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Cross seasonal study on prevalence of ovine babesiosis in Kashmir

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Abstract
A study was undertaken to elucidate the prevalence of ovine babesiosis in Kashmir. A total of 691 sheep reared at different places of central Kashmir and presented to Veterinary Clinical Complex, Faculty of Veterinary Sciences and Animal Husbandry (SKUAST Kashmir), Srinagar were screened from May 2016 to June 2017 for the prevalence of ovine babesiosis. A total of 185 cases of ovine babesiosis was diagnosed with a prevalence rate of 26.77%. The diagnosis was based on clinical manifestations, presence of ticks, demonstration of *Babesia* piroplasms on blood smear, and by PCR amplification. The animals were divided into four groups according to the season. The seasonal prevalence of the disease was highest in summer (42.70%), followed by spring (38.91%), autumn (11.35%) and lowest in winter (7.03%). In all season *Haemaphysalis* ticks were identified as the vectors for the transmission of ovine babesiosis in the study area

Keywords: Prevalence, Sheep, *Babesia*, Season, Kashmir

1. Introduction
Ovine babesiosis is the most important haemoprotozoan tick-borne disease of small ruminants caused by *Babesia ovis*, *Babesia motasi* and *Babesia crassa*, which is characterized by fever, anaemia, icterus and haemoglobinuria [1]. Different tick vectors like *Haemaphysalis*, *Dermacentor* and *Rhipicephalus* can transmit these diverse species [2]. *Babesia ovis*, *Babesia motasi* and *Babesia crassa* are recognized as the species causing ovine babesiosis. In sheep and goats *Babesia ovis* and *Babesia motasi* are known to be pathogenic [3]. Mortality rates in susceptible hosts vary from 30 to 50% in field infections due to *Babesia ovis.* It leads to significant losses among small ruminants due to its drastic effect on the haemobiotic system [4]. The high prevalence rate of tick-borne diseases is seen during summer due to the hot and humid season as the tick infestation is influenced by temperature, rainfall, and relative humidity which in turn directly determine the prevalence [5, 7] Reported *Babesia motasi* infection in 2.2% (1/45) sheep at Mysore. [8] Conducted serological and DNA tests to characterize the prevalence of tick borne pathogens in domestic animals (ruminants, pigs and dogs) in Italy. The most prevalent tick borne pathogen was *Anaplasma* and *Babesia* species. [9] Determined the prevalence of *Babesia* species by PCR amplification in small ruminants. The prevalence varied from 18 -60% in different location of southern Punjab.
The temperature on an average in Kashmir region has shown a rise in 1.45 °C while in Jammu region the rise is 2.32 °C over last two decades [10]. In Kashmir Valley there are only few studies on ovine and caprine haemo-protozoan infection such as *Babesia*, *Theileria* and *Anaplasma* [11-14]. During recent times there has been increase in temperature due to global warming and valley has observed the heat spells which are favourable for proliferation of vectors and hence increase in disease prevalence of livestock. In this study, we investigated the prevalence of ovine babesiosis by clinical evaluation, microscopic examination and molecularly by PCR. The main purpose of the present study was to evaluate prevalence of babesiosis in different seasons of Kashmir valley.

2. Materials and Methods
2.1 Sampling
The screening for ovine babesiosis was carried out at Veterinary Clinical Services Complex, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-Kashmir as well as from different sheep farms located at central part of Kashmir. Total 691 sheep were screened for

ovine babesiosis in the prevalence study. The preliminary screening of disease was made by microscopic examination of peripheral blood smear and through clinical evaluation. Confirmation of the disease was made by molecular diagnosis pertaining to PCR.

Paired peripheral blood smears were made on clean grease free glass slides by pricking ear tip. About 5 ml whole blood was taken in K_3 EDTA vial and stored at -26 °C for PCR. The prevalence rate of ovine babesiosis was calculated in percentage with respect to season.

2.2 Clinical evaluation

Clinical evaluation of sheep was made by distant examination, auscultation of cardiac and lung area and recording vital parameters (rectal temperature, heart rate and respiratory rate), rumen motility, colour of visible mucous membranes and urine.

2.3 Laboratory examination

The blood smears were stained using Giemsa's stain and examined under oil immersion by using a light microscope (1000X) for identification and morphological characterization of *Babesia* piroplasms. The molecular confirmation of the *Babesia* parasite was accomplished by using polymerase chain reaction (PCR) as described by [15] using *Babesia* genus specific oligo-nucleotide primers (Table 1). All the PCR amplified samples which corresponded to a bp size of 408 were considered as *Babesia* positive samples with a comparison to 100 bp ladder and *Babesia* positive control.

