



# First report of *Cryptosporidium* sp. infection in sheep population of Ladakh, India

Adil Majid Bhat<sup>1,5</sup> · H. U. Malik<sup>1</sup> · Nasir Manzoor Wani<sup>2</sup> · Souvik Paul<sup>3</sup> · Saurabh Gupta<sup>3</sup> · Tsewang Dolma<sup>4</sup> · S. V. Singh<sup>3</sup>

Received: 19 December 2018 / Accepted: 23 April 2019 / Published online: 2 May 2019  
© Indian Society for Parasitology 2019

**Abstract** Ladakh is the high altitude (11,184 ft above sea level) cold desert region in the state of Jammu & Kashmir (India), where winter temperature goes below  $-35^{\circ}\text{C}$ . A total of 37 faecal samples were collected from local Changthangi and Russian merino sheep of the Leh region of Ladakh, having the history of diarrhoea and emaciation, followed by death. Of the 37 samples collected, 20 (54.05%) showed presence of *Cryptosporidium* sp. infection on faecal smear examination after Ziehl–Neelsen staining. The number of samples positive for *Cryptosporidium* sp. infection on faecal smear examination was 7 and 13 for Russian Merino and local Changthangi sheep, respectively. The occurrence of *Cryptosporidium* sp. infection was similar in both sexes, 11/20 (55%) in females and 9/20 (45%) for males. The Age-wise occurrence of *Cryptosporidium* sp. infection in different age groups was found to be: 50% in less than 2 year age group and 50% in more than 2 year age group. To the best of our knowledge, this is the first report of *Cryptosporidium* sp. infection in adult sheep from India. The survival of the parasite at such

freezing temperature gives the insight about its cold-resistant nature.

**Keywords** *Cryptosporidium* spp. · Ziehl–Neelsen · Sheep · Ladakh · Diarrhoea · Changthangi

## Introduction

*Cryptosporidium* sp. is the ubiquitous entero-pathogen that affects both humans and livestock (Brar et al. 2017). Cryptosporidiosis significantly affects the farm economy, that is attributed to high morbidity and at times to high mortality of farm animals (Casemore et al. 1985). *Cryptosporidium* sp. has been recognized as an emerging pathogen and causes gastrointestinal disturbances in both domestic animals (cattle, buffalo, sheep, goat, pig, dog, cat and horses), birds, fish, amphibians, reptiles, and humans. *Cryptosporidium* sp. is transmitted by the faecal-oral route (Fayer et al. 2000; Ahamed et al. 2015; Romero-Salas et al. 2001; Bhat et al. 2013) and in sheep and goats, it leads to diarrhoea and decreased weight gain (Foreyt 1990). In young animals, the disease takes severe form and results in high mortality (Chalmers et al. 2002; de Graaf et al. 1999).

*Cryptosporidium* sp. infected animals shed oocysts in large (108–109/g) numbers and these oocysts act as infection source for other animals and humans (Romero-Salas et al. 2001).

The infection in humans causes diarrhoea and in immune-compromised animals, the disease takes a fatal course (Checkley et al. 2015). Infection with *Cryptosporidium* sp. affects epithelial lining of the small intestine, gall bladder, stomach, liver, trachea, and lungs in both animals and humans (Hunter and Thompson 2005).

✉ Adil Majid Bhat  
adil.majid724@gmail.com

<sup>1</sup> Division of Clinical Veterinary Medicine, Ethics and Jurisprudence, F.V.Sc and A.H., SKUAST-K, Srinagar, Jammu and Kashmir, India

<sup>2</sup> Division of Veterinary Pharmacology and Toxicology, F.V.Sc and A.H., SKUAST-J, Jammu, Jammu and Kashmir, India

<sup>3</sup> Microbiology Lab, Animal Health Division, Central Institute for Research on Goats, Mathura, India

<sup>4</sup> Mathu Sheep Farm, Leh Ladakh, Jammu and Kashmir, India

<sup>5</sup> Division of Veterinary Medicine, F.V.Sc and A.H., SKUAST-J, Jammu, Jammu and Kashmir, India

In India, the data related to *Cryptosporidium* sp. in small ruminants is limited to the incidence in calves and young sheep (Ahamed et al. 2015; Singh et al. 2006; Paul et al. 2008; Yadav et al. 2012). There has been no report of *Cryptosporidium* sp. infection from livestock of Ladakh. The present study was conducted to determine the occurrence of *Cryptosporidium* sp. infection in sheep of Ladakh.

