



Periparturient rise in the *Cryptosporidium* oocyst count in Beetal goats and evaluation of infection in new born kids

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ABSTRACT

In cryptosporidial infection, very scarce literature is available about periparturient pattern of oocyst excretion in goats and its implications in the epidemiology of disease in kids; thus the present investigation was done. Faecal samples (160) were examined from 20 pregnant goats, collected at weekly interval, commencing from 4 weeks before kidding up to 3 weeks after kidding. Faecal sample examination by Sheather's floatation sedimentation technique followed by modified Ziehl-Neelsen staining technique revealed an overall positivity of 26.25% for *Cryptosporidium* oocysts. Prevalence of oocysts excretion was high around parturition and the number of oocysts shed oscillated between 20–400 oocysts/g (OPG) of faeces. Examination of 60 faecal samples of 20 kids born to studied goats from 1 to 3 weeks of age revealed 40% samples positive for *Cryptosporidium* oocysts and 65% kids became infected by the end of the 3 weeks. The OPG of kids ranged from 100 to 1,560. Molecular characterization by PCR-RFLP of 18S small subunit (SSU) rRNA gene showed that 73.80% and 26.19% goat samples, and 87.5% and 12.5% kids samples were positive for *C. parvum* and *C. ubiquitum*, respectively. The study indicated periparturient transmission of *Cryptosporidium* spp. from pregnant goats to their new born kids.

Key words: *Cryptosporidium*, Goat, PCR-RFLP, Periparturient rise, Oocyst

Cryptosporidial infection in goat kids is associated with diarrhoea and high mortality in kids aged 1–2 weeks, with prevalence rates ranging from <5% to >35% (Robertson 2009). Periparturient egg rise in helminthic infection is a well known phenomenon in ewes (Gonzalez *et al.* 1990) but very scarce literature is available about periparturient rise in *Cryptosporidium* oocysts (Castro-Hermida *et al.* 2005, Paraud *et al.* 2014). Adult animals can act as asymptomatic carriers shedding small numbers of oocysts to the environment, which increase in number in the perinatal period and contribute in maintaining the infection between lambing periods (Xiao *et al.* 1994, de Graaf *et al.* 1999). A significant relationship has been demonstrated between the environment of the kids during their first hours and their infection, suggesting a predominant role of dams in transmission of infection (Delafosse *et al.* 2006). An increase of *Cryptosporidium* spp. oocyst excretion has previously been described around parturition (Castro Hermida *et al.* 2005).

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In India, although cryptosporidiosis had been reported in does and goat kids (Rajendran *et al.* 2011, Maurya *et al.* 2013) but except Jammu and Kashmir (J&K), no detailed epidemiological studies involving molecular characterization have been documented. Ahmad (2012) reported 40.41% prevalence in goats of Jammu region using acid-fast staining technique. Yadav *et al.* (2016) reported the occurrence of *Cryptosporidium parvum* and *C. ubiquitum* species in goats. Since the adult goats maintain the infection and transmit it to kids, the oocyst shedding pattern is of immense importance to evaluate the oocyst load in barn environment. Moreover, it needs to be validated that the *Cryptosporidium* spp. excreted from the adult goats are infecting the new born kids. In cattle, it was demonstrated that the species of *Cryptosporidium*, which are excreted by adults are different from those infecting calves (Santin *et al.* 2008, Bhat *et al.* 2013) and there is involvement of complex etiologies involving multiple agents (Brar *et al.* 2017). Hence, keeping in view the paucity of information available in India about periparturient *Cryptosporidium* oocyst rise in goats and subsequent transmission to kids, this study was conducted.

MATERIALS AND METHODS

Faecal sample collection: Pregnant Beetal does (20; 2–3 year-old) were selected for the study from October 2015

to August 2016, at Government Goat Breeding Farm, Rajbagh, Kathua, Jammu and Kashmir. The faecal samples were collected at weekly interval commencing from 4 weeks before the planned kidding date to 3 weeks after kidding and stored at 4°C before being analysed.

Examination of faecal samples for *Cryptosporidium* spp. and quantification of oocysts: The faecal samples were processed as per the most sensitive (82.6%) and specific (98.76%) modified Sheather's sucrose floatation technique (Current *et al.* 1983, Paul *et al.* 2009) and examined by modified Ziehl-Neelsen technique (mZN) (Henricksen and Pohlenz 1981) for the presence of *Cryptosporidium* spp. oocysts.

The faecal smears prepared using 50 µl of aliquot and examined by mZN staining technique were also used for counting the number of *Cryptosporidium* oocysts in the faeces. In positive cases, the number of oocysts per gram (OPG) of faeces were calculated by multiplying the total number of oocysts detected in the smear by 20 (Ortega-Mora *et al.* 1999).

