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Original article

Seroprevalence of Haemoprotozoan diseases in ruminants of Kashmir valley

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ABSTRACT

The study was designed to investigate the seroprevalence of haemoparasites (*Babesia*, *Anaplasma* and *Theileria*) among ruminants from Kashmir valley for a period of two years. A total of 585 blood samples were collected from selected small holders and private livestock farms using multistage cluster random sampling technique. EDTA containing, methanol fixed and Giemsa (1:10) stained thin peripheral blood smears were microscopically examined. Microscopic examinations showed the overall 74(29.83%), 9(4.29%) and 3(2.27%) seroprevalence of haemoparasitic infection in cattle, sheep and goats respectively. Prevalence of *Babesia*, *Anaplasma* and *Theileria* in cattle were 33(13.30%), 18(7.25%) and 23(9.27%) and in sheep 5(2.43%), 1(0.487%) and 3(1.46%) respectively. In goats the prevalence of *Babesia* and *Theileria* was 1(0.75%) and 2(1.51%). Age-wise epidemiological observations revealed highest prevalence rate in 1-5 years age group in cattle Overall gender-wise prevalence was not much prominent but female hosts were found to be more infected than males. The highest 56.71% prevalence of haemoparasitic diseases was found in exotic cattle with zero prevalence in local sheep.

1. Introduction

Haemoprotozoan diseases are of considerable economic importance to the agro-animal industry of Jammu and Kashmir (Shaw, 1989). However, it is known that most of blood protozoan parasites cause anaemia by inducing erythrophagocytosis. Most of the haemoprotozoan parasites are tick borne and is of great economic importance in Asia and has been always been a formidable barrier to the survival of exotic and cross bred animals in India. Among the various economically important vector-borne haemoprotozoan infections such as babesiosis, trypanosomosis and theileriosis are recognized as a cause of severe clinical illness in livestock. Studies on 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).

2. Materials and methods

In the present endeavor 585 blood samples (248 of cattle, 205 of sheep and 132 of goats) were collected and studied for protozoan infections by microscopic examinations. The entire positive and some uninfected blood samples were subjected to haematological and biochemical examinations. The present study was carried out in all the districts of Kashmir for a period of 2 years from 2010 to 2012.

The study area has four seasons i.e. autumn, winter, spring and summer. Most of the animals were having stall feeding system and poor management practices with usage of unhygienic needles for injecting treatment of generalized diseases. Multistage cluster random sampling technique was employed for blood sample collection. The data collected was assorted in the categories based on gender (male and female), breed (local, exotic and cross) and age (<1 year, 1-2 years and >2 years) in case of sheep and goats while as in cattle (< 1 year, 1-5 years and > 5years) groups were selected

Blood samples (5ml) were aseptically collected in a sterile syringe from the juglar vein and was transferred into test tubes containing EDTA (Imrovacuter® Improve Laboratory Supply, East-Flanders, Belgium). However, the blood collected from the ear capillaries were used to make blood smears. Thin blood smears were prepared as described by Afridi et al. (2005).The smears were fixed with absolute methanol and stained with dilute Giemsa stain (1:10 ratio) for 25 to 30 min. 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 present study was carried out to present the epidemiological aspect of these diseases in the study area. The results were statistically analyzed by chi square test in MINITAB software. The probability of significance was predetermined at $p < 0.05$.

3. Results and discussion

A total of 585 blood samples (248 from cattle, 205 from sheep and 132 from goats) were examined for the presence of haemoparasitic infection. Micorscopic examination showed that 74(29.83%), 9(4.29%) and 3(2.27%) was the prevalence of haemoparasitic infection in cattle, sheep and goats respectively. In cattle 74(29.83%) were infected with haematozoa comprising *Babesia* 33(13.30%), *Anaplasma* 18(7.25%) and *Theileria* 23(9.27%). Prevalence of Babesiosis in sheep was 5(2.43%), while as in goats it was 1(0.75%). The prevalence of Theileriosis in sheep was 3(1.46%), while in goats it was 2(1.51%). However, Anaplasmosis was found absent in goats 0(0%), while in sheep it was 1(0.487%). Mixed infection was observed in cattle and sheep only. Comparatively goats harbored low infection level as compared to cattle and sheep.

According to age-wise prevalence *Babesia* infection 21(20.38%) was found in the age group 1-5 years in cattle whereas zero infection were observed in < 1 year age group (Table 1). The *Anaplasma* infection was highest 14(13.59%) which was found in cattle of age group 1-5 years whereas *Anaplasma* infection was absent in sheep and goats. *Theileria* infection was highest 11(10.67%) in cattle and all together absent in goats in all age groups.

The differences between prevalence rates of heamoparasitic infection were not much prominent but in female cattle 32.70%, sheep 4.76% and goats 2.38% were found to be more infected than in male cattle 24.71%, sheep 4.22% and goats 2.22% (Table 2).

Table 1
Age wise prevalence of *Babesia*, *Anaplasma* and *Theileria* in ruminants.