## Materials and methods

### Sample collection

The present study was carried out in the Leh region of Ladakh. The region is the part of North Indian state of Jammu and Kashmir and lies at an altitude of 11,184 ft. above sea level. Ladakh is part of cold desert region of India, which spans from Ladakh (Jammu and Kashmir) in the North to Kinnaur (Himachal Pradesh) in the South. The region experiences harsh winters with temperature falling below  $-50^{\circ}\text{C}$  during winters. A total of 37 faecal samples were collected from Matho sheep farm, Leh, Ladakh. The sheep included local Changthangi (also called Changluk) (Fig. 1) and Russian Merino sheep of either sex and in the age group of 0–5 years. Animals had the history of emaciation and intermittent diarrhoea.

### Screening of samples for *Cryptosporidium* sp. oocysts

Faecal samples of animals were collected directly from the rectum and thick faecal smears were prepared and air-dried. The faecal smears were stained by modified Ziehl–Neelsen staining (Henricksen and Pohlenz 1981). The stained smears were observed under high power and oil immersion microscopy.



**Fig. 1** Picture of Changthangi (locally called Changluk) reared in Ladakh

### Identification of *cryptosporidium* parasite and oocysts

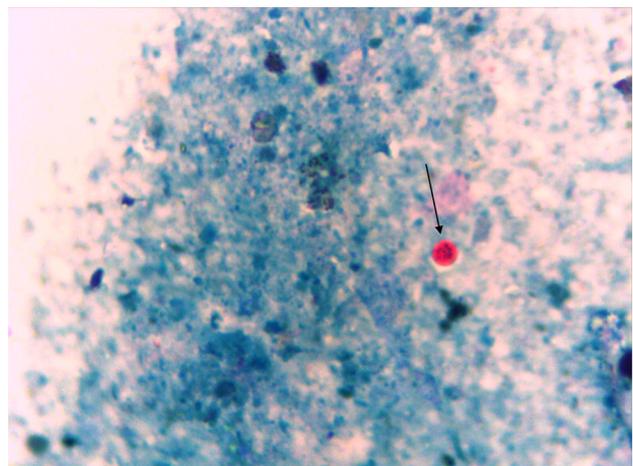
The *Cryptosporidium* sp. oocysts were in the faecal smears visualized as bright red round bodies against blue background, and based on the presence and absence of the oocysts infection was scored as positive or negative. A sample was considered positive if an oocyst was detected bearing a correct morphology.

## Results and discussion

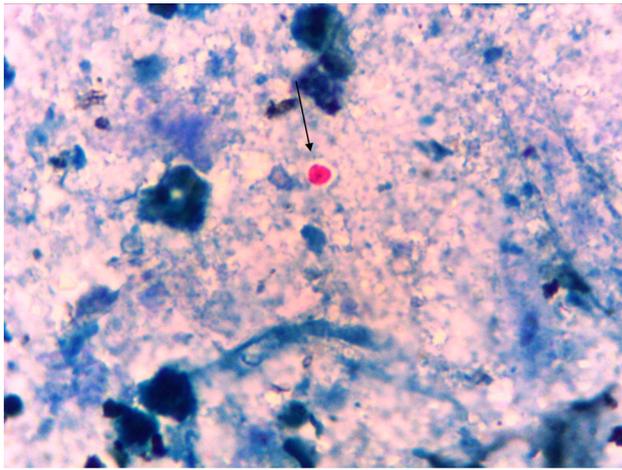
Out of 37 faecal samples screened by microscopy, 20 (54.05%) were found positive for *Cryptosporidium* sp. infection (Figs. 2, 3). The number of samples positive for *Cryptosporidium* sp. infection on faecal smear examination was 7 and 13 for Russian Merino and Changthangi sheep, respectively. The occurrence of *Cryptosporidium* sp. infection was similar in both sexes, 11/20 (55%) in females and 9/20 (45%) for males. The age wise occurrence of *Cryptosporidium* sp. infection was found to be similar in both groups: 50% in less than 2 year age group and 50% in more than 2 year age group. The breed, sex, and age-wise distribution of *Cryptosporidium* sp. infection is provided in Table 1.

The clinical signs of diarrhoea and poor growth was observed in only three animals, that were in age group of  $< 6$  months. Rest of the animals were asymptomatic carriers.

