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Scientific Journal of Veterinary Advances

Journal homepage: www.Sjournals.com



Original article

Seroprevalence of *Babesia bigemina* and *Anaplasma marginale* in domestic animals of district Ganderbal

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ARTICLE INFO

Article history,

Received 13 December 2015

Accepted 12 January 2016

Available online 19 January 2016

iThenticate screening 16 December 2015

English editing 10 January 2016

Quality control 16 January 2016

Keywords,

Seroprevalence

ELISA

Babesia

Smear

TBDs

ABSTRACT

The present study of seroprevalence of *B. bigemina* and *A. marginale* in cattle, sheep and goats was studied in Ganderbal district of Kashmir, between January to December 2012. A total of 153 blood samples were collected randomly from 40 cattle, 52 sheep and 61 goats for the preparation of blood smears and serum samples in four consecutive seasons and tested against *B. bigemina* and *A. marginale* using the SVANOVIR[®] *B. bigemina*-Abs and *A. marginale*-Abs ELISA Kit. Samples were also examined by Giemsa's stained blood smear method. The effect of topography, season, age, gender and breed was observed in cattle during this study. The overall prevalence of *B. bigemina* infection was 3 (7.5%), 2 (3.84%) and 3 (4.91%) in cattle, sheep and goats and for *A. marginale* 2 (5%), 1 (1.92%) and 1 (1.63%) respectively. The mixed infections between *B. bigemina* and *A. marginale* were 5% in cattle, 1.92% in sheep and 3.27% in goats. The seasonal prevalence of *B. bigemina*, *A. marginale* and mixed infection between them peaked in summer as revealed by blood smear examination and ELISA.

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1. Introduction

Tick-Borne Diseases (TBDs) are a constraint to livestock production in many developing countries of the world (Gubbels et al., 1999; Kursat et al., 2004; Oura et al., 2005) and are responsible for high morbidity and mortality resulting in decreased production of meat, milk and other livestock products (Rajput et al., 2005). They are also a significant impediment to the improvement of indigenous breeds of cattle, sheep and goats, since they prevent the introduction of more productive exotic breeds (EFSA). Haemoprotzoan diseases are of considerable economic importance to the agro-animal industry of Jammu and Kashmir. Blood protozoa such as *Babesia*, *Theileria*, *Trypanosoma* and blood rickettsia such as *Anaplasma* has been studied in animals of Jammu and Kashmir (Shaw, 1989; Tufani, 2009). The diseases are frequently characterized by fever, hemolytic anemia, high morbidity and death in severe cases (Uilenberg, 1995; Radostits et al., 2000; Ellis et al., 2003; Schnittger et al., 2012). The prevalence varies from region to region and various factors determine the occurrence of the TBDs including age, sex, breed, tick density, season, geographical area and management (Kivaria, 2006; Magona et al., 2011). Microscopic examination of Giemsa stained blood samples and ELISA was used for the seroprevalence studies. Almost no information has been published on seroprevalence of Babesiosis and Anaplasmosis and its pathogenesis in domestic ruminants in Kashmir except for one farm study (Shaw, 1989) and one clinical diagnosis of Babesiosis (Tufani et al., 2009) in cattle. Therefore, the main purposes of the present study were to detect infection of *Babesia* and *Anaplasma* in cattle, sheep and goats in district Ganderbal of Kashmir by blood smear and ELISA to define their seroprevalence.

2. Material and methods

Blood samples were collected from 40 cattle, 52 sheep and 61 goats in Ganderbal district for a period of a year between January and December 2012. Samples were selected randomly from each animal. Information about age, breed and gender was recorded. Sera were separated by centrifugation and stored at -20 C° and was further used for ELISA studies.

Thin and thick blood smears were prepared from the peripheral blood and jugular vein blood of the goat, sheep and cattle as described by Afridi et al. 2005. Labeled blood samples were transported on ice to the laboratory for further storage and processing. In the laboratory, blood samples were stored at -20°C until required for studies. The smears were air dried, fixed in absolute methanol and stained for 30 min in a 5% dilution of Giemsa solution in PBS having pH 7.2. The slides were examined with oil immersion x100. Twenty microscopic fields were observed in search of blood parasites. The blood parasites were identified as described by various OIE publications (OIE, 2004, 2008a, b)

The sera of animal's samples were detected for presence of antibodies against *B. bigemina* and *A. marginale* using ELISA technique. SVANOVIR® kits for the two micro-organisms were used. The animals were categorized as young or adult according to onsite observation and information from their owners. Animals older than 2 years of age were considered as adult. Information about the breed (Indigenous and cross-breed) was also collected from the farmers and the experts. All the demographic data was noted down.

