



## Review Article

### Cryptosporidiosis in Goats: a Review

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#### ARTICLE HISTORY

Received: 2014-01-20  
Revised: 2014-02-20  
Accepted: 2014-02-22

#### Key Words:

Cryptosporidium,  
Cryptosporidiosis, Goats,  
Neonatal kids, Diarrhoea

#### ABSTRACT

Cryptosporidiosis is a diarrhoeal disease caused by members of the genus *Cryptosporidium*, an obligate intracellular protozoan parasite belonging to the phylum Apicomplexa. It causes diarrhoea in neonatal animals by infecting the intestines in an acute short-term manner. *Cryptosporidium parvum* is regarded as an important etiological agent of diarrhoea in neonatal ruminants, causing substantial economic losses both directly and indirectly. It spreads through the feco-oral route, frequently through contaminated water. The infected animals excrete oocysts in their faeces, which is resistant against inclement weather conditions. Upon ingestion the oocysts presumably excyst in the small intestine and invade the intestinal epithelium. *Cryptosporidium* does not require a vector or intermediate host and is capable of completing its life cycle within a single host, and it is also capable of autoinfection. Typically, neonatal animals below 3 months of age display clinical signs for a period varying from 5–15 days. The predominant symptom of cryptosporidiosis is mild-to-severe diarrhoea, but other clinical symptoms may include depression, dehydration, anorexia, listlessness, unthriftiness and abdominal pain. Cryptosporidiosis is an important zoonotic disease. Infected domestic and feral animals are considered as to be important sources in contaminating the environment and there have been many well-documented outbreaks of human cryptosporidiosis around the world. The diagnosis for *Cryptosporidium* infection is usually carried out through examination of faecal smears for the presence of oocysts by Modified Ziehl Neelsen (mZN) technique. Oral or intravenous fluid therapy to prevent dehydration is the most important treatment to alleviate the clinical signs of disease in both humans and animals due to absence of a single effective drug. At present *Cryptosporidium* is regarded as one of the major enteric pathogen in goat kids and morbidity could be high in outbreaks of cryptosporidiosis in kids. Under these circumstances, the control measures against the disease mainly rely on the knowledge on epidemiology of the disease. But, in India there are only a few published reports of goat cryptosporidiosis therefore more studies are required in the area of caprine cryptosporidiosis.

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ARTICLE CITATION: Paul S, Sharma DK, Boral R, Mishra AK, Shivsharanappa N, Banerjee PS and Pawaiya RVS (2014). Cryptosporidiosis in goats; a review. *Adv. Anim. Vet. Sci.* 2 (3S): 49 – 54.

#### INTRODUCTION

India stands first in goat population in the world which is approximately 154 million along with an annual growth rate of 4 %. Under the existing socio-economic circumstances in the country wherein the per capita land holding is meagerly 0.2 Ha, goat rearing has emerged as an important component of mixed farming system. Goat farming is considered as the best option for the rural farmers in developing countries. Goat farming improves the status of household nutrition and economy as well as boosts capital storage and self employment (Basic Animal Husbandry Statistics, 2006; Kumar, 2007). Ease in animal handling and the ability to efficiently convert limited and non-conventional feed resources into meat and milk are also

decisive factors that had boosted goat farming in rural people and small-scale farmers. A serious constraint to economical and intensive goat production is the mortality of kids as a result of diarrhoea (15–40%) up to the age of 3 months. Among the various diarrhoeal pathogens of goats viz viruses, bacteria and parasites, *Cryptosporidium* spp. is the one principally involved (Noordeen *et al.*, 2000; Ershaduzzaman *et al.*, 2007).

According to the International Commission on Zoological nomenclature (ICZN) there are about 20 valid species of *Cryptosporidium* which infect both warm blooded and cold blooded animals including mammals, birds, reptiles and fishes (Xiao, 2010; Fayer *et al.*, 2010). Cryptosporidiosis in goats is a well-known disease which

