ABSTRACT

Cryptosporidium, 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 up to 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
The genus *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 kangaroos, *C. meleagris* in turkeys and humans, *C. baileyi* in chickens, *C. galli* in adult hens and some wild birds (Pavlasek, 1999 and 2001), *C. varani* in

<table>
<thead>
<tr>
<th>Author</th>
<th>Prevalence (%)</th>
<th>Host species</th>
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<tr>
<td>Dubey et al. (1992)</td>
<td>17.7</td>
<td>Zebu calves</td>
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<td></td>
<td>16.6</td>
<td>buffalo calves</td>
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<td>Khubhamni et al. (1997)</td>
<td>10.9</td>
<td>Adult cattle and calves</td>
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<td>1986-90</td>
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<td>1993-97</td>
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<td>Cattle</td>
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<td>12.9</td>
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<td>20.8</td>
<td>cattle</td>
<td>West Bengal</td>
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<td>Das et al. (2004)</td>
<td>22.4</td>
<td>Winter (45,16), summer (27.2) and rainy season (19.2)</td>
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<td>bovine calves &lt;1 year adult cattle</td>
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<td>2.5</td>
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<td>bovine calves</td>
<td>Tirupati</td>
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<td>&lt; 1 month, 50 in 1-3 month age</td>
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<td>18.04</td>
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<td>25.68 non-diarrhoeic</td>
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<td>Sheikh et al. (2007)</td>
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<td>diarrhoeic; 16.60 non-diarrhoeic</td>
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<td>22.27</td>
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<td>Chhattinggarh</td>
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<td>20.9</td>
<td>in Durg and Rajanandgaon districts, respectively</td>
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<td>Cattle and buffalo</td>
<td>Chhattigarh</td>
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<td>28.1</td>
<td>Cattle and buffaloes</td>
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<td>20.8</td>
<td>cattle</td>
<td>West -Bengal</td>
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<td>(32.9 diarrhoeic; 7.1 non -diarrhoeic)</td>
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<td>18.7</td>
<td>Cattle and buffalo</td>
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<td>Bhat et al. (2012a), Bhat et al. (2012b)</td>
<td>38.3 and 38.9</td>
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<td>Venu et al. (2012)</td>
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<td>Cattle and buffalo</td>
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<td>(Highest Puducherry-86.67 and lowest, Kerala-17.65)</td>
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<td>23.52</td>
<td>buffalo and crossbred cattle calves</td>
<td>Punjab</td>
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<td>12.50 by immunoassay and 17.65 and 6.25</td>
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<td>64</td>
<td>Mithun (Bos frontalis)</td>
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<td>Uttar Pradesh, Uttarakhand, West Bengal, Bihar, Karnataka and Kerala</td>
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<td>Rana et al. (2011)</td>
<td>3.52</td>
<td>Buffalo calves in an organized herd</td>
<td>Haryana</td>
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**Taxonomy**

The genus *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 kangaroos, *C. meleagris* in turkeys and humans, *C. baileyi* in chickens, *C. galli* in adult hens and some wild birds (Pavlasek, 1999 and 2001), *C. varani* in
Cryptosporidiosis has been recognised as an emerging threat worldwide. Reports of cryptosporidiosis have been made in 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 (Hannes et al., 2006), Iran (Meamar et al., 2007; Nikaeen et al., 2005), Turkey (Arslan et al., 2001), Russia (Pashkin 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, anappetence 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 (Hannes et al., 2006), Iran (Meamar et al., 2007; Nikaeen et al., 2005). 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).
Africa (Ayeni et al., 1985), Pakistan (Nasir et al., 2009), Thailand (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 fecal 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; Shohhamani 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 (Ollivet 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 protozoan 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 immunoprophylactic 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 anticyryptosporidial effect.
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