2.4 Statistical analysis

Data generated were subjected to statistical analysis by applying chi-square test as described by [16].

Table 1: Oligo-nucleotide primers* used to amplify 408 bp portion of the small subunit ribosomal DNA of Babesia species

Product size		Primer Sequence[a]	Characteristic
408	F:	AATACCCAATCCTGACACAGGG	*Babesia* specific
	R:	TTAAATACGAATGCCCCCAAC	*Babesia* specific

* *Babesia* genus-specific primer (GCC Biotech)

[a] The 5´ to 3´ primer sequence is given.

3. Results

3.1 Seasonal prevalence

The cases of ovine babesiosis presented during summer (Jun-Aug) and spring (March-May) were higher than the cases presented during other seasons of the year (Table 2). Out of 691 animals screened, the highest prevalence of babesiosis was recorded in summer season (33.19%), followed by spring (31.03%), autumn (18.10%) and lowest in the winter (12.38%). Similarly, out of 185 positive cases of ovine babesiosis the highest prevalence was recorded in summer season-79/185 (42.70%), followed by spring - 72/185 (38.92%), autumn-21/185 (11.35%) and lowest in the winter-13/185 (7.03%) as depicted in Table 2 and 3.

Table 2: Animals screened for ovine babesiosis during May 2016 to June 2017.

Season	Months	No. of animals screened
Autumn	September	50
	October	23
	November	43
Winter	December	22
	January	30
	February	53
Spring	March	54
	April	93
	May	85
Summer	June	68
	July	94
	August	76
Total	691	
Total *Babesia* cases	185/691	
Prevalence	26.77%	

Table 3: Prevalence of ovine babesiosis based on seasons

Season	Months	Animals screened
Autumn		
	September	50
	October	23
	November	43
	Total	116
	Positive case	21[a]/116
	%age	18.10
Winter	December	22
	January	30
	February	53
	Total	105
	Positive case	13[a]/105
	%age	12.38
Spring	March	54
	April	93
	May	85
	Total	232
	Positive case	72[b]/232
	%age	31.03
Summer	June	68
	July	94
	August	76
	Total	238
	Positive case	79[b]/238
Over all		185/691
Prevalence		26.77%

In the last row, for each parameter, the values with different superscripts differ significantly ($P<0.05$)

The values in positive case rows for each season with different superscripts differ significantly ($P<0.05$)

3.2 Clinical presentation
Out of 691 sheep examined, 87 were showing clinical signs resembling ovine babesiosis. The clinical signs and symptoms recorded in majority of the cases were high fever, pale mucous membranes, congested mucous membranes, presence of *Haemaphysalis* ticks gross and microscopic view, respectively in (Fig 1 and 2) on the body surface particularly on ear-pinna, emaciation elevated heart and respiration rates, decreased ruminal contraction and coffee/red coloured urine.

3.3 Microscopic examination
Out of 691 animals screened, blood smears of 185 sheep (26.77%) were found positive for ovine babesiosis by microscopic examination. Positive blood smears showed different intra-erythrocytic forms of haemoprotozoa which were morphologically compatible with *Babesia* piroplasms.

3.4 Polymerase chain reaction (PCR)
Out of 87 clinically and microscopically positive cases, 32 blood samples were taken and subjected to PCR for the detection of ovine babesiosis which showed 408-bp amplified *(Babesia* genus specific) DNA fragment in all the samples by employing specific primers. PCR reactions amplifying a band of 408-bp were considered positive for the *Babesia* infection (Fig 3 Lane 1, 2 and 3). Positive control sample always showed the requisite band size of 408-bp (Fig 3). For negative control, nuclease-free water was used and no band was observed in this process (Fig 3).

4. Discussion
The overall prevalence of ovine babesiosis in sheep recorded in the study area was 26.77%. However, a lower prevalence (2.43%) of ovine babesiosis was reported by [17] in Ganderbal district of Kashmir.

