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BOVINE CRYPTOSPORIDIOSIS: BRIEF REVIEW OF ITS DISTRIBUTION IN INDIA

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ABSTRACT
C*ryptosporidium*, a monoxenous, obligate intracellular-extra-cytoplasmic apicomplexan protozoan, infects humans, domestic, wild animals and birds. The disease caused by this smallest intestinal coccidian generated great public health interest after a large human water borne outbreak in Milwaukee in 1993. Oocysts of this protozoan are resistant, remain viable in the harsh environmental conditions and are transmitted by faeco-oral route, but zoonotic and individual-to-individual transmission is well documented. Bovine cryptosporidiosis is a common cause of neonatal diarrhoea sometimes in the form of outbreaks with mortality and consequent economic loss. With increase in age and immunity infection subsides in the older cattle. Prevalence in bovines ranges from 11.32% to 69.32% with maximum positive cases in diarrhoeic calves upto one month of age. *Cryptosporidium parvum* is the main species in bovines as well as the species of major zoonotic importance. In this review, historic background, taxonomy, public health significance, transmission and distribution of the disease in India are brought up and potential control measures in the prevention of cryptosporidiosis are also discussed.

KEY WORDS: calves, *Cryptosporidium* spp., diarrhoea, prevalence, prevention, public health.

INTRODUCTION

Cryptosporidiosis caused by members of *Cryptosporidium* spp. (a unicellular microscopic apicomplexan protozoan parasite) is a long lasting debilitating diarrheal disease which is usually acute and self-limiting in immunocompetent individuals but can be life threatening to the immunocompromised patients. *Cryptosporidium*, a zoonotic parasite, inhabits the intestinal and respiratory surface epithelium of 152 species of mammals including humans, birds, reptiles, amphibians and fish (Fayer and Xiao, 2008). The first description of *Cryptosporidium* was given by Ernest Edward Tyzzer (1907) who isolated it in the peptic glands of laboratory mice mouse. He regarded this protozoan to be an extracellular species, which resembles the coccidia and named it as *Cryptosporidium muris*. Subsequently, he proposed the genus *Cryptosporidium* with *Cryptosporidium muris* as the type species (Tyzzer, 1910). *Cryptosporidium* spp. was believed to be a commensal until 1950s when its relationship with diarrhoea in young turkeys (*C. meleagridis*) was observed. Since then more than 20 species have been reported from domestic and wild animals, humans, birds, fish and reptiles *Cryptosporidium bovis* has been described recently (Fayer *et al.,* 2005).

The scientific community ignored this parasite for almost 75 years and the report by Panciera *et al.* (1971) on the importance of *Cryptosporidium* in diarrhoeic calves provided a major stimulus to veterinary interest and subsequently led to much experimental work. However, *Cryptosporidium* received veterinary and public health attention in the 1980s due to its increasing impact on human health and association with the newly described acquired immunodeficiency syndrome (AIDS) (Casemore *et al.,* 1985). Cattle and buffaloes are the most important animal group recognized to be infected with *Cryptosporidium.* The first report of calf diarrhea associated with *Cryptosporidium* in India came from Nooruddin and Sarma (1987) and first confirmed case *C. parvum* from India in calves of Uttar Pradesh was reported by Dubey *et al.* (1992).

C. parvum and *C. andersoni* have been recognized as two species that commonly infect cattle throughout the world (Peng *et al.,* 2003). *C. parvum* infects the epithelial lining of the small intestines mostly of pre-weaned calves as well as humans and other animals, frequently instigating diarrhoea syndrome (Casemore *et al.,* 1997). Although calves of 1-3 weeks of age are susceptible but cattle of >2 years age have also been identified to be affected by the disease. With increase in age and immunity infection recedes in the older cattle. Animals that are severely affected die due to malabsorption and dehydration, hence, causing severe economic losses. In addition, prolonged illness results in decreased productivity and increased labour and veterinary costs in the form of drugs and veterinary aid contribute to further losses (de Graaf *et al.,* 1999).