causes neonatal kid diarrhoea and incurs significant production loss to the goat husbandry. The agents responsible for causation of the disease in goats are *Cryptosporidium parvum*, *C. hominis* and *C. xiaoi*; However, *C. bovis* has also been reported from goats and apparently there is report of a *Cryptosporidium* goat genotype (Xiao *et al.*, 2010). It is one of the major health problems among the neonatal goats kids and apart from mortality (up to 40%), cryptosporidiosis causes decline in productivity, retarded growth, decreased feed efficiency, delayed maturity, loss of fertility and overall financial loss in the form of treatment of ailing animals (Paraud and Chartier, 2012). There is no innate resistance to infection and neither is any passive protection transmitted to the newborns through colostrum (Viel *et al.*, 2007, Paul *et al.*, 2009a). Therefore, the impact of the disease varies widely depending on susceptibility of the animals, presence of carrier status and stability of infection in the premises. The situation is further worsened by the lack of any vaccine against the disease and cumbersome diagnostic procedures. Control measures against this disease are not well defined due to the fact that normally used anticoccidial drugs are ineffective against this organism (Xiao *et al.*, 2010). Effective control of the disease is probably possible with integrated programme that utilizes appropriate diagnostic tools and sound sanitary and management practices (Fayer and Ungar, 1986). Absence of an effective drug or vaccine against the disease poses a constraint towards the effective control of the disease.

#### ***Cryptosporidium*: Taxonomy and Life Cycle**

The genus *Cryptosporidium* is classified under the family Cryptosporidiidae sub-order- Eimeriorina, Order- Eucoccidiorida, Subclass- Coccidiasina, and Class- Sporozoasida. Phylum- Apicomplexa (Pellardy, 1965; Hammond and Long, 1973; Levine, 1985). Members of this protozoan genus were previously thought to be closely associated with the coccidians owing to their morphological similarities and presence of organelle resembling mitochondria (Tetley *et al.*, 1999). However, molecular phylogeny data suggested that the members of genus *Cryptosporidium* were more closely related to the gregarines (Barta and Thompson, 2006) which were further supported by the presence of stages like gregarines in their life cycle (Hijjawi *et al.*, 2002).

The member of the genus *Cryptosporidium* parasitizes the microvillous border of the gastrointestinal (G.I) epithelium of a broad range of vertebrates, viz., cattle, buffalo, sheep, goats, reptiles, including humans (Fayer *et al.*, 1997). The infection cycle results in production of a robust encysted oocyst stage which is discharged in the faeces of infected host in magnitude of billions. Transmission of the parasites is direct, either by the faeco-oral route or contamination of water supplies with the oocysts which are the infective stages of the parasite (Fayer, 2004). Oocysts are fully sporulated when excreted. There are two types of oocysts – the thick walled oocysts and the thin walled oocysts. The thick walled oocysts excreted in faeces and are infective to other hosts whereas, the thin-walled oocysts burst while in intestine and the released sporozoites give rise to endogenous autoinfection which is a unique characteristic of *Cryptosporidium* spp. (Solusby, 1982; Levine, 1984; Mehlhorn, 1988). After ingestion by the host the

oocyst excysts, thereby releasing four motile sporozoites that subsequently invade and parasitize the epithelial cells in the gastrointestinal tract (and rarely in extra intestinal sites). Successive endogenous developmental stages are generally seen at the microvillar surface of epithelial host cells and are usually intracellular but extracytoplasmic in location (Fayer, 2004).

#### **Epidemiology of Caprine Cryptosporidiosis**

Neonatal deaths are recognized as an important developmental hurdle which negatively affects the economy of goat husbandry. The generally held assumption that *Escherichia coli* is a major cause of diarrhoea in goat kids was confronted by some reports wherein comprehensive laboratory analysis revealed the fact that *Cryptosporidium* spp. were one of the most common etiological agents in diarrhoeic neonatal kids which had previously been overlooked. The first case of *Cryptosporidium* infection in goats was documented from Australia, in which a two week old Angora goat kid died after suffering from diarrhoea for a short period; histopathology and electron microscopy revealed the endogenous developmental stages in the intestine (Mason *et al.*, 1981). In subsequent epidemiological studies *Cryptosporidium parvum* was found as one of the most common etiological agent causing diarrhoea neonatal kids (Smith and Sherman, 1994; Ozmen *et al.*, 2006). Caprine cryptosporidiosis has been reported all over the globe viz., Australia (Tzipori *et al.*, 1982), South America (Viera *et al.*, 1997, Bomfim *et al.*, 2005) France (Castro-Hermida *et al.*, 2005), Spain (Castro-Hermida *et al.*, 2007; Diaz *et al.*, 2010) and other European countries (Thamsborg *et al.*, 1990; Molina *et al.*, 1994; Molina *et al.*, 1996). In the Indian subcontinent the disease has been reported from Sri Lanka (Noordeen *et al.*, 2000, 2001). Lately, cryptosporidiosis in goats has also been reported from India (Paul *et al.*, 2013; Maurya *et al.*, 2013).

