Prevalence and molecular characterisation of Cryptosporidium spp. in goat kids


Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur - 482 001, India.

Received: 23-05-2018 Accepted: 20-06-2018 DOI: 10.18805/ijar.B-3642

ABSTRACT
Prevalence and molecular characterisation of Cryptosporidium species was done in kids belonging to organised and non-organised goat farms at Jabalpur. The overall prevalence of Cryptosporidium was 14.63%. The prevalence was non-significantly higher in male kids (16.16%) as compared to that of female kids (13.21%). Age wise prevalence was higher in kids up to one month age (16.13%) than that of kids up to 3 months age (13.99%). No significant difference was found in prevalence among different breeds and in kids kept in farm or field conditions. The prevalence was non-significantly higher in non-diarrhoeic kids than diarrhoeic kids. Most of the infections were of one score (76.6%). Molecular characterisation by PCR-RFLP of 18S SSU rRNA gene revealed presence of Cryptosporidium parvum species in positive faecal samples.

Key words: Cryptosporidium, Goat kids, Molecular characterisation, PCR-RFLP, Prevalence.

INTRODUCTION
Cryptosporidiosis, caused by an intracellular and extracytoplasmic apicomplexan enteric protozoan parasite, is one of the important causes of neonatal mortality and it affects more to young and immune-compromised animals (Bhat et al., 2012). This important zoonotic disease affecting wide variety of vertebrates and humans is prevalent in different parts of the world. In most of the studies, the diagnosis of infection was done based only on differential staining of oocysts in positive faecal samples or serologically (Randhawa et al., 2012; Brar et al., 2016). As there exists antigenic and morphometric similarities between oocysts of different Cryptosporidium species, molecular characterisation becomes important to know the species involved (Xiao, 2010). Most of the studies on cryptosporidiosis in India are on calf cryptosporidiosis (Agrawal, 2013; Brar et al., 2017). Literature on caprine cryptosporidiosis from all over the world is scarce (Geurden et al., 2008) and only a few studies have been done on molecular characterisation of Cryptosporidium in goats in India with smaller sample size (Rajendran et al., 2011; Maurya et al., 2013). As the cryptosporidium infection has its importance in young and immune-compromised neonates and very scarce literature is available on pattern of oocyst excretion and its implications in the epidemiology of disease in kids thus the present investigation has been carried out to investigate the prevalence and molecular characterisation of Cryptosporidium spp. in goat kids of Jabalpur.

MATERIALS AND METHODS
Collection, staining and scoring of faecal samples: A total of 205 goat kids belonging to different goat farms, as well as different goat rearers were included in the study. Faecal smears were stained with modified Zeil Neelson staining (Henriksen and Pholenz, 1981) and scoring of infection in positive cases was done as described by Castro Hermida et al. (2002).

DNA extraction and Nested PCR: Oocysts were purified from positive samples by modified sucrose floatation technique (Current et al., 1983) and discontinuous step gradient centrifugation technique (Arrowood and Sterling, 1987). DNA was extracted from these purified oocysts using QIAGEN DNA stool minikit with some modifications in the method such as five prior freeze thaw cycles in Liquid Nitrogen and boiling water, increasing the time of action of inhibitory tablet from one min to five min, increasing the time of action of proteinase K from 10 min to 20 min and decreasing the elution volume for DNA from 200µl to 50µl. The 18S SSU rRNA gene was amplified from each sample using the oligonucleotide primers CRP-DIAG1 forward and CRP-DIAG1 reverse in the primary PCR For nested PCR, 2 µl of the primary product was used as template and primers CRP-DIAG2 forward and CRP-DIAG2 reverse were used (Xiao et al., 1999, 2001). The PCR product was resolved by gel electrophoresis in 1% agarose.

PCR – RFLP: Five µl of the PCR product (834 bp) was separately subjected to restriction endonuclease enzyme digestion with Ssp1 and Vsp1 in 20 µl reaction mixture for 3 hrs (Xiao et al., 1999; Feng et al., 2007). The 20 µl reaction mixture contained ampiclon DNA 10.0 µl, 10x RE buffer 2.0 µl, enzyme (Ssp1/Vsp1) 1.0 µl. The digested product was resolved in 2.5% agarose gel.

*Corresponding author’s e-mail: alokdixit7@yahoo.com
**Statistical analysis:** Prevalence of *Cryptosporidium* infection was calculated using Chi-square analysis (Snedecor and Cochran, 1994).