The high prevalence of ovine babesiosis recorded in the present work may be primarily due to the import of sheep from other states of India, where they might have been previously infected with babesiosis. The climate change due to global warming may favour the propagation of tick vectors and transmission of the disease. Stress due to increased environmental temperature makes sheep susceptible to haemo-protozoan infections. In Kashmir Valley due to climatic alterations, the vector population is consistently expanding to unforeseen areas like high altitude or temperate zones and the temperature on an average has shown a rise in 1.45 °C while in Jammu region the rise is 2.32 °C over last two decades [10]. Therefore, it is anticipated that climate change and import of carrier animals from neighboring states (Punjab, Himachal Pradesh, Rajasthan and Haryana) may be attributed to the occurrence of babesiosis in the valley. This finding is in close agreement with the observation of [18]. The highest prevalence of ovine babesiosis in summer (June to August) stands in agreement with the observation of [19-21]. The high prevalence rate during summer might be due to hot and humid climate as the tick infestation is influenced by the temperature, rainfall and relative humidity which in turn directly determine the prevalence of haemo-protozoan diseases [5]. Higher prevalence of haemo-protozoan diseases was also reported during the rainy season [21, 22]. Lower temperature and humidity in winter months do not favour the growth and multiplication of tick vectors and thereby haemo-protozoan diseases [23].

Variable prevalence's were reported by many authors, 51.96% [24], 48% [25], 6.36% [26] and 23.5% [27]. Such variation can be attributed to climatic conditions which affect the vector activity. The highest rate was in summer followed by spring, autumn and winter, similar results were reported by many other authors where spring and summer represented the season of high activity of the tick vector [28, 29].

5. Conclusion
The overall prevalence of ovine babesiosis was 26.77%, being highest in the summer followed by spring season. The higher prevalence of disease may be due to climate change and vector proliferation in summer season. So, need of an hour is to address the issue and plan prophylactic measures according to the seasonal prevalence of disease. The study summarizes the fact that season has an important role in disease prevalence.

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Fig 1: Ticks adherent to ear of sheep suffering from babesiosis

Fig 2: *Haemaphysalis* tick identified in *Babesia* affected sheep

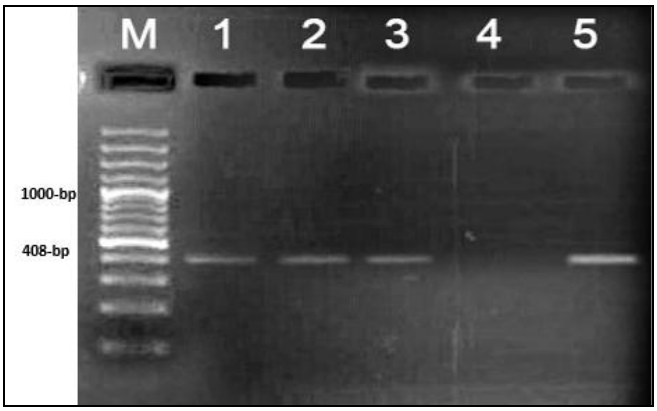

Fig 3: Agar gel electrophoresis PCR products of *Babesia* positive samples DNA extracts with primers specific for *Babesia* genus (408-bp)

Lane M	:	100-bp ladder
Lane M	:	100-bp ladder
Lane 1	:	408-bp Babesia +ve
Lane 2	:	408-bp Babesia +ve
Lane 3	:	408-bp Babesia +ve
Lane 4	:	Babesia -ve control
Lane 5	:	Babesia +ve control