3. Results

Microscopic and serological test for *B. bigemina* and *A. marginale* showed that 5, 3 and 4 were positive in cattle, sheep and goats respectively. Sero-positive for *B. bigemina* and *A. marginale* antibodies and their mixed infection is showed in Table 1.

The Figure 1 showed that highest prevalence of *B. bigemina* was found in cattle (7.5%) and lower value (3.84%) was detected in sheep, also the highest rates of positive prevalence *A. marginale* (5%) were diagnosed in cattle while lower value (1.63%) in goats.

Result of Figure 2 show that both *B. bigemina* and *A. marginale* was observed more frequently in summer followed by autumn in all the three study animals. However, neither *B. bigemina* nor *A. marginale* could be identified in any of the blood sera analyzed in the other two seasons (spring and autumn).

It was found that's out of the 12 positive infections there was only 5 animals infected by *B. bigemina* and *A. marginale* in the same time (Mixed infection between *B. bigemina* and *A. marginale*) and which represented of

(5%) cattle, (1.92%) sheep and (3.27%) goats as showed in Figure 3, on the other hand the highest prevalence of co-infection was in June (40%) Figure 3.

Table 1

Number of infected animals by *B. bigemina* and *A. marginale* and mixed infection between them.

Animal	No. of examined	No. of Sero-positive with		Total	Mixed infection of <i>Babesia</i> and <i>Anaplasma</i> in same animals
		<i>B. bigemina</i>	<i>A. marginale</i>		
Cattle	40	3 (7.5%)	2 (5%)	5	2 (5%)
Sheep	52	2 (3.84%)	1 (1.92%)	3	1 (1.92%)
Goats	61	3 (4.91%)	1 (1.63%)	4	2 (3.27%)
Total	153	8	4	12	5

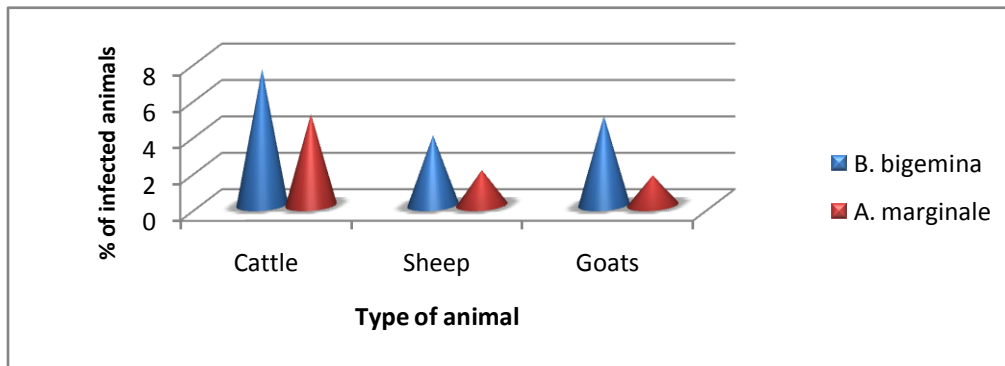


Fig. 1. Percentage of infection by *B. bigemina* and *A. marginale* in domestic animals.

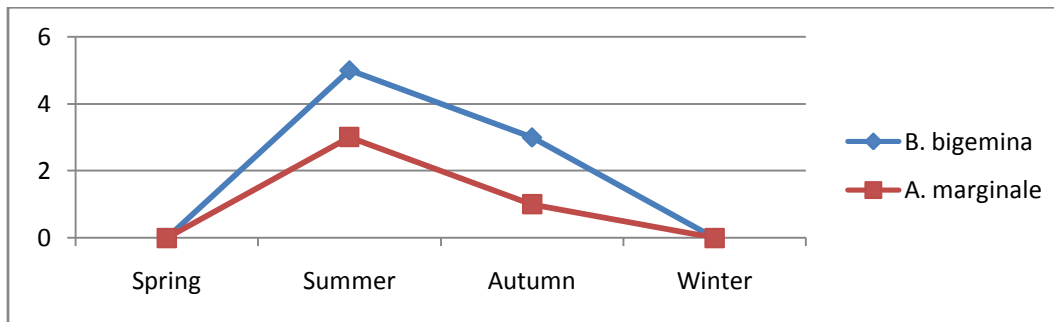


Fig. 2. Seasonal dynamic infection by *B. bigemina* and *A. marginale* in study animals.