Table 1. Distribution of bovine cryptosporidiosis in India

Author	Prevalence (%)	Host species	Place
Dubey *et al.* (1992)	17.7 16.6	Zebu calves buffalo calves	Uttar Pradesh
Khubnani *et al.* (1997)	10.9	Adult cattle and claves	Maharastra
Jithendran and Bhat (1999)	1986-90 Cattle (1.5) and buffalo (2.1) 1993-97 Cattle (0.7) and buffalo(5.6)	Cattle and buffalo	Himachal Pradesh
Chattopadhyay *et al.* (2000)	11.3 12.9	Cattle buffaloes	West Bengal
Das *et al.* (2003)	20.8 with higher prevalence in diarrhoeic cases	cattle	West Bengal
Das *et al.* (2004)	22.4 Winter (45.16), summer (27.2) and rainy season (19.2)	cattle	West Bengal
Kumar *et al.* (2004)	25 2.5	bovine calves <1 year adult cattle	Pondicherry
Shobhamani *et al.* (2005)	19.52 non-diarrhoeic; 48.38 diarrhoeic	bovine calves	Tirupati
Jeyabal and Ray (2005)	35.7 < 1 month, 50 in 1-3 month age	calves	Uttar Pradesh
Shobhamani and Singari (2006)	31.8	cattle	Tirupati, Andhra Pradesh
Roy *et al.* (2006)	17.46 and 18.04 in first and second year study	cattle	West Bengal
Singh *et al.* (2006)	50 diarrhoeic; 25.68 non-diarrhoeic	Cattle calves	Punjab
Gunjan Das *et al.* (2006)	32.9	cattle	West Bengal
Sheikh *et al.* (2007)	29.37 between 1 day to 6 months	calves	Kashmir valley
Paul *et al.* (2008)	38.02	Cattle calves	Bareilly, U.P.
Pradeep (2008)	18.6 and 27.0	Cattle and buffalo	Uttar Pradesh, Karnataka and Kerala
John and kapoor (2009)	45 .00	calves	Hisar, Haryana
Mallinath *et al.* (2009)	24.20 diarrhoeic; 16.60 non-diarrhoeic	cattle	Bangalore, Karnataka
Prakash *et al.* (2009)	9.05	dairy calves	Chennai, Tamilnadu
Sahu and Maiti (2009)	22.27 and 20.9 in Durg and Rajnandgaon districts, respectively	bovine calves	Chhattisgarh
Sahu *et al.* (2010)	21.9	Cattle and buffalo	Chhattigarh
Khan *et al.* (2010)	11.7	Cattle	West Bengal
Yadav *et al.* (2010)	28.1	Cattle and buffaloes	
Das *et al.* (2011)	20.8 (32.9 diarroeic; 7.1 non -diarrhoeic)	cattle	West -Bengal
Maurya (2011)	18.7	Cattle and buffalo	Uttar Pradesh, Uttarakhand, Bihar
Bhat *et al.* (2012a), Bhat *et al.* (2012b)	38.3 and 38.9	Buffalo and Cattle	Ludhiana, Punjab
Venu *et al.* (2012)	Overall 39.65 (Highest Puducherry-86.67 and lowest, Kerela-17.65)	Cattle and buffalo	South staets viz.Andhra Pradesh, Karnataka, Kerala, Tamil Nadu and union territory, Puducherry)
Singla *et al.* (2013)	23.52 and 12.50 by immunoassay and 17.65 and 6.25 by modified Ziehl-Neelsen staining	buffalo and crossbred cattle calves	Punjab
Rajkhowa *et al.* (2006)	64 in semi-intensive system and 40 in free-range conditions.	Mithun (*Bos frontalis*)	Nagaland
Maurya *et al.* (2013)	16.3 and 24.2	Cattle and buffalo calves	Uttar Pradesh, Uttarakhand, West Bengal, Bihar, Karnataka and Kerala.
Rana *et al.* (2011)	3.52	Buffalo calves in an organized herd	Haryana

