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CLINICO-EPIDEMIOLOGY AND THERAPEUTICAL TRIALS ON BABESIOSIS IN SHEEP AND GOATS IN LAHORE, PAKISTAN

M. Ijaz, A. Rehman[*], M.M. Ali, M. Umair[**], S. Khalid[****], K. Mehmood and A. Hanif[***]

Department of Clinical Medicine and Surgery; [*] Department of Epidemiology and Public Health; University of Veterinary and Animal Sciences Lahore, 54000, Punjab, Pakistan; [**]Department of Epidemiology & Public Health, University of Salford, Manchester, United Kingdom; [***]King Edward Medical University, Lahore
[****]University College of Veterinary & Animal Sciences, Islamia University of Bahawalpur
Corresponding author's email: mijaz@uvas.edu.pk

ABSTRACT

The prevalence of babesiosis in sheep and goat in Lahore and its peri-urban areas was investigated and the efficacy of three different treatments was measured. A total of 620 blood samples (n=243 sheep; n=377 goats) were collected and examined microscopically. *Babesia* infection was found in 57(23.46%) sheep and 51(13.53%) goats. Haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBCs) and thrombocytes were found to be significantly decreased ($P<0.05$) while there was no effect on other blood parameters. Efficacy of imidocarb dipropionate along with oxytetracycline, imidocarb dipropionate alone, diminazene aceturate along with oxytetracycline and diminazene aceturate alone was 100, 80, 80 and 70 percent in sheep whereas in goats 100, 80, 90 and 70 percent against babesia respectively, making imidocarb dipropionate along with oxytetracycline the most effective combination in both sheep and goats.

Key words: Hb; RBCs; *Babesia*; Imidocarb dipropionate; Diminazene aceturate.

INTRODUCTION

Babesiosis is a disease of economic importance which inflicts significant losses to small ruminants production potential in tropical and subtropical regions of the world (Mehlhorn and Shein, 1985). Babesiosis is the third most important disease of sheep in Pakistan from an epidemiological point of view (Anonymous, 1994-95). Babesiosis is a haemoparasitic disease of domestic and wild animals. *Babesia* spp. is tick-borne apicomplexan parasites which infect a wide range of vertebrate hosts. The *Babesia* spp. are transmitted by hard ticks (family: Ixodidae) and animals develop fever concurrent with parasitemia within 2 to 4 days; the clinical signs of the disease are severe and include varying degrees of anorexia, listlessness, anemia, moderate jaundice and hemoglobinuria (Rahbari *et al*., 2008). Chronically infected sheep are usually symptomless, except for parasitemia and unthriftiness (Kimberling *et al*., 1988). Diagnosis of piroplasmosis in small ruminants is mainly based on the microscopic examination of Giemsa-stained blood smears and clinical symptoms (Aktas, 2007). *Babesia ovis* and *Babesia motasi* are known to be pathogenic in sheep and goats (Soulsby, 1986). The effects of *B. ovis* are usually less severe than *B. motasi* (Morel, 1989). Mixed infection with *B. ovis* and *B. motasi* is a highly-pathogenic disease syndrome in sheep (Rafyi and Maghami, 1966). Keeping in view the economic losses rendered by babesiosis, the present study was conducted to describe the prevalence of babesiosis, clinical features, chemotherapy and effect on various blood parameters in sheep and goats.

MATERIALS AND METHODS

Experimental design: A total of 620 animals (n=243 sheep; n=377 goats) of all age groups and both sexes, which were brought at outdoor teaching hospital, University of Veterinary and Animal Sciences and various Govt. and private hospitals in urban and peri-urban areas of Lahore during August 2010 to November 2010, were included in the study. Thin blood smears were prepared from all sheep and goats, brought to the above mentioned hospitals, by taking one drop of blood from the ear tip after proper disinfection of the area. The smears were air-dried, fixed in methanol and stained with 10% Giemsa stain. The slides were examined with an oil immersion lens at a total magnification of 1000x. Prevalence of babesiosis was calculated as per formula described by Thrusfield (2002).