Host	Age	Examined	*Babesia* (%)	*Anaplasma* (%)	*Theileria* (%)	Overall Prevalence (%)
Cattle	< 1 year	87	0(0)	3(3.44)	4(4.59)	7(8.04)
	1-5 year	103	21(20.38)	14(13.59)	11(10.67)	46(44.66)
	>5years	58	12(20.68)	6(10.34)	3(5.17)	21(36.20)
	Total	248	33(13.30)	23(9.27)	18(7.25)	74(29.83)
Sheep	< 1 year	50	1(2)	0(0)	1(2)	2(4)
	1-2 year	83	3(3.61)	0(0)	1(1.20)	4(4.81)
	>2years	27	2(7.40)	0(0)	1(3.70)	3(4.16)
	Total	160	6(3.75)	0(0)	3(1.83)	9(5.62)
Goat	< 1 year	38	1(2.63)	0(0)	0(0)	1(2.63)
	1-2 year	51	1(1.96)	0(0)	0(0)	1(1.96)
	>2years	43	1(2.08)	0(0)	0(0)	1(2.32)
	Total	132	3(2.27)	0(0)	0(0)	3(2.27)

Table 2
Gender wise prevalence of *Babesia, Anaplasma* and *Theileria* in ruminants.

Host	Gender	Examined	*Babesia* (%)	*Anaplasma* (%)	*Theileria* (%)	Overall Prevalence (%)
Cattle	Male	89	7(7.86)	8(8.98)	7(7.86)	22(24.71)
	Female	159	26(16.35)	10 (6.28)	16(10.02)	52(32.70)
	Total	248	33 (13.30)	18 (7.25)	23(9.27)	74(29.83)
Sheep	Male	142	3(2.11)	1(0.70)	2(1.40)	6(4.22)
	Female	63	2(3.17)	0(0)	1(1.58)	3(4.76)
	Total	205	5(2.43)	1(0.48)	3(1.46)	9(5.62)
Goat	Male	90	1(1.11)	0(0)	1(1.11)	2(2.22)
	Female	42	0(0)	0(0)	1(2.38)	1(2.38)
	Total	132	1(0.75)	0(0)	2(1.515)	3(2.27)

Breed-wise observations showed that in all the three (cattle, sheep and goats); exotic breed were more infected followed by cross breed and local breed ruminants (Table 3). The highest 56.71% prevalence of haemoparasitic infection was found in exotic cattle. *Babesia* infection was highest 20.89% in exotic cattle; however, altogether absent in cross breed and local goats respectively. *Anaplasma* was highest 14.92% in exotic cattle were as absent in goats, local and cross breed sheep respectively. *Theileria* infectivity was highest in cattle 20.89% and absent in goats and local sheep.

The present study of breed-wise prevalence indicated that in all the three cattle, sheep and goats exotic breed were more infected followed by cross breed and local breed ruminants (Table 4).The highest 56.71% prevalence of haemoparasitic diseases was found in exotic cattle. *Babesia* infection was highest 20.89% in exotic cattle were as altogether absent in cross breed and local goats respectively. *Anaplasma* was highest 14.92% in exotic cattle were as absent in goats, local and cross breed sheep respectively. *Theileria* infectivity was highest in cattle 20.89% and absent in goats and local sheep.

Table 3
Breed wise prevalence of *Babesia*, *Anaplasma* and *Theileria* in ruminants.

Host	Breed	Examined	*Babesia* (%)	*Anaplasma* (%)	*Theileria* (%)	Overall Prevalence (%)
Cattle	Local	79	7(8.86)	3(3.79)	3(3.79)	13(16.45)
	Exotic	67	14(20.89)	10(14.92)	14(20.89)	38(56.71)
	Cross Breed	102	12(11.76)	5(4.90)	6(5.88)	23(22.54)
	Total	248	33(13.30)	18(7.25)	23(9.27)	74(29.83)
Sheep	Local	70	1(1.42)	0(0)	0(0)	1(1.42)
	Exotic	67	3(4.47)	1(1.49)	2(2.98)	6(8.95)
	Cross Breed	68	1(1.47)	0(0)	1(1.47)	2(2.94)
	Total	205	5(2.43)	1(0.48)	3(1.46)	9(4.39)
Goat	Local	39	0(0)	0(0)	0(0)	0(0)
	Exotic	48	1(2.08)	0(0)	1(2.08)	2(4.16)
	Cross Breed	45	0(0)	0(0)	1(2.22)	1(2.22)
	Total	132	1(0.57)	0(0)	2(1.515)	3(2.27)

Table 4
Breed wise prevalence of *Babesia*, *Anaplasma* and *Theileria* in ruminants.