Characterization of *Cryptosporidium* spp.: DNA was extracted from *Cryptosporidium* positive faecal samples by using faecal stool DNA extraction kit (Qiagen) as per the manufacturer's protocol, with the addition of 8 freeze-thaw (freezing in liquid nitrogen and immediate thawing at 90°C) cycles prior to re-suspension in lysis solution to rupture *Cryptosporidium* oocysts. DNA was stored at -20°C before being used in polymerase chain reaction (PCR).

The 18S rRNA nested PCR protocol was adopted for detection of *Cryptosporidium* spp. (Xiao *et al.* 1999, 2001). For genotyping of *Cryptosporidium* spp., a restriction fragment length polymorphism (RFLP) pattern analysis of nested PCR products was conducted by using the restriction enzymes *SspI* and *VspI* fast digest (Xiao *et al.* 2001). The digested products were analyzed by agarose gel electrophoresis for identification of the species of *Cryptosporidium*.

The results were analysed using one-way ANOVA (Snedecor and Cochran 1994) statistical software packages for social science (SPSS). In the analyses, $P \leq 0.05$ was set for significance.

RESULTS AND DISCUSSION

As in other farm animals, cryptosporidiosis in goats is considered as an economically important disease with

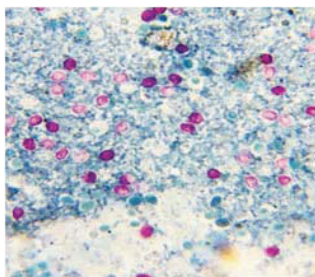


Fig. 1. *Cryptosporidium* oocyst in faeces using modified Ziehl - Neelsen staining. $\times 1000$.

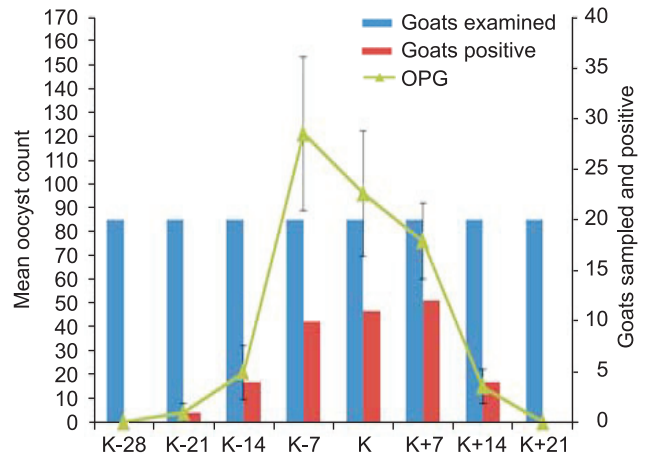


Fig. 2. Mean OPG of *Cryptosporidium* oocysts excreted by goats before, during and after kidding (where K is kidding and \pm days after kidding).

clinical manifestations and death in kids (Vieira *et al.* 1997) and adult goats (Johnson *et al.* 1999). In the new millennium, cryptosporidiosis has been recorded in lambs and goat kids throughout the globe (Mirhashemi *et al.* 2016). In India, *Cryptosporidium* has been reported in does and goat kids (Rajendran *et al.* 2011, Maurya *et al.* 2013). In the present study, examination of 160 faecal samples of goats before, after and during kidding revealed an overall positivity of 42 samples (26.25%) for *Cryptosporidium* oocysts (Fig. 1). Ahamed (2012) recorded 40.41% prevalence of *Cryptosporidium* oocysts in goats aged < 6 months in Jammu region (J&K), while Yadav *et al.* (2016) recorded 27.09% prevalence in faecal samples of 262 pre-weaned lambs and goat kids of the same area. In our study, the number of oocysts shed oscillated between 20–400 oocysts per gram (OPG) of faeces in the does in and around kidding. The mean number of oocysts excreted was significantly ($P < 0.05$) higher on 3 sampling dates, i.e. 1 week before and after kidding, on the day of kidding in comparison to other sampling dates (Fig. 2). This study confirmed the periparturient rise of oocyst excretion as previously described in goats (Paraud *et al.* 2014). In nematode infections, the PPR was attributed to immunosuppression caused by increased plasma prolactin levels around parturition (Gibbs 1986). Ortega-Mora *et al.* (1999) reported that PPR has been associated with the altered state of immunoreactivity during late pregnancy and lactation and thought to be associated with sex hormones and pregnancy associated proteins impacting on regulatory T-cell function or effector B-cell activity. Because administration of corticosteroids induces a recrudescence of cryptosporidiosis in previously infected mice, gerbils or cats (Asahi *et al.* 1991), increased progesterone and estrogen levels during late pregnancy may also be involved in the PPR of *C. parvum* oocysts, especially for the increased excretion at parturition.

