Cross seasonal study on prevalence of ovine babesiosis in Kashmir

Abrar Ul Haq, NA Tufani, HU Malik, SA Hussain, Idrees Mehraj Allaie and SU Nabi

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, Shuhama, SKUAST-K, 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 haemoprotezoon 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-proteozoon 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

<table>
<thead>
<tr>
<th>Product size</th>
<th>Primer Sequence$^a$</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>408</td>
<td>F: AATACCCAATCCTGACACAGGG</td>
<td>Babesia specific</td>
</tr>
<tr>
<td></td>
<td>R: TTTAATACGAATGCCCCCAAC</td>
<td>Babesia specific</td>
</tr>
</tbody>
</table>

$^a$ Babesia genus-specific primer (GCC Biotech)

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.

<table>
<thead>
<tr>
<th>Season</th>
<th>Months</th>
<th>No. of animals screened</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>September</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>November</td>
<td>43</td>
</tr>
<tr>
<td>Winter</td>
<td>December</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>January</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>February</td>
<td>53</td>
</tr>
<tr>
<td>Spring</td>
<td>March</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>April</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>232</td>
</tr>
<tr>
<td>Summer</td>
<td>June</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>76</td>
</tr>
<tr>
<td>Total</td>
<td>691</td>
<td>185/691</td>
</tr>
<tr>
<td></td>
<td>Babesia cases</td>
<td>26.77%</td>
</tr>
</tbody>
</table>

Table 3: Prevalence of ovine babesiosis based on seasons

<table>
<thead>
<tr>
<th>Season</th>
<th>Months</th>
<th>Animals screened</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>September</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>November</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Positive case</td>
<td>21/116</td>
</tr>
<tr>
<td></td>
<td>%age</td>
<td>18.10</td>
</tr>
<tr>
<td>Winter</td>
<td>December</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>January</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>February</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>Positive case</td>
<td>13/105</td>
</tr>
<tr>
<td></td>
<td>%age</td>
<td>12.38</td>
</tr>
<tr>
<td>Spring</td>
<td>March</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>April</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>232</td>
</tr>
<tr>
<td></td>
<td>Positive case</td>
<td>72/232</td>
</tr>
<tr>
<td></td>
<td>%age</td>
<td>31.03</td>
</tr>
<tr>
<td>Summer</td>
<td>June</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>238</td>
</tr>
<tr>
<td></td>
<td>Positive case</td>
<td>79/238</td>
</tr>
<tr>
<td></td>
<td>%age</td>
<td>31.03</td>
</tr>
</tbody>
</table>

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-pinnia, 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 [21].

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.

6. Acknowledgment
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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