques. Molecular diagnosis of *C.parvum* through polymerase chain reaction (PCR) using genomic DNA extracted directly from faecal samples of cattle was reported (Das *et al.* 2004). All the faecal samples assayed showed a DNA band at 825 bp specific for *C.parvum* confirming the sensitivity and specificity of nested PCR. A commercially available ELISA kit (Rajkhowa *et al.* 2006) was satisfactory for detection in mithuns. Antigenc detection by simple and rapid chromatographic lateral flow immunoassay detected higher percent positivity for C. parvum from buffalo (23.52) and cross bred cattle calves (12.50) as compared to modified Ziehl-Neelsen (mZN) staining in buffalo (17.65) and crossbred cattle calves (6.25) (Singla et al. 2010). In an evaluation of microscopy and ELISA for cryptosporidiosis in remote areas (Barua *et al.* 2008) microscopy was considered more efficacious and modified ZN staining technique proved superior diagnostic method. Polymerase chain reaction (PCR) offers a useful alternative to conventional coprological techniques. In a comparative study on modified ZN, Safronin methylene blue (SM), antigen detection ELISA and a nested PCR assay for detection of C*ryptosporidium* in HIV seropositive and seronegative patients with diarrhoea (Kaushik *et al.* 2008), while sensitivity of PCR was 100% in both HIV seropositive and seronegative patients, sensitivity of Ag-ELISA was 92.6% and 94.1% respectively. The ZN and SM techniques were much less sensitive than these two. Specificity of PCR, ZN and SM was 100% while specificity of Ag-ELiSA was 92.2% and 96.3% in HIV seropositive and seronegative patients. A comparative evaluation of 4 coprological diagnostic techniques, viz. direct faecal smear staining (DFSS), normal saline sedimentation staining (NSSS), Sheather's floatation (SF) and Sheather's floatation sedimentation staining (SFSS) with PCR directed against the 185 SSU æRNA gene as standard reference test for the diagnosis of cryptosporidiosis, was carried out (Paul *et al.* 2009b). Out of 457 faecal samples from neonatal bovine calves, PCR amplification was achieved in 138 whereas positivity was 65 samples by DFSS, 92 by NSSS, 82 by SF and 109 by SFSS. Sheather's flotation sedimentation was found to be the most sensitive (82.6%) and specific (98.76%) among the coprological methods. On the basis of per sample cost analysis, DFSS was found to be the most economical. Tuli *et al.* (2010) found significant increase in sensitivity of microscopy in detecting C*ryptosporidium* after formal-ether concentration. They also ranked Kinyoun's staining as better than ELISA due to ease and cost-effectiveness.

Treatment and Control

In immunocompetent patients the infection is usually self-limiting, with possible need only for restoration of fluid and electrolyte levels. Short-term azithromycin treatment for cryptosporidial diarrhoea in AIDs patients gave good clinical improvement but parasitological benefit was doubtful (Kadappu *et al*. 2002). Among anti-diarrhoeals for management of childhood diarrhoea, nitazoxanide had been reported to have high efficacy against cryptosporidial diarrhoea only (Alam and Bhatnagar 2006). Oral azithromycin for 5 days (Karanth *et al.* 2007) resulted in reduced frequency of stools, and negative results of stool examination in a severe human case of cryptosporidiosis concurrent with relapsed Hodgkin's disease. Paramomycin, often used in human patients with HIV-associated cryptosporidiosis, had been shown to give good response in infected calves (Sabu 2005). Most clinical cases in calves respond to supportive therapy with I.V. fluid supplementation with sodium bicarbonate. Therapeutic management of C*ryptosporidium*—related diarrhoea in bovine calves with anti-coccidial (furazolidone and sulfadimidine) and antidiarrhoeal agents with parenteral fluid therapy gave better recovery (71.4%) compared with untreated control (Gunjan Das *et al.* 2006). A clinical trial in C*ryptosporidium* positive bovine calves (Shobhamani *et al.* 2007b) found that the antibiotic azithromycin and the anticoccidial tylosin, both were able to combat the cryptosporidial infection. The dehydrated calves in both the groups received supportive therapy of Ringer's lactate intravenously. However,

azithromycin was found to be more efficacious on the basis of clinical improvement, absence of oocysts in faecal samples and return of haemato-biochemical parameters to normalcy.

Cryptosporidium oocysts are extremely resistant to a variety of commonly used disinfectants, but infectivity of oocysts can be destroyed after heating to 73°C for one minute. Effective removal of C*ryptosporidium* by a bio-flocculant (extracellular biopolymer produced from *Klebsiella terrigena)* as alternative to routinely used chemical flocculants for removal of C*ryptosporidium* was reported (Ghosh *et al.* 2009).

CONCLUSION

A number of studies have established *Cryptosporidium* spp. as a major cause of diarrhea in neonatal calves in India. Apart from *C.parvum* which is the major zoonotic species found mainly in pre-weaned calves, *C.bovis, C. muris* and *C.andersoni* also affect bovines. The disease was more severe in European breeds and their crosses, with high morbidity and mortality. Clinical cryptosporidiosis in neonatal lambs appears to be equally serious. In the background of a solitary report from poultry, investigators need to be primed to look for C*ryptosporidium* in this livestock species as molecular biology and genotyping assays have indicated the presence of *C.baileyi* in India. Cryptosporidial prevalence should also be explored in cats and pigs in view of the proven existence of *C.felis*. Pets do not seem to have a role in zoonotic cryptosporidiosis. In HIV-seropositive patients with diarrhoea, early detection of enteric pathogens and prompt treatment is of paramount importance. At organized farms, with history of endemicity, periparturient cows should be isolated and new born calves should be reared separate from adult cattle.

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