**RESULTS AND DISCUSSION**

Faecal smears showing pinkish red oocysts against a blue/green background were considered positive for *Cryptosporidium* infection (Fig. 1). Results of prevalence study are shown in Table 1. The overall prevalence of *Cryptosporidium* was 14.63% (30/205) which was slightly higher in kids kept at farm conditions than that of kids kept in field conditions but this difference was non-significant. Season wise prevalence of *Cryptosporidium* was highest in monsoon (21.31%) followed by that of winter (12.15%) and summers season (10.81%) but the difference was non-significant. Male kids had higher prevalence (16.16%) than female kids (13.21%). Kids upto the age of one month had higher prevalence (16.13%) than that of kids upto three months age (13.99%). No significant difference was found in *Cryptosporidium* prevalence among different breeds i.e. barbari (26.67%), sirohi (8.75%), jamunapari (16.67%), black Bengal (11.11%) and non-descript (16.25%). The prevalence was non-significantly higher in non-diarrhoeic kids (18%) as compared to that of diarrhoeic kids (11.71%). Scoring of infection intensity revealed that most of the infections were of one score (76.6%) while only 23.3% kids had two score.

DNA yield was between 38-102 ng/ml. Agarose gel electrophoresis at 0.8% gel revealed compact strong bands of genomic DNA. Agarose gel electrophoresis of PCR product of all the samples showed a clear 1317 bp band in primary PCR and 834 bp band in secondary (nested) PCR. The *Ssp1* digestion of 834 bp nested PCR product showed three bands at 449 bp, 267 bp and 108 bp. *Vsp1* digestion of 834 bp nested PCR product revealed two bands at 628 and 105 bp (Fig. 2). These band patterns represented *Cryptosporidium parvum*.

The overall prevalence (14.63%) of *Cryptosporidium* spp. was much higher than the prevalence reported by Wang et al. (2014), Jafari et al. (2012), Maurya et al. (2013) and Marreros et al. (2012) who reported the prevalence up to 3.48%, 3.54%, 3.5% and 2.5% in goats from Spain, Switzerland, India and Iran respectively. This prevalence rate in the present study was lesser than the prevalence reported from Siberia (31%) by Misic et al. (2006) in kids and comparable to the prevalence reported from Iran (18.86%) by Khezri and Khezri (2013). Bejan et al. (2009) reported the prevalence of *Cryptosporidium* spp. as 24% in kids up to 6 weeks age from north western part of Romania. In India, caprine cryptosporidiosis prevalence has been reported as low as 3.5% (Maurya et al., 2013) and as high as 48% (Paul et al., 2013).

Seasonal dynamics of *Cryptosporidium* revealed highest seasonal prevalence in monsoon. The reason behind this could be overcrowding of animals in shelters leading to more pickup of infection from the floor in rainy season. Wetness of the floor also favours oocyst survival. Studies on seasonality in the prevalence of cryptosporidiosis in goats are limited (Paul et al., 2013).

Sex wise prevalence was only slightly higher in male kids as compared to that of female kids. Statistically insignificant higher prevalence of *Cryptosporidium* in male kids was also reported by Paul et al. (2013) but Bhat et al. (2013) reported higher prevalence in female calves than that of male calves. Bejan et al. (2009) reported that the distribution of *Cryptosporidium* infection was almost uniform in male and female kids up to six weeks age.

Age wise prevalence of *Cryptosporidium* did not differed significantly between kids of different age though it was slightly higher in kids upto one month age than that of kids up to three months age. Causape et al. (2002) also found a decrease in percentage of *Cryptosporidium* prevalence in lambs with increasing age. The parasite’s lifecycle is approximately four days and peri-parturient does, infected kidding area and less immunocompetence may be the reasons for more prevalence of this infection in young animals.

**Table 1:** Prevalence of *Cryptosporidium* in kids

<table>
<thead>
<tr>
<th>Age</th>
<th>Prevalence (%)</th>
<th>Sex</th>
<th>Prevalence (%)</th>
<th>Farm/field</th>
<th>Prevalence (%)</th>
<th>Season</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 1 month</td>
<td>10 (16.13)</td>
<td>Female kid (106)</td>
<td>14 (13.21)</td>
<td>Field (113)</td>
<td>16 (14.16)</td>
<td>Winter</td>
<td>13 (12.15)</td>
</tr>
<tr>
<td>More than 1 month</td>
<td>20 (13.99)</td>
<td>Male kid (99)</td>
<td>16 (16.16)</td>
<td>Farm (92)</td>
<td>14 (15.22)</td>
<td>Summer</td>
<td>4 (10.81)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Monsoon</td>
<td>13 (21.31)</td>
</tr>
<tr>
<td><strong>χ² value</strong></td>
<td>0.16</td>
<td>0.36</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
<td>3.14</td>
</tr>
</tbody>
</table>
The breed wise difference in prevalence of *Cryptosporidium* was non-significant. No breed differences in prevalence of *Cryptosporidium* infection are reported by other workers.

