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Haemato-biochemical and Therapeutic Studies on Ovine Babesiosis in Kashmir

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ABSTRACT

Background: The clinical study was designed to evaluate the haemato-biochemical changes in sheep affected with babesiosis and therapeutic regimes of various drugs used against babesiosis.

Methods: A total of 24 clinically affected sheep with 6 animals in each group were selected for the haemato-biochemical and therapeutic study. The diagnosis was based on clinical manifestations, microscopic examination of blood smears and molecular confirmation of *Babesia* by PCR technique. For therapeutic study Group I, II, III and IV were treated with imidocarb dipropionate, imidocarb dipropionate+oxytetracycline, diminazene aceturate and diminazene aceturate+oxytetracycline, respectively.

Result: The clinical signs recorded were high fever, pale mucous membranes, presence of ticks, inappetence, coffee-coloured urine and diarrhoea. The vectors identified were *Haemaphysalis* ticks. Hb, PCV, TEC, total protein, albumin, calcium, phosphorus and blood glucose were significantly (P<0.05) lower in the diseased group as compared to healthy animals while TLC, globulin and iron levels were significantly (P<0.05) higher compared to the healthy control group. The study revealed that the combination of imidocarb dipropionate and oxytetracycline was highly effective for ovine babesiosis.

Key words: Babesiosis, Haemato-biochemical, Sheep, Therapy.

INTRODUCTION

Babesiosis is a tick borne disease transmitted by vectors like *Haemaphysalis*, *Dermacentor* and *Rhipicephalus* in sheep (Taylor *et al*., 2007). In acute cases, pyrexia, anaemia, haemoglobinuria, constipation, recumbency, pale mucous membrane, icterus are common.

Next to recovery from the infection, immunity is incomplete and most animals hold a latent infection. Diagnosis is mainly based on the microscopic examination of Giemsa-stained blood smears and clinical symptoms (Aktas *et al*., 2007). Serological tests like immunofluorescent antibody test, complement fixation test or enzyme-linked immunosorbent assay can also confirm a primary diagnosis or be used for epidemiological surveys. However, PCR which is more sensitive and specific than other methods have been developed and used for the detection of *Babesia* (Aktas *et al*., 2005 and Oliveira-Sequeira *et al*., 2005). The disease has a seasonal influence when tick activity is high. In the Kashmir valley due to climatic alterations, the vector population is consistently expanding to unforeseen areas like high altitude or temperate zones in recent years. A few studies on animal's blood protozoa such as *Babesia*, *Theileria* and *Rickettsia* such as *Anaplasma* have been carried out in Kashmir Valley in recent years (Tufani *et al*., 2009 and Haq *et al*., 2017). The present paper deals with the haemato-biochemical and therapeutic studies on ovine babesiosis in Kashmir.

MATERIALS AND METHODS

Sampling

The study was carried out in the Division of Clinical Veterinary Medicine, SKUAST Kashmir, Srinagar, during the year 2017.

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About 10 ml of blood was collected out of which 2 ml was transferred to EDTA vial for CBC, 5 ml in EDTA vial for PCR and 3 ml in clot activator vial for biochemical estimation using a spectrophotometer. Blood glucose was measured by using ACCU-CHEK®. The disease was confirmed through microscopic examination and PCR.

Therapeutic trial

A total of 24 sheep showing clinical signs were taken for therapeutic study irrespective of their age, breed, sex and six healthy sheep were selected under the control group for the comparative therapeutic study. All animals were randomly divided into four equal groups consisting of 6 animals in each. Animals of group I were treated with imidocarb dipropionate @ 2 mg/kg body weight, i/m as a single dose. Animals of group II were offered imidocarb

dipropionate @ 2 mg/kg body weight as a single dose and oxytetracycline @ 10 mg/kg body weight daily for 5 days, i/m. Group III animals were administered diminazene aceturate @ 12 mg/kg body weight, deep i/m as a single dose. Group IV was treated by diminazene aceturate @ 12 mg/kg body weight, deep i/m as a single dose and oxytetracycline @ 10 mg/kg body weight i/m daily for 5 consecutive days. The therapeutic efficacy of the drugs was accessed based on clinical recovery, blood smear examination and restoration of haemato-biochemical parameters.

Statistical analysis

Data generated were subjected to statistical analysis by applying analysis of variance. The difference was considered significant when $P<0.05$, using SPSS.

RESULTS AND DISCUSSION

Clinical examination and diagnosis

Clinical signs recorded were pale mucous membranes (Fig 1), inappetence, high fever, coffee-coloured urine, diarrhoea and *Haemaphysalis* tick infestation in the ear (Fig 2). On microscopic examination, the blood smears of all 24 clinically affected sheep were positive for babesiosis showing different intra-erythrocytic forms morphologically compatible with *Babesia* piroplasms (Fig 3). PCR revealed 408-bp amplified (*Babesia* genus-specific) DNA fragments in all 24 blood samples that were clinically and microscopically positive. The positive control sample always showed the necessary band size of 408-bp (Fig 4).