#### **Sources and Transmission of Infection**

Being a monoxenous parasite with direct life cycle, the cryptosporidial infection is transmitted through feco-oral route by the ingestion of oocysts through contaminated feed and fodder or drinking water. Young animals account for the main source of environmental contamination. The rate of excretion of oocysts depends upon the severity of infection as well as the age of the animal (Paraud *et al.*, 2009). However, adult animals also excrete oocysts in the environment but the magnitude varies. Studies have revealed the evidence of rise in oocyst excretion three weeks around parturition in adult goats (Castro-Hermida *et al.*, 2005). Nevertheless, the main environmental contamination is contributed by the young kids. The infectious dose is very low for neonates and the minimum infectious dose in gnotobiotic lambs varies between one to five oocysts (Blewett *et al.*, 1993).

#### **Pathogenesis and Pathology of Cryptosporidiosis**

The pathogenesis of cryptosporidiosis is rather unclear. The characteristic diarrhoea results from maldigestion and malabsorption owing to the reduction in both enzymatic action and absorptive area in the gastrointestinal tract due to diminution of microvilli and destruction of intestinal epithelia by the parasite. The mucosal damage inflicted by *Cryptosporidium* is linked with increase in paracellular

permeability of the gastrointestinal tract and destruction of the functional mucosal barrier system (Klein *et al.*, 2008).

Studies of *C. parvum* infection in calves have shown that jejunum and ileum is mainly affected and the diarrhoea occurs either due to the hindrance in sodium absorption coupled with increased prostaglandin production in the intestinal mucosa or due to the increase in the mucosal permeability (Foster and Smith, 2009).

There are no pathognomonic lesions of cryptosporidiosis. However, the intracellular but extracytoplasmic location of the endogenous stages in intestinal mucosa is quite characteristic. Macroscopical lesions may include catarrhal enteritis. Histological examination may show stunting and fusion of villi as well as replacement of enterocytes by immature cells. The characteristic oocysts of *Cryptosporidium* spp are seen on the microvillar epithelium of jejunum and ileum (Klein *et al.*, 2008).

#### Clinical Signs

In outbreaks of cryptosporidiosis in goat kids the morbidity varies from 80–100% and the mortality may exceed 50% in young kids (Thamsborg *et al.*, 1990; Chartier *et al.*, 1996, 1999). Flock mortality may increase with concomitant infections with other pathogens, nutritional deficiencies and unhygienic managemental practices that facilitate contamination and propagation of oocysts in the environment. Aggravating factors include stress, concurrent infections with other enteropathogens and infective dose. A positive correlation exists among the rate of excretion of oocysts and the severity of clinical symptoms (Paraud and Chartier, 2012).

Caprine cryptosporidiosis is mainly a disease of young kids of 0–2 months old, the prepatent period is around 4 days and clinical symptoms are more prominent in young kids. The predominant symptom of cryptosporidiosis is mild-to-moderate or severe diarrhoea, but other clinical symptoms may include depression, dehydration, anorexia, listlessness, unthriftiness and abdominal pain. The diarrhoeic faeces is yellow in colour, pasty to liquid in consistency, have an offensive odour and contains large number of oocysts ( $10^5$  to  $10^7$  oocysts/g). The infection subsides with attainment of immunological maturity; the recovered animals become a carrier, thereby serving as a potential source of infection to susceptible population. In the adults, the disease runs a chronic course characterized by progressive loss in body weight but most of the infected animals remain asymptomatic. The rate of excretion of oocysts and OPG (oocyst per gram of faeces) counts were considerably higher in goat kids below 6 months of age followed by those below 12 months of age as compared with kids above 12 months of age and adults (Paraud and Chartier, 2012).

#### Diagnosis

There are no special techniques for diagnosis of cryptosporidiosis in goats; the diagnostic procedures for bovine or human cryptosporidiosis are applicable for the detection of *Cryptosporidium* spp in goats.

#### Differential Staining Techniques

There are several differential staining techniques; However, the “gold standard” and most widely used staining technique for the detection of *Cryptosporidium* oocyst in stool is the modified Ziehl–Neelsen staining (Henricksen and

Pohlenz, 1981) or modified Kinyoun staining (Fayer *et al.*, 2000). The detection limit of modified Ziehl–Neelsen staining was reported to be 50,000 oocyst per gram of faeces (Balatbat *et al.*, 1996) whereas, that of modified Kinyoun technique was  $1-5 \times 10^4$  oocyst per gram of faeces (Weber *et al.*, 1991).