Haematological Examination: For this purpose 5 ml blood sample was collected directly from the jugular vein of sheep and goats, infected with *Babesia*, into sterilized plastic bottles coated with EDTA @ 1 mg/ml of blood. Haematological examination was carried out by using haematological analyzer.

Therapeutic Trials: A total of 80 animals (n=40 sheep and n=40 goats) positive for babesiosis, were divided into four groups A, B, C and D, each group comprised of 20 animals (n=10 sheep; n=10 goats). The animals of group A were treated with imidocarb dipropionate (Imizol®,

ICI, Pakistan) @ 2 mg/kg BW i.m. plus oxytetracycline @ 10 mg/kg BW i.m., group B was treated with imidocarb dipropionate (Imizol®, ICI, Pakistan) @ 2 mg/kg BW i.m. alone, group C was treated with diminazene aceturate (Fa-try.banil; Prix Pharma, Pakistan) @ 3.5 mg/kg BW i.m. and oxytetracycline @ 10 mg/kg BW i.m. while group D was treated with diminazene aceturate (Fa-try.banil; Prix Pharma, Pakistan) @ 3.5 mg/kg BW i.m. alone. Efficacy of drugs was measured through recovery rate of the animals on the basis of disappearance of clinical signs and negative blood smear examination at day 2, 4, 6 and 10 post-medication.

Statistical Analysis: Data on prevalence of babesiosis was analyzed by Pearson's chi-square test for significance whereas data on clinical signs was analyzed by Non-parametric, Chi-square test for proportion while data on haematology was analyzed by independent sample T-test using statistical software package STATA 9.1 (College Station T×77845, USA). $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Data on prevalence of babesiosis, clinical signs, haematology and therapeutic trials in sheep and goats are shown in table 1, 2, 3 and 4, respectively. The overall prevalence of babesiosis in sheep and goat was 17.42 percent while prevalence was 23.46 and 13.53 percent in sheep and goats respectively. The results of the present study are completely in line with the findings of Razmi *et al.*, 2003, who examined 391 sheep and 385 goats, and reported prevalence in sheep and goats 26.1 and 14.8 percent respectively. Similarly the findings of the present study are broadly consistent with karatepe *et al.*, 2003 who reported 23.63 percent ovine babesiosis. Month wise prevalence was also calculated and found highest prevalence during the month of August which is congruent with the findings of Yeruham *et al.*, 1998. They reported the highest prevalence of babesiosis in August (56%), corresponding to the most active season of the adult vector ticks because there is strong correlation between infection of *Babesia* and presence of ticks. High rise of body temperature, anorexia, dyspnea, haemoglobinurea, emaciation, pale mucous membrane, jaundice, constipation and recumbency were the main clinical signs in both sheep and goats. The results correlate with the findings of Sulaiman *et al*., 2010, who reported that clinically infected goats showed different signs graduated from loss of appetite, emaciation, pale mucous membranes, jaundice, fever, coughing, nasal discharge, recumbency and haemoglobinurea.

Results of the present study showed significant decrease ($P<0.05$) in Hb, PCV, RBCs, thrombocytes and WBCs count in the infected sheep as compared to healthy sheep, while in goats Hb, PCV, RBCs and thrombocytes count were significantly decreased ($P<0.05$) with non-significant ($P>0.05$) effect on WBCs count. The study correlates with the findings of Rahbari *et al.*, 2008, who reported that haematological values in animals infected with *B. ovis* were significantly different in most of the cases. RBCs, haematocrit and haemoglobin values clearly suggested that anemia was almost a constant characteristic of the infection. Similarly, Baby *et al.*, 2001 and Yeruham *et al.* 1998, reported significant decrease in Hb concentration and RBCs count resulting in severe anemia which might be due to destruction of RBCs by *Babesia.* The present results also agree with the findings of Voyvoda *et al.*, 1997 and Banerjee *et al.*, 1987 who reported decreased Hb concentration in sheep and goats, respectively.

The combination of imidocarb dipropionate and oxytetracycline was the most effective treatment regime of babesiosis in sheep and goats followed by combination of diminazene aceturate with oxytetracycline. These results are in agreement with those of Ramin, 2000 and McHardy *et al.*, 1986, who recorded 97.28% and 100% imidocarb efficacy respectively while treating sheep with babesiosis. Similarly, Banerjee *et al.*, 1987 and Rao *et al.*, 1989 reported diminazene to be an effective drug against ovine babesiosis.