Examination of 60 faecal samples of kids revealed that 40% of faecal samples were positive for *Cryptosporidium* oocysts. Romero-Sales *et al.* (2016) reported varying extent

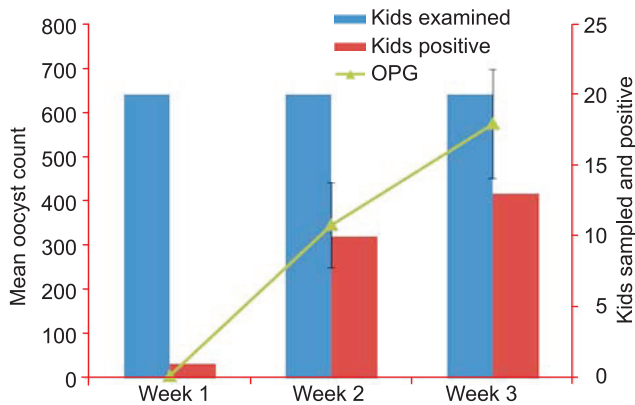


Fig. 3. Mean OPG of *Cryptosporidium* oocysts excreted by kids.

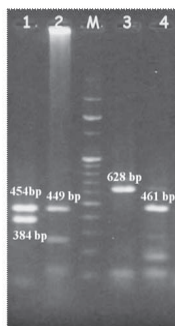


Fig. 4. PCR-RFLP pattern of *C. parvum* and *C. ubiquitum*. Lane M, 100 bp ladder; lane 2, 3, digestion with *SspI* and *VspI*, respectively positive for *C. parvum*; lanes 1, 4, digestion with *SspI* and *VspI*, respectively positive for *C. ubiquitum*.

of prevalence of *Cryptosporidium* in kids. Analysis of the data according to age revealed that a large proportion of the kids (65%; 13/20) born on the farm became infected by the end of the 3 weeks (Fig. 3). The percentage of kids infected in the farm increased with age from 1 week old (5%) to 2-week old (50%) and then 3-week old (65%). Paraud *et al.* (2010) reported that excretion of oocysts may start very early, at age of 4–5 days, with a peak between 7–10 days and declines after 3 weeks. Statistical analysis revealed significant ($P < 0.05$) difference in oocysts count at 1 week of age in comparison to other age group animals.

Because of the ubiquitous presence of *Cryptosporidium* oocysts in the environment, it is highly significant to elucidate the source of infection to kids and identify the infected dams to understand the epidemiology of cryptosporidiosis in goat kids. Genotypes of *Cryptosporidium* isolated from 42 faecal samples of pregnant goats and 24 faecal samples of kids in the present study were differentiated by PCR-RFLP technique targeting the amplified 830 bp fragments of SSU rRNA gene (Fig. 4). Our findings were in agreement with Xiao *et al.* (1999, 2001) and Paul *et al.* (2009). Similarly, Das *et al.* (2011) identified 2 visible bands at 628 bp and 104 bp when nested PCR products of bovine *Cryptosporidium* strain were digested with *VspI* restricted enzyme.

The management of kids and pregnant does is of immense importance in the epidemiology of the disease.

At the farm under study, the new born kids were kept with the dams for 10–15 days; hence they acquire the infection, which attained a peak after 3 weeks of birth. Results of genotyping in this study support the conclusion that periparturient rise in the shedding of *Cryptosporidium* oocyst is a source of infection in goat kids. The two *Cryptosporidium* species (*C. parvum* and *C. ubiquitum*) identified in kids in this study were all present in periparturient goats during the periparturient period. *C. parvum* was the predominant species in both periparturient goats (31 of 42 *Cryptosporidium* positive samples) as well as kids of 1–3 weeks of age (21 of 24 *Cryptosporidium* positive samples). The other species in kids in this study, *C. ubiquitum*, was found at lower frequency in periparturient goats (11 of 42 *Cryptosporidium* positive samples), which could be responsible for less prevalence in kids (3 of 24 *Cryptosporidium* positive samples). Many workers have reported both adult goat and kids are infected by *C. parvum* (Drumo *et al.* 2012) and *C. xiaoi* (Rieux *et al.* 2013); however, meager reports of infection of goats by *C. ubiquitum* are available (Yadav *et al.* 2016).

Similar studies encompassing wide geographical area can give new insights into periparturient rise in oocyst count in goats. Moreover, scientific studies on the physiology of gestation may help in explaining the reasons for this phenomenon. To substantiate our results and determine if adults can be a source of *Cryptosporidium* oocysts for their kids, more sensitive methods like quantitative real-time PCR allowing the detection of light excretions of the species of *Cryptosporidium* involved are further needed.

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