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Cross sectional study on prevalence of ovine babesiosis in different breeds of Kashmir valley

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Abstract
A study was undertaken to elucidate the prevalence of ovine babesiosis in Kashmir. Present study evaluated 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. Animals were screened from May 2016 to June 2017 for the prevalence of ovine babesiosis. A total of 185 cases of ovine babesiosis were 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 based on the major breeds present in Kashmir valley. Group A comprised of cross bred sheep, group B comprised of corriedale breed of sheep, group C comprised of south down breed of sheep and group D comprised of local bakarwal breed of sheep. The higehest prevalence of diseases (babesiosis) was found in cross bred sheep (91.35%), followed by Corriedale (8.11%), South Down (0.54%) and Bakerwal (0.00%) breeds. The disease was more predominant in females (78.37%) than males (21.62%). *Haemaphysalis* ticks were identified as the vectors for the transmission of ovine babesiosis in the study area.

Keywords: Cross sectional study, prevalence, different breeds, Kashmir valley

Introduction
Tick-borne diseases are important constraints on livestock industries especially in the production of ruminants in tropical and subtropical areas. In India the economic loss due to tick-borne diseases has been estimated US$ 498.7 million per annum [1]. *Babesia ovis* and *Babesia motasi* commonly occur in Southern Europe, former Soviet State, Middle East, Asia and Africa. Mixed infection with *Babesia ovis* and *Babesia motasi* is highly-pathogenic to sheep [2]. The combination of imidocarb dipropionate and oxytetracycline is the most effective treatment of babesiosis in sheep and goats [3]. To aid recovery, supportive treatments are often administered, such as iron and vitamins. Blood transfusion is highly recommended for animals with acute anaemic anoxia [4].

In Kashmir Valley there is little historical information on ovine haemo-protozoan infection such as *Babesia*, *Theileria* and *Anaplasma* [5, 6, 7]. However, ovine babesiosis has not been established earlier and is often undiagnosed in the Valley which can cause serious economic loss to the farmers. During recent times there has been a surge in import of livestock from outside Kashmir which has made the introduction of new germ pool in Kashmir and introduction of new infection. 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 major sheep breeds of Kashmir valley.

Materials and Methods
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 breed.

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. 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 [8] 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.

Statistical analysis

Data generated were subjected to statistical analysis by applying chi-square test as described by [9].

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.

Results

Out of 691 sheep, the number of Corriedale, South down, Crossbred and Bakerwal was 50, 24, 607 and 10, respectively. Among different breeds of sheep cross bred (169, 27.84%) was highly positive for *Babesia* infection, followed by South-down (1, 4.17%), Corriedale (15, 30.00%) and Bakerwal (0, 0.00%) as shown in Table 2. Out of 185 positive cases (Table 3), the highest percentage of *Babesia* infection was recorded in Crossbred sheep (91.35%), followed by Corriedale (8.11%), South down (0.54%) and Bakerwal (0%), respectively.

Out of 691 cases, an overall prevalence of ovine babesiosis was highest in female (37.66%) than in male (13.07%). Similarly, among 185 positive cases, the prevalence of ovine babesiosis was highest in female (78.37%) as compared to male (21.62%) as shown in Table 3.

Clinical Presentation

Out of 691 sheep examined, 87 were showing clinical signs resembling ovine babesiosis. The frequency distributions of clinical signs observed in this study are depicted in Table 4. The clinical signs and symptoms recorded in majority of the cases were high fever, pale mucous membranes (Fig 1).

Table 2: different breeds of sheep screened for ovine babesiosis during May 2016 to June 2017

Season	Months	Corriedale	South Down	Cross bred	Bakerwal
Autumn		12	8	25	5
	September	0	0	23	0
	October	0	0	43	0
	November	3	2	16	1
Winter	December	0	0	30	0
	January	0	0	53	0
	February	6	0	48	0
Spring	March	9	7	76	1
	April	1	O	83	1
	May	6	O	62	0
Summer	June	9	6	77	2
	July	4	1	71	0
	August	50	24	607	10
Total		15/50	1/24	169/607	0/10
Total *Babesia* cases		30.00%	4.17%	27.84%	0.00%

Table 3: Prevalence of ovine babesiosis based on breed

Season	Months	Corriedale	South Down	Cross bred	Bakerwal
Autumn	September	12	8	25	5
	October	0	0	23	0
	November	0	0	43	0
	Total	12	8	91	5
	Positive case	2/12	0/8	19/91	0/5
	%age	16.67	0.00	20.88	0.00
Winter	December	3	2	16	1
	January	0	0	30	0
	February	0	0	53	0
	Total	3	2	99	1
	Positive case	1/3	0/2	12/99	0/1
	%age	33.33	0.00	12.12	0.00
Spring	March	6	0	48	0
	April	9	7	76	1
	May	1	0	83	1

	Total	16	7	207	2
	Positive case	6/16	1/7	65/207	0/2
	%age	37.50	14.29	31.40	0.00
Summer	June	6	0	62	0
	July	9	6	77	2
	August	4	1	71	0
	Total	19	7	210	2
	Positive case	6/19	0/7	73/210	0/2
	%age	31.58	0.00	34.76	0.00
overall		15^{b}/185	1^{a}/185	169^{a}/185	0/185
	% age	8.11%	0.54%	91.35%	0.00%

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 ($P<0.05$)

Table 4: Frequency distribution of clinical signs and symptoms in 87 cases of ovine babesiosis.