Taxonomy

The genus of *Cryptosporidium* was classified under the family cryptosporidiidae, sub-order – eimeriorina, order – eucoccidiorida, subclass- coccidiasina, class- sporozoasida, phylum- apicomplexa (Levine 1985, Fayer and Ungar 1986). More than 20 species of this coccidian protozoon have been described on the basis of the animal hosts from which they were isolated. *C. hominis* found primarily in humans, *C. parvum*, found in humans and other mammals, *C. andersoni* and *C. bovis* in cattle, *C. canis* in dogs, *C. muris* in mice, *C. felis* in cats, *C. wrairi* in guinea-pigs, *C. suis* in pigs, *C. fayeri* in red kangaroo (Ryan *et al.*, 2008), *C. macropodium* in grey kangaroo, *C. meleagridis* in turkeys and humans, *C. baileyi* in chickens, *C. galli* in adult hens and some wild birds (Pavlasek, 1999 and 2001), *C. varanii* in

emerald monitor lizards, *C. serpentis* in snakes and lizards, *C. molnari* and *C. scophthalmi* in fish (Fayer, 2008)., C. xiaoi from sheep and goat (Fayer and Santin, 2009), *C. ubiquitum* from wild and domesticated ruminants, rodents, carnivores and primates including humans (Fayer *et al.*, 2010) and *C. ducismarci* from tortoises (Traversa, 2010) *C. parvum*, *C. andersoni*, *C. baileyi* and *C. meleagridis* have been reported to cause morbidity and outbreaks of disease in livestock (OIE, 2008).

Public health significance

Cryptosporidiosis is an emerging waterborne zoonotic disease of great public health importance. In immunodeficient individuals, the disease may run as a life threatening prolonged diarrhoea, which does not respond to antibiotic treatment. *Cryptosporidium* is progressively inviting attention as a zoonotic protozoan largely due to its overriding involvement in worldwide waterborne outbreaks (Karanis *et al.*, 2007). After the large human water borne outbreak occurred in 1993 in which estimated 403,000 residents of the greater Milwaukee, Wisconsin, area of USA, got infected *Cryptosporidium* generated great public health interest worldwide (MacKenzie *et al.*, 1994). Eight *Cryptosporidium* species i.e. *C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, *C. canis*, *C. suis*, *C. muris* and *C. andersoni* infect immunocompetent and immunocompromised humans (Xiao *et al.*, 2004; Cacciò *et al.*, 2005; Nichols *et al.*, 2006, Feltus *et al.*, 2006), but *C. hominis* and *C. parvum* are the most commonly detected (Cacciò *et al.*, 2005). The sequel as well as pathogenesis of the disease depend on the host immune status, ranging from a severe but self-limiting diarrhea in immunocompetent individuals (DuPont *et al.*,1995) to a debilitating, life-threatening, prolonged infection in immunocompromised individuals such as AIDS patients (Chalmers and Davies, 2010). In India, Human cases of cryptosporidiosis with diarrhoea for the first time appeared in the mid-1980's. (Mathan *et al.*, 1985; Das *et al.*, 1987; Malla *et al.*, 1987).

Life cycle and transmission

Cryptosporidium spp. has monoxenous type of life cycle and begins with the intake of the sporulated oocyst which is roughly 5 µm in diameter and contains four naked sporozoites each measuring 5 x 1 µm. Oocysts of this protozoan are resistant and remain viable in the harsh environmental conditions and are transmitted by faeco-oral route (Fayer, 2004), but zoonotic and individual-to-individual transmission is well documented (O`Donoghue, 1995). The prepatent period is generally 4 days. An oocyst contains four infective sporozoites, which are released through a suture situated along one side of the oocyst. Sporozoites upon reaching their favourite site, ileum penetrate individual epithelial cells. Following developmental forms are usually found at the microvillous surface of epithelial cells which are intracellular but extracytoplasmic (Fayer, 2004). Extensive recycling of merozoites from type first schizont and production of thin walled auto-infective oocysts is responsible for massive number of *Cryptosporidium* in clinically affected host. Affected animals upon recovery become carriers and hence act as source of infection to the susceptible individuals (OIE, 2008).