Haematological parameters

Haematological parameters of different groups of animals were evaluated and are depicted in (Tables 1 and 2). Pre-treatment Hb, PCV, TEC values were significantly ($P<0.05$) lower than the healthy group since babesiosis increases erythrophagocytosis (Sevinc *et al*., 2013; Esmaeilnejad *et al*., 2014 and Mohamed, 2017). After treatment, a significant increase in haematological values were recorded, but significantly lower as compared to the control group (Table 1). Before treatment, significantly ($P<0.05$) higher TLC values were recorded which is in agreement with the findings of Sulaiman *et al*., 2010 and Kumar *et al*., 2010 and after treatment, a significant decreasing trend in TLC was recorded (Table 1). Such an increase in TLC levels may

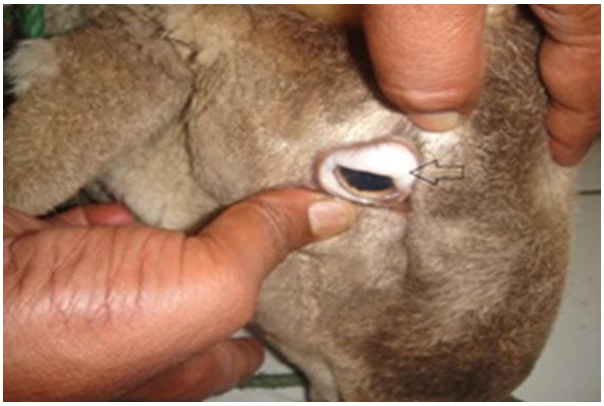

Fig 1: Pale mucous membrane in *Babesia* affected sheep.

Fig 2: Tick infestation (*Haemaphysalis*) in sheep and its magnified view.

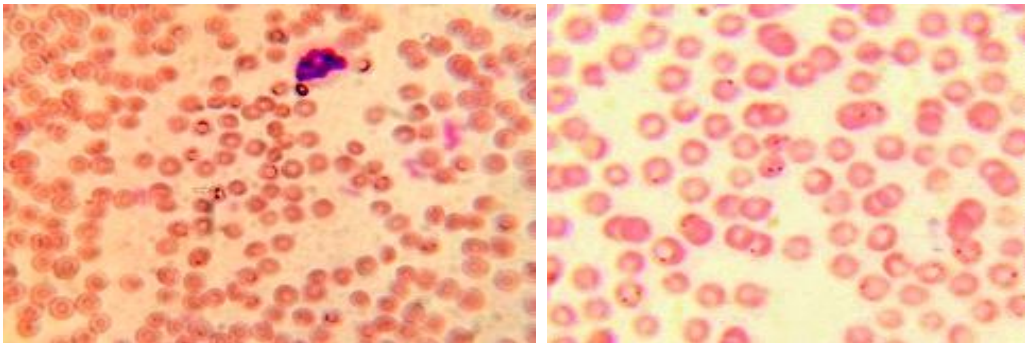

Fig 3: Microphotograph showing intra-erythrocytic *Babesia* piroplasms in sheep (Giemsa-stained, 1000X).

occur due to the defensive response during the initial stages of the infection (Constable *et al*., 2017). Differential leukocyte count showed a significant (P<0.05) increase and a decrease in neutrophils and lymphocyte percentage, respectively in the babesiosis affected animals (Table 2). After treatment, the neutrophil values decreased and lymphocyte values increased significantly compared to pre-treatment values (Table 2). The pre-treatment values of monocytes, eosinophils and basophils did not differ significantly from the post-treatment values (Table 2).

Biochemical profile

The pre-treatment mean glucose, total protein levels were significantly (P<0.05) lower in all the groups as compared to the control group, attributed to inappetence and metabolic disturbances of the diseased animals. After treatment, a significant increase in blood glucose and total protein levels were recorded (Table 3a). The pre-treatment mean albumin levels differed significantly (P<0.05) in all groups compared to the control group and after treatment, a significant rise in albumin levels was recorded (Table 3a). A significant decrease in the protein and albumin levels occurs due to liver dysfunction, renal failure and anorexia (Esmaeilnejad *et al*., 2012). Globulin levels were significantly (P<0.05) higher in all the groups compared to the control group that can be attributed in response to parasitic antigen and haemoglobin release from the destructed erythrocytes. After therapy, all groups showed a significant (P<0.05) reduction in the globulin levels except for group I (Table 3a). The pre-treatment mean A/G ratio showed no significant difference (P>0.05) and after treatment; a significant (P<0.05) increase in the A/G ratio was recorded in all the diseased groups (Table 3a).

Serum Ca and P levels revealed significantly (P<0.05) lower values in all groups compared to the control group and similar results were reported by Zintl *et al*. (2003) and Mohamed (2017). The reason for decreased concentrations of calcium might be due to the interdependency of calcium and albumin metabolisms. The decreased serum phosphorus levels is the result of anorexia, renal wasting and the presence of diarrhoea (Moe, 2008).