Clinical signs	No. of animals affected	Percentage (%)
Fever	75/87	86.21
Ticks present	49/87	56.32
In appetence	43/87	49.43
Pale mucous membranes	39/87	44.83
Congested mucous membranes	46/87	52.87
Icteric mucous membranes	2/87	2.30
Normal colour urine	65/87	74.71
Coffee/red coloured urine	20/87	22.99
Dark yellow coloured urine	2/87	2.30
Diarrhoea	17/87	19.54
Constipation	4/87	4.60
Emaciation	6/87	6.90

Microscopic Examination
Positive blood smears showed different intra-erythrocytic forms of haemoprotozoa which were morphologically compatible with *Babesia* piroplasms (fig 2).

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 (Fig 3).

Discussion
The *Babesia* infection was highest in Corriedale and lowest in Bakerwal are in close agreement with [10] who reported a higher prevalence of infection in exotic and cross-bred ruminants compared to local breeds. The results are supported by [11]. The local animals have genetic makeup which makes them resistant to these parasites. Moreover, change in climate does not affect the normal physiology of the local breeds and make them resistant to the infection.
Female sheep (37.66%) had a higher prevalence of infection as compared to males (13.07%). Similar findings were also observed by [10] in Ganderbal district, Kashmir. Such an observation agrees with the findings of [12, 13] in babesiosis affected sheep and goats. Female sheep are subjected to stress during different physiological conditions which increases their susceptibility to haemo-protozoan infection. Adult sheep particularly females come into stress that could be attributed to pregnancy or act of parturition.
The clinical signs of fever, inappetence, pale mucous membranes, haemoglobinuria and diarrhoea (19.54%) and presence of ticks are in agreement with the studies of [14-16]. Inappetence observed in diseased sheep correlates with the findings of [17, 14]. The inappetence and anorexia could be attributed to the persistent fever and decreased rumen motility and the pale mucous membranes were noticed due to the development of anaemia [18].
Haemoglobinuria observed in our study correlates with the findings of [19, 17, 20-22, 16]. Haemoglobinuria could be attributed to sever haemolytic process associated with the presence of *Babesia* piroplasms within red blood cells [20]. Diarrhoea recorded in the present study corroborates with the findings of [19].
Haemaphysalis ticks were identified as the vectors for the transmission of ovine babesiosis in the area of investigation. Similar findings were reported by [23, 24, 16] that identified *Haemaphysalis* ticks responsible for the transmission of *Babesia* infection in sheep and goats.

Conclusion
The overall prevalence of ovine babesiosis was 26.77%, being highest Cross-bred sheep above 12 months were mostly affected compared to other breeds. Major clinical observations were high fever, pale to congested mucous membranes, dark yellow to coffee coloured urine, inappetence and presence of ticks. PCR is the best tool for the confirmation as well as diagnosis of asymptomatic cases of ovine babesiosis.

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Fig 1: Pale mucous membrane in babesiosis affected sheep

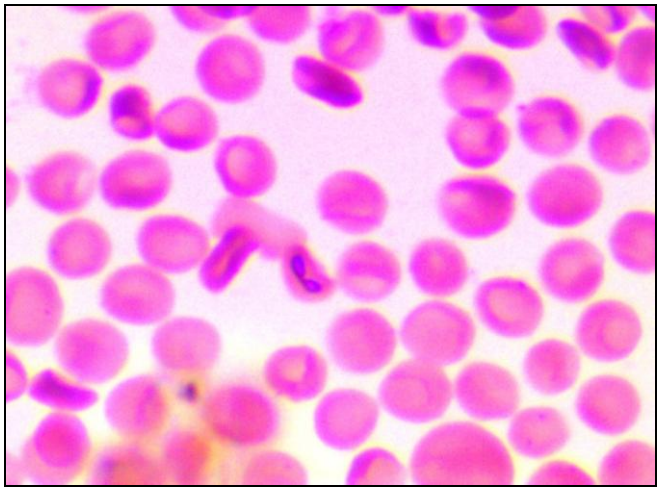

Fig 2: Microphotograph showing intraerythrocytic *Babesia* piroplasms in sheep (Giemsa-stained, 1000X)

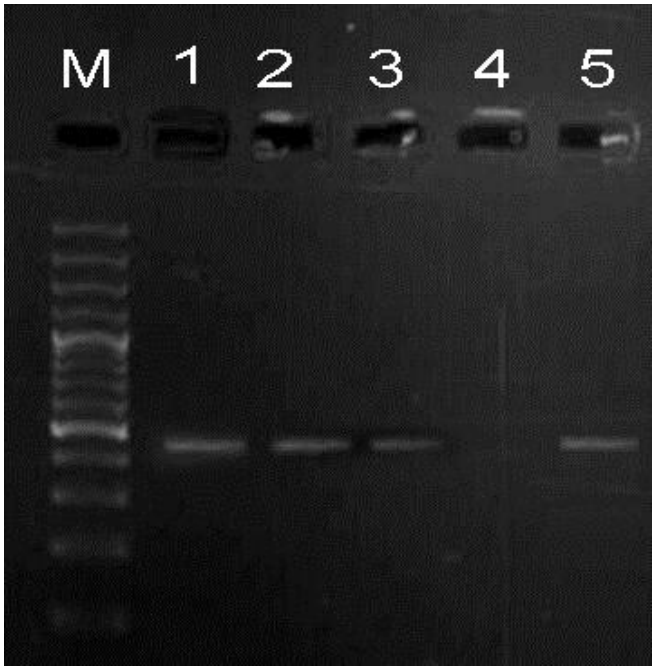

Fig 3: Agar gel electrophoresis PCR products of *Babesia* positive samples DNA extracts with primers specific for *Babesia* genus (408-bp)

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 Babes*ia* +ve
Lane 4 : *Babesia* -ve control
Lane 5 : *Babesia +ve* control

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