Clinical signs

In India, *Cryptosporidium* is associated with calf hood morbidity and diarrhoea since the initial reports by Noorudin and Sarma (1987) and Dubey *et al.* (1992). The clinical symptoms in cattle include dehydration, diarrhoea which is accompanied with mucus, dullness, inappetance and fever. Affected calves do not respond to antibiotic therapy and in more severe cases, dehydration and cardiovascular collapse lead to mortality (Olson *et al.*, 2003). Clinically duration of diarrhoea in neonatal calves varied from 2-23 days with dehydration, mild fever, depression and varying degree of anorexia (Shobhamani *et al.*, 2006). 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 neonatal calves (Singh *et al.*, 2006). Sandford and Josephsen (1982) observed varying degree of morbidity as high as 40% but generally low mortality during *Cryptosporidium parvum* infection. Affected calves show reduced growth rate (OIE, 2008). Mortality rate of upto 35% was observed by Singh *et al.* (2006) in calves less than 1 month age. *C. andersoni* that inhabits the digestive glands of the abomasum of post weaned calves and adult cattle has been reported to cause reduced milk yield in dairy cattle (Lindsay *et al.*, 2000). The endogenous stages of the protozoa destroy microvilli of peptic glands which may justify the increased quantity of plasma pepsinogen detected in diseased cattle (Anderson, 1998).

Epidemiology

Cryptosporidiosis has been recognised as an emerging threat worldwide. Reports of cryptosporidiosis have been made from many countries including United States (Sischo *et al.*, 2000; Santin *et al.*, 2004; Fayer *et al.*, 2006), Canada (Olson *et al.*, 1997), South America (Xiao *et al.*, 2001), United Kingdom (Sturdee *et al.*, 2003), Norway (Hamnes *et al.*, 2006), Iran (Meamar *et al.*, 2007; Nikaeen *et al.*, 2005),Turkey (Arslan *et al.*, 2001), Russia (Pashkin *et al.*,1988),

Africa (Ayeni *et al.*,1985), Pakistan (Nasir *et al.*, 2009), Thialand (Nuchjangreed *et al.*, 2008), Australia (Jerrett and Snodgrass, 1981) and Japan (Sakai *et al.*, 2003). In India bovine cryptosporidiosis was first reported by Nooruddin and Sarma (1987) and later, it was reported in Uttar Pradesh by Dubey *et al.* (1992) and different other parts of the country by several workers (Dubey *et al.*, 1992; Das *et al.*, 2004; Jeyabal and Ray, 2005; Roy *et al.*, 2006; Singh *et al.*, 2006; Singh *et al.*, 2006; Sheikh *et al.*, 2007; Paul *et al.*, 2008; Mallinath *et al.*, 2009; Yadav *et al.*, 2010; Das *et al.*, 2011; Bhat *et al.*, 2012a and 2012b; Venu *et al.*, 201; Maurya *et al.*, 2013). Prevalence of bovine cryptosporidiosis from different parts of the country is mentioned in Table 1.

Diagnosis

Diagnosis is made conventionally by microscopy after staining feacal smears with Modified Ziehl-Neelsen or Auromine Phenol methods for detection of round, sporulated oocysts of 4 to 5 µm in size. In India, this technique has been widely in use for diagnosis of cryptosporidiosis in animals (Chattopadhyay *et al.*, 2000; Jeyabal and Ray, 2005; Singh *et al.*, 2006; Shobhamani *et al.*, 2009; Bhat *et al.*, 2012a; Randhawa *et al.*, 2012a; Randhawa *et al.*, 2012b; Singla *et al.*, 2013). Kumar *et al.* (2004) found mucus portion of the faecal sample ideal material for diagnosis. The immunological approaches like direct immunofluorescence, enzyme linked immunosorbent assay and immunochromatography for the detection of *Cryptosporidium* oocysts are useful but inherit the limitation of species identification (OIE, 2008). Antigenc based simple and rapid chromatographic lateral flow immunoassay has got more efficiency for detection of *C. parvum* from buffalo and cross bred cattle calves as compared to modified Ziehl-Neelsen staining (Singla *et al.*, 2013).