The study is the first report of *Cryptosporidium* sp. infection in sheep of Ladakh. The study is also the first report of *Cryptosporidium* sp. infection in adult sheep of India. Ahamed et al. (2015) for the first time reported the *Cryptosporidium* sp. infection in lambs of India. Majewska



**Fig. 2** Picture of *Cryptosporidium* parasite in faecal smear of Changthangi Sheep reared in Ladakh



**Fig. 3** Picture of *Cryptosporidium* Oocyst in the faecal smear of Changthangi Sheep reared in Ladakh

**Table 1** Age, breed and sex profile of sheep positive for *Cryptosporidium* sp. infection

Animal details	No. of positive animals ZN-staining
<b>Breed</b>	
Merino	7
Changthang	13
<b>Age</b>	
0–2 years	5
> 2 years	15
<b>Sex</b>	
Female	11
Male	9

et al. (2000) reported *Cryptosporidium* sp. infection in sheep of Poland.

In our study, the occurrence of infection was similar in lambs and adult sheep. Xiao et al. (1993) reported occurrence of *Cryptosporidium* sp. infection high in lambs as compared to adult sheep. However, Olson et al. (1997) demonstrated that the prevalence of *Cryptosporidium* sp. was greater in animals falling in age group of > 6 months.

The animals examined in our study were mostly asymptomatic (85%) and included both adults and lambs. Similar findings have been reported by other workers (Tzipori et al. 1981; Xiao et al. 1993). The asymptomatic infected animals are important reservoir of *Cryptosporidium* sp. infection.

The symptoms of cryptosporidiosis infection are found mostly in lambs below 1 month of age (Ahamed et al. 2015) and the clinical manifestations include diarrhoea followed by mortality in neonates (Tzipori et al. 1981;

Angus et al. 1982), however, recovery of infected lambs has also been reported (Xiao et al. 1993).

The occurrence of *Cryptosporidium* sp. parasite at such freezing temperature demonstrates its ability to survive freezing temperatures. The freezing of aqueous suspension of *Cryptosporidium* sp. oocysts at  $-70\text{ }^{\circ}\text{C}$  for one hour and  $-20\text{ }^{\circ}\text{C}$  for 24 h or longer has rendered oocysts non infectious for mice (Fayer and Nerad 1996).

The study is the preliminary report on *Cryptosporidium* sp. infection in sheep of Ladakh. Presence of *Cryptosporidium* sp. infection in sheep population not only adversely affects per animal productivity but is also potential threat to other livestock, wild-life and human population. Further work is needed to determine the molecular epidemiology of MAP and its impact on livestock of the region.

**Acknowledgements** The authors are highly thankful to Dean, Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, Alusteng, Srinagar, J&K, India for providing funds and necessary infrastructure required for the study.

**Author's contribution** AMB, SP and SG did sample collection and processing, while as HUM, NM and SVS wrote the manuscript.

#### Compliance with ethical standards

**Conflict of interest** The authors declare no conflicts of interest regarding the information provided in this manuscript.

**Ethical approval** The study was approved by Institutional Animal Ethics Committee of Faculty of veterinary and animal sciences, Shuhama, Srinagar, J&K, India.

#### References

- Ahamed I, Yadav A, Katoch R, Godara R, Saleem T, Nisar NA (2015) Prevalence and analysis of associated risk factors for *Cryptosporidium* infection in lambs in Jammu district. *J Parasit Dis* 39(3):414–417. <https://doi.org/10.1007/s12639-013-0353-y>
- Angus KW, Appleyard WT, Menzies JD, Campbell I, Sherwood D (1982) An outbreak of diarrhea associated with cryptosporidiosis in naturally reared lambs. *Vet Rec* 110:129–130
- Bhat SA, Juyal PD, Singla LD (2013) Bovine cryptosporidiosis: brief review of its distribution in India. *Trends Parasitol Res* 2(2):5–13
- Brar APS, Sood NK, Kaur P, Singla LD, Sandhu BS, Gupta K, Narang D, Singh CK, Chandra M (2017) Periurban outbreaks of bovine calf scours in Northern India caused by *Cryptosporidium* in association with other enteropathogens. *Epidemiol Infect* 145(13):2717–2726
- Casemore DP, Armstrong M, Sands RL (1985) Laboratory diagnosis of cryptosporidiosis. *J Clin Pathol* 38:1337–1341. <https://doi.org/10.1136/jcp.38.12.1337>
- Chalmers RM, Elwin K, Reilly WJ, Irvine H, Thomas AL, Hunter PR (2002) *Cryptosporidium* in farmed animals: the detection of a novel isolate in sheep. *Int J Parasitol* 32:21–26. [https://doi.org/10.1016/S0020-7519\(01\)00309-5](https://doi.org/10.1016/S0020-7519(01)00309-5)
- Checkley W, White AC Jr, Jaganath D, Arrowood MJ, Chalmers RM, Chen XM (2015) A review of the global burden, novel

