

Prevalence and analysis of associated risk factors for *Cryptosporidium* infection in lambs in Jammu district

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Abstract An epidemiologic study was carried out to investigate the prevalence and analysis of risk of *Cryptosporidium* infection in lambs in Jammu district. Faecal samples of 120 lambs of different age groups viz., <1 month, 1–3 months and 3–6 months were assessed. *Cryptosporidium* oocysts were identified by using modified Zeihl Neelsen technique. Statistical analysis showed that infection rates were significantly higher in lambs of <1 month age group (65 %) than other two age groups ($p < 0.05$). Similarly, cryptosporidial infection rates were significantly higher in diarrhoeic (54.41 %) than in non diarrhoeic lambs (34.61 %). Winter records highest prevalence (73.33 %) which varied significantly. Sex wise higher prevalence was observed in females (51.56 %) as compared to males (39.28 %). The relationship between intensity of infection and various epidemiological factors showed that highest intensity was observed in lambs of 0–1 month age group, having diarrhoea, in winter season.

Keywords *Cryptosporidium* · Epidemiology · Lambs

Introduction

Cryptosporidium is considered one of the major gastrointestinal pathogens in young ruminants and has been associated with diarrhoea. Mortality is low in naturally reared suckling lambs, although it increases when the disease is associated with concurrent infections or deficiencies in nutrition or husbandry (de Graaf et al. 1999). Cryptosporidial infection in small ruminants was first diagnosed in 1987, in diarrhoeic lambs (Rojo-Vázquez et al. 1987). Later, infection was reported in sheep from different geographical areas of the world as reviewed by Thompson et al. (2005). In India, most data on the incidence of *Cryptosporidium* infection in animals are mostly related to calves (Singh et al. 2006; Paul et al. 2008; Yadav et al. 2012) and till date no report has been published regarding status of the infection in lambs.

The very limited options for treatment of the disease and the potential hazard that *Cryptosporidium* poses to public health have led to interest in understanding the factors that lead to the transmission and spread of infection in animals and design strategies to prevent the infection, especially on farm management practices. Nevertheless, most studies focused on identifying factors that may play a role in the risk of *Cryptosporidium* infection in ruminant farms have been conducted in dairy cattle (Garber et al. 1994; Mohammed et al. 1999). Thus, the present study is to find the prevalence and analysis of risk factor for *Cryptosporidium* infection in lambs of Jammu district.

Materials and methods

The year wise study (October 2010 to September 2011) was performed in the Jammu district of the Jammu and

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Kashmir state wherein the faecal samples were collected from various sheep flocks. Ten faecal samples each of different age group lambs (<1 month, 1–3 months and 3–6 months) were collected per season viz., winter (December to February), summer (March to June), rainy (July to September) and post monsoon (October to November) for a year. For each animal, faecal sample was taken directly from the rectum by using a disposable plastic bag and disposable plastic containers were used in lambs with diarrhoea. Faecal samples were examined by preparing smears on the glass slides and then were stained by the modified Ziehl Neelsen technique (Henricksen and Pohlenz 1981). Data regarding consistency of faeces, age and sex of the animal were also recorded. The entire surface of the smear was examined for the presence of *Cryptosporidium* oocysts at 400× and 1,000× magnifications and, if positive, infection intensity was evaluated as per OIE (2008) i.e., 1+ ; less than 5 oocysts per slide, 2+ ; 1–10 oocysts per field of view and 3+ ; 11 or more oocysts per field of view.

Chi square test was used to compare infection rates between different age groups and between diarrhoeic and non diarrhoeic animals (Samad et al. 1994). A *p* value <0.05 was required for significance. Odd's ratio (OR) and 95 % confidence interval (CI) for *Cryptosporidium* infection in the different age groups were calculated using each age group.

Results

The overall prevalence of *Cryptosporidium* spp. in lambs of Jammu district was found out to be 45 %. The highest incidence was found in <1 month age group (65 %) followed by 1–3 months (37.5 %) and 3–6 months (35 %). Statistical analysis revealed that per cent prevalence in <1 month age group varied significantly ($p < 0.05$) with other two age groups. Further, statistically it was observed that the chances of occurrence of *Cryptosporidium* spp. in animals of <1 month age group was 3.09 times higher (OR: 3.09, 95 % CI: 1.252–9.657) than 3–6 months age group animals. Diarrhoeic lambs revealed significantly ($p < 0.05$) higher prevalence (54.41 %) than non diarrhoeic lambs (34.61 %) and 2.25 times higher risk (Odd ratio: 2.25; 95 % CI 1.004–5.093) of susceptibility to *Cryptosporidium* infection was observed. Higher prevalence was observed in female lambs (51.56 %) as compared to male lambs (39.28 %). Results of analysis of risk factors revealed that female lambs show 0.60 times more infection as compared to male lambs (OR 0.60, 95 % CI 0.275–1.339). The detailed prevalence and analysis has been shown in Table 1. Seasonal prevalence revealed highest infection during the winter months (73.33 %) and it varied significantly ($p < 0.05$) when compared with other

seasons and the probability of occurrence was 6.41 times higher (OR 6.41, 95 % CI 1.832–23.505) than summer months. The seasonal prevalence of *Cryptosporidium* spp. in lambs has been depicted in Fig. 1.

The intensity of infection for positive samples were measured based on the number of oocysts observed under 40× objective lens (OIE 2008) whereas minimum intensity (1+) was observed in 36.84 % of lambs whereas highest oocyst intensity (3+) was observed in diarrhoeic lambs (38.57 %). It was observed that higher oocyst intensity (3+) in winter season than other seasons Fig. 2.