7. References

1. Liu AH, Yin H, Guan GQ, Schnittger L, Liu ZJ, Ma ML *et al*. At least two genetically distinct large *Babesia* spp. infective to sheep and goats in China. Veterinary Parasitology. 2007; (147):246-251.
2. Taylor MA, Coop RL, Wall RL. Veterinary Parasitology, 3rd Ed, Blackwell Publishing, 2007.
3. Soulsby EYL. The Helminths, Arthoropods and Protozoa of Domestic Animal. Bailliere & Tindall, London, 1986, 809.
4. Radostitis OM, Gay CC, Blood DC, Hinchcliff KW. Veterinary medicine, A text of the diseases of cattle, sheep, pigs, goats and horse. 9th Ed, Saunders Company, Philadelphia: WB, 2000, 1289-1296.
5. Ghosh S, Bansal GC, Gupta SC, Ray D, Khan MQ, Irshad H *et al*. Status of tick distribution in Bangladesh, India and Pakistan. Parasitology Research. 2007; (2):207-216.
6. Naz S, Maqbool A, Ahmed S, Ashraf K, Ahmed N, Saeed K *et al*. Prevalence of theileriosis in small ruminants in Lahore-Pakistan. Journal of Veterinary and Animal Sciences. 2012; (2):16-20.
7. Muraleedharan K, Ziauddin KS, Margoob Hussain P, Puttabytappa B, Seshadri SJ. Prevalence of parasitic infection among small domestic animals. Karnataka Journal of Agricultural Sciences 1994; 7:64-68.
8. Torina A, Vicente J, Alongi A, Scimeca S, Turla R, Nicosia S *et al*. Observed Prevalence of tick-borne pathogens in domestic animals in Sicily, Italy during 2003-2005. Zoonoses Public Health. 2007; 54:8-15.
9. Iqbal F, Fatima M, Shahnawaz S, Naeem M, Shaikh RS, Ali M *et al*. A study on the determination of risk factors associated with babesiosis and prevalence of *Babesia* spp. by PCR amplification, in small ruminants from southern Punjab (Pakistan). Parasite. 2011; 18:229-234.
10. Anonymous. Action Aid Report. Community concerns on climate change, 2007. www.actionaidorg/India.
11. Shaw AA. Investigation on some infections in the exotic, pure and crossbred cattle of Kashmir valley. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases. 1989; (10):33-38.
12. Tufani NA, Hafiz A, Malik HU, Peer FU, Makhdoomi DM. Clinico-therapeutic management of acute babesiosis in bovine. Intas Polivet. 2009; 10(1):49-50.
13. Tufani NA, Fazili MR, Malik HU, Beigh SA, Dar KH. Clinico haematological Profile and therapeutic management of acute babesiosis in a Holstein-friesian crossbred cow. Veterinary Clinical Science. 2015; 3(3):11-14.
14. Haq AU, Tufani NA, Gugjoo MB, Nabi SU, Malik HU. Therapeutic amelioration of severely anaemic local Kashmiri goats affected with babesiosis. Advances in Animal and Veterinary Sciences. 2017; 5(11):463-467.
15. Olmeda AS, Armstrong PM, Rosenthal BM, Valladares B, Castillo AD, Armas FD *et al.* A subtropical case of human babesiosis. Acta Tropica. 1997; (67):229-234.
16. Snedecor GW, Cochran WG. Statistical Methods, 8th Ed, Iowa State University Press, Ames, Iowa, 1994.
17. Rather SA, Tak H, Kakru DK. Seroprevalence of haemoprotozoan diseases in ruminants of Kashmir valley. Agricultural Advances. 2015; 4(11):122-128.
18. Khan A, Rehman AU, Hisham M, Khan AZ, Rehman HU, Khan MI *et al*. Burden of babesiosis among domestic cattle of southern Khyber Pakhtunkhwa, Pakistan. Journal of Entomology and Zoology Studies. 2016; 4(5):305-307.
19. Rashid A, Khan JA, Khan MS, Rasheed K, Maqbool A, Iqbal J. Prevalence and chemotherapy of babesiosis among Lohi sheep in the livestock experiment station, Qadirabad, Pakistan, and environs. The Journal of Venomous Animals and Toxins including Tropical Diseases. 2010; 16(4):587-591.
20. Mahmud AAM, Belal SSH, Hossain MA. Prevalence of theileriosis and babesiosis in cattle in Sirajganj district of Bangladesh. Research in Agriculture, Livestock and Fisheries. 2015; 2(1):79-86.
21. Radostits OM, Gay CC, Hinchcliff KW, Constable PD. Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats. 10th Ed, Elsevier, Philadelphia, 2007.
22. Bhatnagar CS, Bhardawaj B, Sharma DK, Meena SK. Incidence of Haemoprotozoan diseases in Cattle in Southern Rajasthan, India. International Journal of Current Microbiology and Applied Sciences. 2015; 4(3):509-514.
23. Zahid IA, Latif M, Baloch KB. Incidence and treatment of theileriosis and babesiosis. Pakistan Veterinary Journal. 2005; 25(3):137-139.
24. Ali D ZG, Zibam. Seroprevalance of Babesia ovis in sheep around Afyon. Turky, Journal of Veterinary and Animal Science. 2004; 28:638-686.
25. Jurasek V. Results of the laboratory examinations of parasitoses in the animals of Bilimleri Dergisi, Firant Universitesi Veteriner. 1986; 16(1):79-83.
26. Inci A, Karer Z, Ica A. Babesiosis in sheep and goat around kayseri. Saglik-Bilimleri Dergisi, Firant Universitesi Veteriner. 2002: 16(1):79-83.
27. Razmi GR, Naghibi A, Aslani MR, Datjerdiand K, Hossieni H. An epidemiological study on Babesia infection in small ruminants in Mashhad suburb; Khorasan province; Iranian Small Ruminant Research. 2003; 9500:39-44.
28. Pipano E. Observation on the seasonal distribution of blood parasites in Veterinary Medicine. 9th Ed. WH Sounders Co. Ltd, London, New York, 1991.

29. Yeryham I, Hadani A, Gafker F, Rosen SH Schlien J. A field study of haemoparasites in two flocks of sheep in Israel. Journal of Veterinary Medicine. 1992; 47(3):107-111.