#### Concentration Techniques

Various procedures for concentration of *Cryptosporidium* oocyst from faeces have been discussed in literature among which Sheather’s sugar floatation is the most widely used and sensitive technique (Fayer *et al.*, 1997). A modified Sheather’s floatation technique was reported by Current *et al.* (1983) which was highly sensitive for selective purification and recovery of oocysts from faeces. Arrowood and Sterling (1987) reported up to 72% recovery of oocyst from crude faeces by discontinuous sucrose step–gradient centrifugation technique. This method was comparable to isopycnic percoll gradient centrifugation which yielded 79% recovery of oocysts from crude faeces. Of the various density gradient methods, Caesium chloride (CsCl) density gradient centrifugation was reported to be the most sensitive technique (Fayer *et al.*, 1997). A sophisticated procedure for concentration and purification of oocyst is immuno–magnetic separation (IMS) using magnetisable particles coated with antibodies; this method is used to obtain highly purified oocysts for subsequent biological studies (Parker and Smith, 1994).

#### Immunological Diagnosis

A number of immunological diagnostic tests have been described for cryptosporidiosis.

The sensitivity and specificity of direct fluorescent antibody (DFA) test were reported to be 96–100% and 99.8–100% respectively, and was equal to conventional faecal smear examination following concentration (Johnston *et al.*, 2003). A number of antigen capture ELISAs were reported with detection limit in the range of  $3 \times 10^5$ – $10^6$  oocysts per gram of faeces, which indicated that the assays did not appear to have superior sensitivity over microscopical methods (Anusz *et al.*, 1990; Robert *et al.*, 1990). The monoclonal antibody based immunofluorescence test was found to be more efficient than modified Kinyoun technique (Alles *et al.*, 1995), whereas equal sensitivity and specificity of direct fluorescent antibody (DFA) test and modified Ziehl Neelsen staining (mZN) technique was reported by Kehl *et al.* (1995). The Solid phase qualitative immune chromatographic assay (Garcia *et al.*, 2003) and immuno–chromatographic dip strip test (crypto–strip) used monoclonal antibody and a gold conjugate to give a sensitive and specific diagnosis (Llorente *et al.*, 2002).

#### Nucleic Acid Based Diagnosis

Molecular or DNA based diagnostic methods target the DNA instead of parasite protein antigens, and is more stable and free from phenotypic variations. The PCR protocols so far described could detect as few as 10–50 *Cryptosporidium* oocysts per sample, while the most sensitive PCR assays can detect as low as 1 oocyst per sample (Gibbons *et al.*, 1998; Xiao *et al.*, 1999; Coupe *et al.*, 2005). One of the drawbacks of these PCR assays is that they demand preparatory DNA purification protocols, which are time consuming and tedious. Also, the presence of ubiquitous PCR inhibitors in faecal samples can cause great problems (Wilson, 1997).

Alternate protocols for direct PCR assays for faecal samples without DNA purification requirements although described, are far from satisfactory for use as routine diagnostic assays with respect to fidelity considerations and ease of test protocols. Various genetic loci have been targeted for PCR assays but the PCR–RFLP method based on 18S small sub unit rRNA gene for *Cryptosporidium* identification is a sensitive method, both for diagnosis and genetic characterization of species, and is the most used assay for differentiation of *Cryptosporidium* spp. (Xiao *et al.*, 1999, 2004).

A comparative evaluation of four 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 18S SSU rRNA gene as standard reference test for the diagnosis of bovine cryptosporidiosis, revealed that SFSS was the most sensitive (82.6%) and specific (98.76%) among the coprological methods; whereas, DFSS was found to be the most economical one (Paul *et al.* 2009).

#### Treatment of Caprine Cryptosporidiosis

Supportive therapy includes rehydration, feed supplementation and administration of anti-diarrhoeals. There is no specific cure for cryptosporidiosis in goats, several drugs have been analyzed for their cryptosporicidal activities, which includes  $\alpha$ -Cyclodextrin (Castro–Hermida *et al.*, 2001),  $\beta$ -Cyclodextrin (Castro–Hermida *et al.*, 2004), Decoquinat (Ferre *et al.*, 2005), Nitazoxanide (Viel *et al.*, 2007), Tilmicosin (paraud *et al.*, 2010), Halofuginone lactate (Giadinis *et al.*, 2007, 2008) and Paromomycin sulphate (Johnson *et al.*, 2000; Viu *et al.*, 2000).