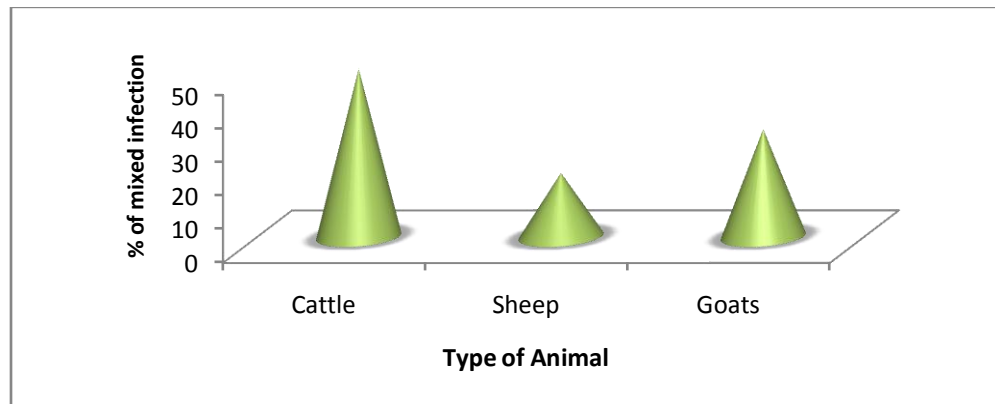


Fig. 3. Percentage of mixed infection between *B. bigemina* and *A. marginale* in domestic animals.

Table 2Age-wise Seroprevalence of *B. bigemina* and *A. marginale* in domestic animals.

Parameter	(Sero-positive for <i>B. bigemina</i> / <i>A. marginale</i>)			
	Cattle	Sheep	Goats	Total
Age				
Young (<2 years) 0/0	0.0	1.0	1.0	2
Adults (>2 years) 3/2	3.2	1.1	2.1	10
Gender				
Male	1	1	2	4
Female	4	2	2	8
Breed				
Indigenous	1	0	0	1
Cross-breed	4	3	4	11

The adults of the domestic animals were found to be more infected than the young individuals. Out of the total 12 positive animals only 2 young, one from each sheep and goats were infected. Females were two times more infected than male as shown in Table 2. However, a clear cut indication from the results shows that cross-breed animals are predominantly becoming hosts of *B. bigemina* and *A. marginale*.

4. Discussion

It was hard to find any previous reference regarding sero-prevalence of *B. bigemina* and *A. marginale* infection from the entire Kashmir valley. Major parts of study districts have distinct agro-ecological zones, justifying the significant similarity in seroprevalence among study sites from the district Ganderbal. Moderate climate of Kashmir favours the growth and multiplication of vector ticks in summer months only. The life cycle of vectors plays an important role in the epidemiology of Babesiosis and Anaplasmosis. No work has been previously done in regard to sero-prevalence by ELISA of *B. bigemina* and *A. marginale* in cattle, sheep and goat in the entire Kashmir valley. However, studies on blood protozoa such as *Babesia*, *Theileria*, *Trypanosoma* and blood rickettsia such as *Anaplasma* have been carried out in animals of Jammu and Kashmir (Shaw, 1989; Tufani, 2009).