The infection was slightly higher in kids kept in farm conditions than that of kids kept in field conditions but the difference was non-significant. Ahamed *et al.* (2014) reported the prevalence of *Cryptosporidium* 35% in lambs on an unorganized farm at Jammu. Paul *et al.* (2013) also reported higher prevalence up to 48.14% in kids kept in farm conditions.

There was no significant differences between prevalence of *Cryptosporidium* spp. infection in diarrhoeic and non-diarrhoeic kids. However, infection percentage was more in non diarrhoeic kids. Most of the workers reported higher prevalence of *Cryptosporidium* infection in diarrhoeic than non diarrhoeic calves (Roy *et al.*, 2006; Paul *et al.*, 2008) but in pigs there is one report (Maurya *et al.*, 2013) indicating higher prevalence of infection in non-diarrhoeic piglets. Bejan *et al.* (2009) also reported higher prevalence of the infection in diarrhoeic kids (82.5%) than that of non-diarrhoeic kids (18.5%). In one study Noordeen *et al.* (2000) found that out of 291 goats suffering from *Cryptosporidium* infection only nine were suffering with diarrhoea. Causape *et al.* (2002) also reported that diarrhoea along with *Cryptosporidium* infection is common only in younger lambs and the percentage decreases as their age increases. They found significantly higher infection rates in diarrhoeic (87.7%) than non diarrhoeic (22.4%) lambs up to 21 days of age. Some workers have also reported that this protozoan is not always associated with diarrhoea (Quilez *et al.*, 1996; Olson *et al.*, 1997).

On scoring of intensity of infection, the infections were of 1 score in 76.6% kids while in 23.3% kids only the infection intensity was of 2 score. Many workers have done scoring of infection in faeces of neonates (Castro-Hermida *et al.*, 2002; Sanz-Ceballos *et al.*, 2009). Ahamed *et al.*
(2014) had reported that in 60% of lambs oocyst excretion was of 2 score in lambs from birth to one year of age in an unorganized farm at Jammu. Causape et al. (2002) also developed a method of scoring of oocyst in faecal samples of lambs.

Freeze-thaw cycles prior to DNA isolation was done to maximize oocyst lysis resulting to more amount of nuclear DNA of Cryptosporidium oocyst. Many workers recommended freeze thaw cycles prior to DNA isolation (Maurya et al., 2013). Use of commercial kits as used in the study yielded good quality DNA with less chance of inhibitors of PCR in eluted DNA (Inpankaew et al., 2014). Further the addition of BSA in primary PCR resulted in more effective binding of primers with template to amplify the product of comparatively higher size. Use of BSA like agents in primary PCR for cryptosporidiosis has been suggested (Jiang et al. 2005).

In molecular characterisation, PCR-RFLP pattern revealed the presence of C. parvum in goat kids in the area. In goats, initially three species of Cryptosporidium i.e. C. parvum, C. xiaoai and C. hominis have been described. Later C. ubiquitum and C. andersoni were also identified in goats (Wang et al., 2014). In one study in kids, in Belgium, C. parvum was reported (Geurden et al., 2008; Koinari et al., 2014) also reported rat genotype along with Cryptosporidium parvum in goats.

Agrawal (2013) reported two species of Cryptosporidium i.e. C. parvum and C. andersoni in calves of Jabalpur. Out of these two species, C. parvum is widely associated with neonatal diarrhoea in ruminants (Lorenz et al., 2011; Singla et al., 2013) while C. andersoni is associated with abomasal cryptosporidiosis (Putignani and Menichella, 2010).

In this study, restriction fragment length polymorphism is considered as discriminatory, less time consuming and more cost effective alternative to sequencing for typing of Cryptosporidium isolates from domestic animals.

CONCLUSION

The findings indicate that Cryptosporidium parvum infection is present in goats of this region. As the zoonotic importance of the parasite is well known, its prevalence in kids of the area indicated the necessity of some collaborative studies on human cryptosporidiosis for devising control and prevention strategies for the parasite.

REFERENCES