Table 1. Month wise prevalence of babesiosis in sheep and goats

Months	Sheep				Goats			
	No. of animals examined	No. of animals infected	%age of infection	P (F)	No. of animals examined	No. of animals infected	%age of infection	P (F)
August	48	35	72.92	X^2=87.0571 P=0.001	77	33	42.86	X^2=80.2648 P=0.001
September	59	13	22.03		96	14	14.58	
October	63	05	07.94		95	03	03.16	
November	73	04	05.48		109	01	00.92	
Total	243	57	23.46		377	51	13.53	

Chi-square test for significance analysis showed significant difference ($P<0.05$) in prevalence of babesiosis in different months in both sheep and goats

Table 2. Haematological values of healthy and *Babesia* positive sheep and goats (Mean ± S.E)

Haematological Parameters	Sheep		Goat	
	Infected	Healthy	Infected	Healthy
	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E
Hb g/dl	**6.36±00.30	**12.43±0.31	**06.75±0.09	**10.38±0.26
PCV %	**25.30±0.91	**35.50±1.16	**27.30±0.51	**30.60±0.74
RBC (x 10^6/µl)	**6.23±00.33	**12.00±0.39	**05.48±0.10	**13.40±0.40
Thrombocytes (x 10^3/ µl)	**270±19.55	**500±31.59	**296±16.14	**440±21.65
WBC (x 10^3/ µl)	**12.50±0.45	**07.0±00.47	*08.17±00.40	*08.10±00.64
Neutrophils (× 10^9/L)	*03.41±00.22	*03.29±00.38	*04.49±00.27	*04.65±00.42
Lymphocytes (× 10^9/L)	*05.10±00.45	*05.50±00.50	*05.30±00.47	*05.70±00.47
Monocytes (× 10^9/L)	*0.145±00.04	*00.29±00.07	*00.249±0.06	*00.27±00.05
Eosinophils (× 10^9/L)	**0.067±0.02	**0.56±00.06	*00.341±0.07	*00.38±00.06

** indicates significant difference ($p<0.05$) among healthy and infected groups, by independent sample *t*- test.
* indicates non-significant difference ($p>0.05$) between healthy and infected groups, by independent sample *t*- test.

Table 3. Clinical signs observed in sheep and goats infected with *Babesia*

Clinical Signs	Sheep n=57		Goats n=51	
	No. of animals	%age	No. of animals	%age
High rise of temperature (>105^{o}F)	55	96.49	46	90.20
Pale mucous membrane	52	91.23	44	86.27
Anorexia	50	87.72	35	68.63
Dyspnea	48	84.21	34	66.67
Haemoglobinurea	47	82.46	31	60.78
Emaciation	22	38.60	26	50.98
Jaundice	21	36.84	23	45.10
Constipation	11	19.30	12	23.53
Recumbency	05	08.77	04	07.84
Ticks	35	61.40	22	43.14

Non-parametric, Chi-square test for proportion= 88.62, p-value 0.0000
56.68, p-value = 0.0000
P-value 0.764

Table 4. Comparative efficacy of various drugs against babesiosis at different days in sheep and goats

Groups	Drug used	Efficacy (%) of drugs							
		2nd day		4th day		6th day		10th day	
		Sheep n=10	Goat n=10	Sheep n=10	Goat n=10	Sheep n=10	Goat n=10	Sheep n=10	Goat n=10
A	Imidocarb dipropionate + Oxytetracycline	80	80	100	90	100	100	100	100
B	Imidocarb dipropionate	70	60	80	70	80	80	80	80
C	Diminazine aceturate + Oxytetracycline	60	70	80	80	80	90	80	90
D	Diminazine aceturate	40	30	60	50	60	60	70	70

It is concluded that babesiosis in sheep and goats is of considerable importance in the study area along with significant decrease in Hb concentration, PCV, RBCs and thrombocytes count whereas the combination of imidocarb dipropionate and oxytetracycline is the most effective treatment.

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