Host	Breed	Examined	*Babesia* (%)	*Anaplasma* (%)	*Theileria* (%)	Overall Prevalence (%)
Cattle	Local	79	7(8.86)	3(3.79)	3(3.79)	13(16.45)
	Exotic	67	14(20.89)	10(14.92)	14(20.89)	38(56.71)
	Cross Breed	102	12(11.76)	5(4.90)	6(5.88)	23(22.54)
	Total	248	33(13.30)	18(7.25)	23(9.27)	74(29.83)
Sheep	Local	70	1(1.42)	0(0)	0(0)	1(1.42)
	Exotic	67	3(4.47)	1(1.49)	2(2.98)	6(8.95)
	Cross Breed	68	1(1.47)	0(0)	1(1.47)	2(2.94)
	Total	205	5(2.43)	1(0.48)	3(1.46)	9(4.39)
Goat	Local	39	0(0)	0(0)	0(0)	0(0)
	Exotic	48	1(2.08)	0(0)	1(2.08)	2(4.16)
	Cross Breed	45	0(0)	0(0)	1(2.22)	1(2.22)
	Total	132	1(0.57)	0(0)	2(1.515)	3(2.27)

The overall seasonal prevalence of haemoparasitic infections revealed a definite seasonal trend in all the three species of ruminants, with highest infection in summer and lowest in winter. There was a gradual increase in the prevalence rate from spring to summer and falls down with onset of autumn and least observed prevalence during winter season (Fig 1).

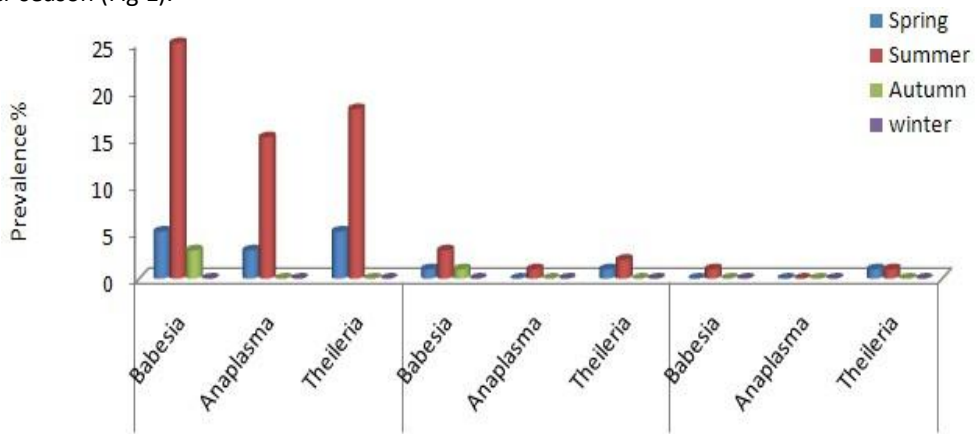

Fig. 1. Seasonal prevalence of *Babesia, Anaplasma* and *Theileria* in ruminants.

It was hard to find any previous reference regarding sero-prevalence of haemoparasitic infection in Kashmir valley. There were variations in the distribution of haemoparasitic infection in different geographical regions but seroprevalence was statistically significant among all study areas. Climate condition favours the growth and multiplication of vector ticks only in summer months. However, lack of quarantine measures, veterinarian availability and pastural migrations of flocks and herds from other hot, humid areas in the valley resulted in increase of infectivity of haemoparasites.

Babesiosis in cattle was found 13.30% which is 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 and Singh et al. 2012). However Shaw (1989) has reported babesiosis (1.8%) in cattle of Kashmir valley. Lower prevalence in Shaw studies may be due to reason that the samples were collected from the Intensive cattle development centers. Prevalence of the *Anaplasma* infection was found (9.27%) in cattle which is in line with the work of (Sing and Gill, 1977; Yadav et al., 1985; Shaw, 1997; Ogden et al., 2002; Rajput et al., 2005; Ruybal et al., 2009; Haque et al., 2011; Singh et al., 2012). Theileriosis (7.25%) is the least prevalent among the cattle of Kashmir valley. Shaw (1989) has reported low prevalence of Theileriosis in cattle of Kashmir valley. The high incidence in the present work may be primarily due to imported high yielding varieties of cattle from other states of India into the valley, which there might have been previously infected with Theileriosis (Jithendra, 1997; Sharma et al., 1999; Khan et al., 2004; Afridi et al., 2005; Zahid et al., 2005). The prevalence *Theileria* was more in sheep compared with goats ($p < 0.05$) that might be due to the nature of the skin and seems to be more resistant for the tick compared to sheep (Rehman et al., 2010; (Durrani et al., 2012).

Babesia infections in cattle,(<2 year) were found uninfected (0%).Also low prevalence of these parasites in lambs below six month of age could be attributed to transfer of maternal immunity to lambs. This effect could not be observed in 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. 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 immunosupression in advanced pregnancy and or lactation in high producing study animals were the possible reasons for higher prevalence of infection in ruminants (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 incidence of haemoparasitic diseases were found high in exotic followed crossbred and local study animals. From the observations of present study and findings of other authors in various parts of the world; it is implicated that different cattle, sheep and goat breeds (genotypes) show varied susceptibility/resistance to haemoparasitic infections. Local animals may have the different genetic makeup which makes them resistant to these parasites and also different climatic conditions of this region also may not favour the disease in local animals. Crossbred cattle were more susceptible to tick-borne diseases (TBDs) as compared to the indigenous cattle. 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.

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 (Gosh et al., 2007; Naz et al., 2012). Hot and humid season favors 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).

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