The polymerase chain reaction has revolutionized the field of diagnosis in parasitology. The advantages of PCR for the detection of *Cryptosporidium* in clinical and environmental samples include sensitivity, ease of use, ability to analyse large numbers of samples at one time, relatively low cost, ability to speciate (thus eliminating false positives encountered with cross reactions of antibodies to non-pathogenic protozoan species) and 'strain typing' potential (thus allowing the source of infection to be determined). The application of genetic tools has led to enhanced understanding of the, biology, epidemiology, ecology and population genetics of *Cryptosporidium* species (Jiang and Xiao, 2003; Monis *et al.*, 2005; Gasser, 2006; Smith *et al.*, 2006). Molecular methods have been used for identifying *Cryptosporidium* species/genotypes in human and non-human hosts (Fayer, 2008; Xiao and Ryan, 2008). *Cryptosporidium* 18s rRNA gene loci, 20 copies of which are present per oocyst, provide proper targets (Smith *et al.*, 2009).

A higher degree of sensitivity of nested PCR assay was reported by different workers from different parts of the world (Xiao *et al.*, 1999; Sturbaum *et al.*, 2001; Nikaeen *et al.*, 2005) and India (Roy *et al.*, 2006; Paul *et al.*, 2009; Bhat *et al.*, 2014).

Prevention

Control of the bovine cryptosporidiosis is challenging because so far no drug or vaccine has been found effective against the disease (Woods *et al.*, 1996). A lot of chemotherapeutic trials have been attempted but no successful treatment for cryptosporidiosis has been identified (Mead, 2002); however some agents may be encouraging (Ollivett *et al.*, 2009). This has restricted the path of control in dairy farms to the utilisation of effective management practices, hygiene and sanitation of dairy premises along with diagnostic tools. The most helpful approach to control this protozoon is to keep preventive measures in place. Oocysts of *Cryptosporidium* can be destroyed by applying 5% ammonia solutions on surfaces of housing facilities (Campbell *et al.*, 1982), especially if coupled with heat. One of good preventive actions is to isolate the diseased animals and feed adequate colostrum to the newborn calves. Passively acquired antibodies have not been effective in protecting calves against *Cryptosporidium* infections (Harp *et al.*, 1989) but calves that were fed with hyperimmune colostrum from immunized dams established less severe diarrhea and released fewer oocysts than calves fed "non-hyperimmune" colostrum (Harp *et al.*, 1989). Some research groups have published data on passive immunization and immunotherapy against *C. parvum* using different zoite surface glycoproteins expressed during, and involved in, invasion and infection of host epithelial in mice, goats and cattle cells (Fayer *et al.*, 1989; Harp and Goff, 1995; Sagodira *et al.*, 1999). However, a successful vaccine has yet to become commercially available. The 15 kDa 123 amino acid antigen of *C. parvum* designated CP15/60 (GenBank Accession No. L34568) was identified by Jenkins and Fayer (1995). Attempts to design effective chemotherapeutic or immunoprophylatic agents have been unsuccessful due to a lack of understanding of basic cellular and molecular biology of the opportunistic parasite. However, halofuginone lactate was found to have anticryptosporidial effect

(Jarvie *et al.*, 2005; Klein, 2007). Combination of metronidazole and furazolidone induced clinically and parasitological recovery in dairy cattle calves of Punjab, India (Randhawa *et al.*, 2012a).

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