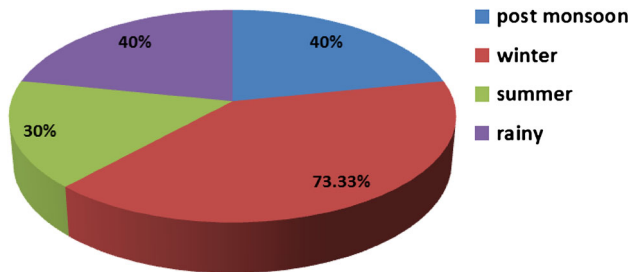
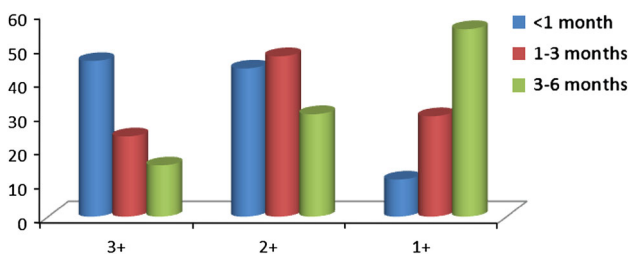
Discussion

In this study, out of 120 lambs examined, 55 lambs (45 %) were found to be infected with *Cryptosporidium* species. This finding is probably the first documented report regarding the prevalence of *Cryptosporidium* infection in lambs in India. The high prevalence of lambs shedding oocysts in the first month stresses on the fact that most of them might be infected immediately after birth. This observation is consistent with the duration of the parasite's life cycle which is around 4 days and suggestive of heavy environmental contamination in the lambing area and/or infection from post parturient dams which remain permanently with lambs and responsible for infection at suckling (Causape et al. 2002).

The presence of diarrhoeic lambs was significantly associated with increased risk of infection in the flock and the parasite was strongly associated with significantly high probability of diarrhoea though the evidence is insufficient to conclude that this protozoan was the primary cause of diarrhoea as many other agents (*Escherichia coli*, Rota virus, *Corona virus*) are also involved in diarrhoea of neonatal ruminants which were not examined in the instant study. Nevertheless, Munoz-fernandez et al. (1996) had earlier reported that *Cryptosporidium* was the most frequent agent found in 45 % of diarrhoeic lambs in contrast to potentially pathogenic *E. coli* (30 %) and Rota virus (2.1 %). The association of diarrhoea with prevalence of cryptosporidiosis showed positive correlation as higher prevalence was observed in diarrhoeic lambs (54.41 %) in comparison to non diarrhoeic lambs (34.61 %). The constant association of diarrhoea and the presence of oocysts of *Cryptosporidium* in the faeces have been recorded by other workers (Causape et al. 2002). In Serbia, diarrhoea was observed more frequently in younger lambs positive for *Cryptosporidium* (69 %) than older lambs where infection was frequently asymptomatic (70.8 %) (Zorana et al. 2006). As many as 93.3 % of lambs in the first week infected by *Cryptosporidium* had diarrhoea and this was found to be statistically associated with cryptosporidial

Table 1 Prevalence and analysis of risk factors in lambs infected with *Cryptosporidium* spp

Parameters	Number of samples Positive/examined	% Prevalence	Odd ratio	95 % Confidence interval	p-value
Faecal consistency	37/68	54.41			
	18/52	34.61	2.254	1.004–5.093	0.049
Sex	22/56	39.28	0.608	0.275–1.339	0.245
	33/64	51.56			

**Fig. 1** Season wise prevalence of *Cryptosporidium* in lambs of Jammu district**Fig. 2** Intensity of *Cryptosporidium* oocysts in different age groups of lambs of Jammu district

infection in all age groups even in lambs of 22–90 days of age (Causape et al. 2002).

The age related susceptibility of lambs to cryptosporidial infection showed higher prevalence in lambs of <1 month age. The presence of *Cryptosporidium* infection in clinically asymptomatic animals indicated that particular age group of animals might be reservoir for the parasite (Mtambo et al. 1997) and the prevalence rate decreases with increase in age and lower prevalence was recorded in lambs of 3–6 months of age. Ulutas and Voyvoda (2004) reported 46.5 % cryptosporidial infection in lambs (aged from 1 to 30 days) in Turkey using Carbol fuschin staining. The lower prevalence of infection with advancing age may be due to age related immunity (Zu et al. 1992).

A significant association was observed between the season and prevalence of *Cryptosporidium* in lambs during the present study. Winter season showed highest prevalence 73.33 %. This is because lambing generally occurs in winter season (late November and December months), therefore, higher number of lambs were in the risk age

group i.e., 1–3 weeks of age (Santin et al. 2007). In winter, the temperature in study area is suitable for viability and survival of *Cryptosporidium* oocysts. The present findings are also supported by observation of Fayer et al. (2000) and Jenkins et al. (2003) who observed that *Cryptosporidium* oocysts can remain viable and infective for 4–5 months at 5–20 °C. The studies on seasonality in the prevalence of cryptosporidiosis in lambs are limited.

As per intensity of *Cryptosporidium* infection is concerned, more numbers of lambs of <1 month age group, having diarrhoea showed highest intensity (3+) in acid fast smear. The highest intensity of infection in <1 month age group diarrhoeic animals, mainly in winter season demonstrates that these animals are important from an epidemiological point of view and play an important source of contamination to water and soil of the area which has resulted in several water borne outbreaks of cryptosporidiosis in man and animals (Fayer et al. 2000).

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