#### Control and Management

In the absence of any innate resistance and marked effect of passive protection transmitted to the new born kids through colostrum (Current *et al.*, 1985) the impact of the disease varies widely depending on susceptibility of the animals, presence of carrier status and stability of infection in the premises. The situation is further worsened by the fact that no satisfactory vaccine or specific cryptosporicidal drugs are still available. Under these circumstances, effective control of the disease is probably possible with the knowledge of epidemiology of the disease coupled with the use of appropriate diagnostic tools, supportive treatment and segregation of affected animals along with sound sanitary and management practices.

#### Zoonotic Aspect of Cryptosporidiosis

Cryptosporidiosis is a highly zoonotic disease and *C. parvum* is the only parasite under Bioterrorism grp. B pathogen. *Cryptosporidium* infection has been reported from 3 day old neonates to 95 years old persons, but clinical data imply that young children constitute the principal risk group (Fayer *et al.*, 1997). The first report of extensive human cryptosporidiosis surfaced in 1982, in the United States (U.S) with the advent of acquired immune deficiency syndrome or AIDS. Within two years it became obvious that another high risk group were the immunocompromised patients. In 1986 the U.S. Center for Disease Control (CDC) described that 3.6% of 19,817 AIDS cases were positive for cryptosporidiosis and the case fatality rate was 61% (Fayer, 2004). In 1993 an extensive outbreak of cryptosporidiosis

occurred in Milwaukee, Wisconsin, U.S when one of the two water treatment plants for the city became contaminated. During a period of two weeks 403,000 people developed the disease among which 103 patients died showing symptoms like fever, diarrhoea, dehydration and abdominal cramps. The 1993 Milwaukee outbreak is documented as the largest waterborne disease outbreak in the history of U.S (Mckenzie *et al.*, 1994).

Human cryptosporidiosis runs a short course and is ordinarily a self-limiting disease in immunocompetent individuals. In humans, cryptosporidiosis, for immunocompetent hosts, is usually a self-limiting disease (Arrowood, 1997). However, in pediatric, geriatric and immunocompromised patients, *Cryptosporidium* spp. infection is accompanied by a high mortality rate (Casemore *et al.* 1997; Fayer *et al.* 1997; O'Donoghue 1995). In immunocompromised patients, the infection may also spread to extra-intestinal site, in such patients the diarrhoea often become persistent and the resultant dehydration may be life threatening (O'Donoghue 1995). Cryptosporidiosis is also regarded as an important cause of diminished growth rate and weakened cognitive function among the children in developing countries (Cacciò 2004; O'Donoghue 1995).

#### CONCLUSIONS

Goat husbandry constitutes an integral part of the agrarian economy of India. Goat meat is free from religious taboos and widely consumed and relished all over the country. Additionally, the demands for goat milk and goat milk products are also increasing in the country. There had been few studies related to prevalence of cryptosporidiosis in goats, but a thorough and detailed investigation towards the epidemiology and genetic characterization of *Cryptosporidium* spp. in goats is very important to India to assess the potential risk of zoonotic transmission of goat *Cryptosporidium* spp. to human as the importance of goat as food animal is ever increasing.

#### ACKNOWLEDGEMENTS

The authors thankfully acknowledge Director, CIRG, Makhdoom; ICAR and SERC, DST Govt. of India.

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PDF | Cryptosporidiosis is a diarrhoeal disease caused by members of the genus *Cryptosporidium*, an obligate intracellular protozoan parasite belonging to the phylum Apicomplexa. It causes diarrhoea in neonatal animals by infecting the intestines in an acute short-term manner. . . .  
Cryptosporidiosis in Goats: a Review. *Advances in Animal and Veterinary Sciences*. 2 (3S): 49 - 54.  
Purpose of Review. *Cryptosporidium* is an intestinal apicomplexan recently classified as a gregarine. It is associated with diarrhea both in immunocompromised and immunocompetent patients. Direct person-to-person, zoonotic, and waterborne are the principal transmission routes. Despite the significant impact on public health of cryptosporidiosis, especially in developing countries, there are few studies on this parasitic disease. . . . More genetic studies on circulating species and subtypes are required in order to characterize the transmission dynamics of cryptosporidiosis in the country, including host range, reservoirs, and infection sources. **Keywords.**  
*Cryptosporidium* Prevalence Species Subtypes Colombia.