- diagnostics, therapeutics, and vaccine targets for *Cryptosporidium*. *Lancet Infect Dis* 15:85–94. [https://doi.org/10.1016/S1473-3099\(14\)70772-8](https://doi.org/10.1016/S1473-3099(14)70772-8)
- De Graaf DC, Vanopdenbosch E, Ortega-Mora LM, Abbassi H, Peeters JE (1999) A review of the importance of cryptosporidiosis in farm animals. *Int J Parasitol* 29:1269–1287. [https://doi.org/10.1016/S0020-7519\(99\)00076-4](https://doi.org/10.1016/S0020-7519(99)00076-4)
- Fayer R, Nerad T (1996) Effects of low temperatures on viability of *Cryptosporidium parvum* oocysts. *Appl Environ Microbiol* 62(4):1431–1433
- Fayer R, Morgan U, Upton SJ (2000) Epidemiology of *Cryptosporidium*: transmission, detection and identification. *Int J Parasitol* 30:1305–1322. [https://doi.org/10.1016/S0020-7519\(00\)00135-1](https://doi.org/10.1016/S0020-7519(00)00135-1)
- Foreyt WJ (1990) Coccidiosis and cryptosporidiosis in sheep and goats. *Vet Clin N Am Food Anim Pract* 6:655–670. [https://doi.org/10.1016/S0749-0720\(15\)30838-0](https://doi.org/10.1016/S0749-0720(15)30838-0)
- Henricksen SA, Pohlenz JFL (1981) Staining of cryptosporidia by a modified Ziehl–Neelsen technique. *Acta Parasitol* 22:594
- Hunter PR, Thompson RC (2005) The zoonotic transmission of *Giardia* and *Cryptosporidium*. *Int J Parasitol* 35:1181–1190. <https://doi.org/10.1016/j.ijpara.2005.07.009>
- Majewska AC, Werner Sulima P, Luty T (2000) Prevalence of *Cryptosporidium* in sheep and goats bred on five farms in west-central region of Poland. *Vet Parasitol* 89:269–275. [https://doi.org/10.1016/S0304-4017\(00\)00212-0](https://doi.org/10.1016/S0304-4017(00)00212-0)
- Olson ME, Thorlakson CL, Deselliers L, Morck DW, McAllister TA (1997) *Giardia* and *Cryptosporidium* in Canadian farm animals. *Vet Parasitol* 68:375–381. [https://doi.org/10.1016/S0304-4017\(96\)01072-2](https://doi.org/10.1016/S0304-4017(96)01072-2)
- Paul S, Chandra D, Ray DD, Tewari AK, Rao JR, Banerjee PS, Baidya S, Raina OK (2008) Prevalence and molecular characterisation of bovine *Cryptosporidium* isolates in India. *Vet Parasitol* 153:143–146. <https://doi.org/10.1016/j.vetpar.2008.01.044>
- Romero-Salas D, Alvarado-Esquivel C, Cruz-Romero A, Aguilar-Domínguez M, Ibarra-Lee YM, Johnson PW et al (2001) Development and application of a quantitative, specific assay for *Cryptosporidium parvum* oocyst detection in high-turbidity environmental water samples. *Am J Trop Med Hyg* 65:1–9. <https://doi.org/10.1186/s12917-016-0638-3>
- Singh BB, Sharma R, Kumar H, Banga HS, Aulakh RS, Gill JPS, Sharma JK (2006) Prevalence of *Cryptosporidium parvum* infection in Punjab and its association with diarrhoea in neonatal dairy calves. *Vet Parasitol* 140:162–165. <https://doi.org/10.1016/j.vetpar.2006.03.029>
- Tzipori S, Angus KW, Campbell I, Clerihew LW (1981) Diarrhea due to *Cryptosporidium* infection in artificially reared lambs. *J Clin Microbiol* 14:100–105
- Xiao L, Herd RP, Rings DM (1993) Diagnosis of *Cryptosporidium* on sheep farm with neonatal diarrhea by immunofluorescence assays. *Vet Parasitol* 47:17–23. [https://doi.org/10.1016/0304-4017\(93\)90172-J](https://doi.org/10.1016/0304-4017(93)90172-J)
- Yadav A, Katoch R, Katoch M, Aggarwal R, Khajuria JK, Godara R, Kalha R (2012) Cross sectional study and analysis of potential risk factors for *Cryptosporidium* sp. infection in buffalo calves in Jammu. *Vet Pract* 13(2):278–281

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.