The low incidence of both *B. bigemina* and *A. marginale* indicates the low spread distribution of Babesiosis and Anaplasmosis in the area of study, this might be due to the geographical distribution of tick vectors transmitted these diseases and the low distribution of the principle vectors. However, lack of quarantine measures, veterinarian availability and pastoral migrations of flocks and herds from other hot, humid areas in the valley resulted in an increase in the infectivity of haemoparasites. Present findings are in agreement with the previous work done in the other parts of the world having similar or close environmental conditions as of the Kashmir valley (Jithendra, 1997; Sharma et al., 2000; Saud et al., 2004; Zahid et al., 2005; Ahmad and Hashmi, 2007; Anand et al., 2009; Singh et al., 2012). However, Shaw (1989) has reported babesiosis (1.8%) in cattle of Kashmir valley. Lower prevalence in Shaw (1989) studies may be due to the reason that the samples were collected from the intensive cattle development centers. This present study also demonstrated the mixed infection between *B. bigemina* and *A. marginale* in all infected animals. Competitive ELISA technique as recommended by World Animal Health Organization for the serodiagnosis of Anaplasmosis in cattle (OIE, 2004) allowed us to detect parasites at low parasitaemia while discriminating various species of mixed infecting agents (Adejinmi et al., 2004; Shayan et al., 2005). The cELISA uses a 19-kDa antigen based on recombinant major surface protein (MSP5) which is highly conserved among *Anaplasma* species (Knowles et al., 1996; Palmer et al., 2000).

The prevalence of infection between seasons was found to be different in the infection by *B. bigemina* and *A. marginale* in domestic animals (Hunfield et al., 2008). The high prevalence rate during summer may be due to hot and humid season prevalent during summer months as the tick infestation is influenced by temperature, rainfall and relative humidity which in turn directly determine the prevalence (Uilenberg et al., 2006; Gosh et al., 2007; Naz et al., 2012). Hot and humid season favours the propagation and multiplication of ticks (Soulsby, 1982). The prevalence also varies from region to region, host, management and environmental factors (agro-ecological and geo-climatic conditions) influence the prevalence of ticks and tick-borne diseases (Kivaria, 2006). In order to determine the epidemiology of tick-borne diseases, it is crucial to know the seasonal activities of the ticks. So in the

present study all infection by tick and TBDs arise in summer and carried to the autumn. Seasonal distribution of *B. bigemina* and *A. marginale* antibodies peaked in summer in cattle, sheep and goats respectively followed by autumn and absent in both in winter and spring (Kocan et al., 2000).

B. bigemina and *A. marginale* infections in cattle (young; <2 year) were found uninfected (Table 2). This effect could not be observed in sheep and goats. Although the young calves below two years of age are resistant to *Babesia* infection but such animals may act as carriers for considerable periods of time. Also low prevalence of these parasites in lambs below six month of age could be attributed to transfer of maternal immunity to lambs. Above mentioned results are also in argument with results of (Shaw, 1987; Kocan et al., 2010; Atif et al., 2010; Singh et al., 2012), but contradictory to the findings of Naz et al. 2012 who observed that there is no effect of age of these diseases in goats.

In the present study female hosts appeared more prone to tick-borne diseases (TBDs) than males. Similar results were shown on gender-wise prevalence by Atif et al., (2012), although these differences were not statistically significant. The immunosuppression in advanced pregnancy and or lactation in high producing study animals were the possible reasons for higher prevalence of infection in domestic animals (Kocan et al., 2010). These findings are also in agreement with the results of (Shaw, 1989; Rajput et al., 2005; Durrani, 2008; Rehman et al., 2010).

The higher seropositivity of *B. bigemina* and *A. marginale* in crossbreed than study animals may be due to the different genetic makeup which makes indigenous more resistant to these parasites and also different climatic conditions of this region also may not favour the disease indigenous animals. Similar findings are observed in the studies of (Yadav et al., 1985; Radostits et al., 2000; Urquhart et al., 2003; Chaudhry et al., 2010). There have been reports of genetic differences among breeds and within-breed variation in resistance to infection by haemoparasites.

5. Conclusion

It was found that 18.36% of domestic small and large ruminants in district Ganderbal using of microscopic and ELISA technique were infected by *B. bigemina* and *A. marginale*. There is a need for further investigations using modern serological and molecular techniques for the complete epidemiological picture of TBDs and the identification of the carriers of the tick-borne pathogens.

Acknowledgement

Authors are highly thankful to the authorities of Department of Zoology, University of Kashmir for the facilities, they provide.

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How to cite this article: Shakeel Ahmad Rather, Hidayatullah Tak, Dalip K. Kakru 2016. Seroprevalence of *Babesia bigemina* and *Anaplasma marginale* in domestic animals of district Ganderbal. Scientific Journal of Veterinary Advance, 5(1), 74-79.

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