After treatment values of Ca and P increased significantly (P<0.05) in all the groups but remained significantly (P<0.05) lower than the control group before and after treatment (Table 3b). The pre-treatment means Ca/P ratio in groups II and III differ significantly (P<0.05) with the control group on day-0 and decreased significantly (P<0.05) in group II after treatment (Table 3b). Iron levels in our study revealed a significant (P<0.05) increase which was similar to the observations of Kozat *et al*. (2003). The post-treatment serum iron concentration decreased significantly (P<0.05) in all the groups as compared to pre-treatment concentrations (Table 3b). The elevated serum iron in the infected sheep can probably be ascribed to haemolytic anaemia.

Therapeutic study

Pre and post-treatment clinical parameters

The rectal temperature varied from 104-105ºF with a mean value of 104.45±0.10°F (Table 4). The mean rectal temperature, respiration rate was significantly elevated (P<0.05) before treatment as was the case with heart rate except in group I (Table 4). The post-treatment rectal temperature, heart rate and respiration rate decreased significantly (P<0.05) as compared to pre-treatment values. Post-treatment temperature almost became normal in groups I and II only but remained significantly (P<0.05) higher in other groups (III and IV) compared to the control group. The post-treatment values of respiration rate in all the groups did not differ significantly (P>0.05) from the control group. The pre-treatment mean rumen motility in all animals was significantly (P<0.05) lower as compared to the control group. After treatment, there was a significant (P<0.05) increase in rumen

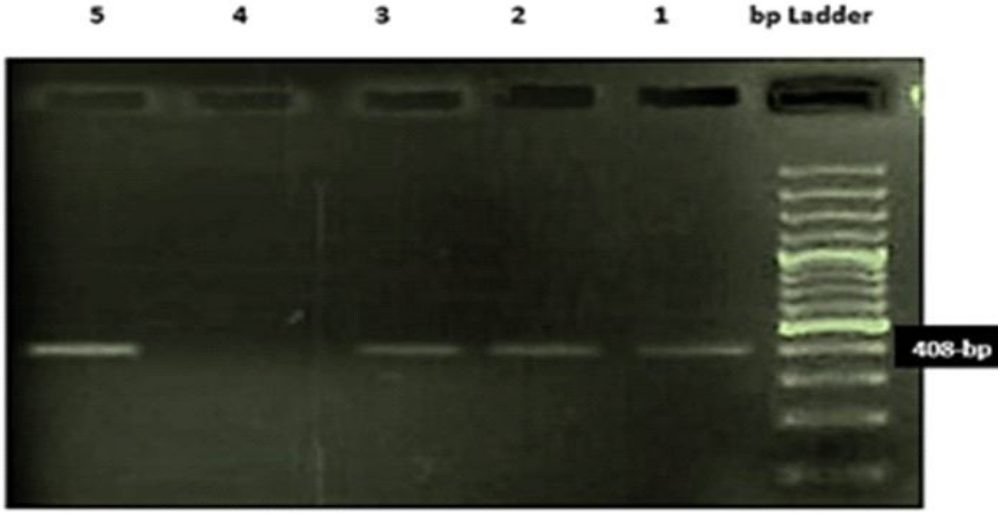

Fig 4: PCR positive result of *Babesia* samples with primers specific for *Babesia* genus (408-bp). (Lane 1-3, 408-bp *Babesia* +ve control), (Lane 4, *Babesia* -ve control and Lane 5, *Babesia* +ve control).

Table 1: Pre and post treatment haematological parameters of sheep affected with babesiosis.

Groups	Haemoglobin (g/dl)		PCV (%)		TEC (10^6cells/µl)		TLC (10^3cells/µl)	
N=6	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
I	7.05 ± 0.93^{a}	8.85 ± 0.67*^{b}	22.66 ± 2.72^{a}	25.83 ± 2.08*^{b}	5.96 ± 0.65^{b}	7.00 ± 0.38*^{b}	11.79 ± 2.07^{a}	9.44 ± 1.32*^{bc}
II	6.86 ± 0.83^{a}	9.01 ± 0.66*^{b}	21.33 ± 2.53^{a}	27.50 ± 1.97*^{b}	5.51 ± 0.62^{ab}	8.01 ± 0.55*^{b}	11.61 ± 1.22^{a}	8.51 ± 0.63*^{ab}
III	5.01 ± 0.69^{a}	6.13 ± 0.61*^{a}	16.16 ± 2.38^{a}	18.50 ± 2.10*^{a}	3.85 ± 0.74^{a}	4.86 ± 0.67*^{a}	13.80 ± 1.16^{a}	11.48 ± 0.98*^{c}
IV	6.35 ± 0.89^{a}	7.61 ± 1.04*^{b}	18.83 ± 2.95^{a}	22.66 ± 3.14*^{b}	5.00 ± 0.71^{ab}	7.01 ± 0.71*^{b}	14.31 ± 0.89^{a}	110.65 ± 0.95*^{bc}
V	11.76 ± 0.43^{b}	12.03 ± 0.40^{c}	36.33 ± 1.33^{b}	36.33 ± 1.23^{c}	11.65 ± 0.40^{c}	11.83 ± 0.38^{c}	6.22 ± 0.06^{b}	6.20 ±0.04^{a}

Mean values bearing different superscripts in rows and * between columns differ significantly (P<0.05).

Table 2: Differential leukocyte count of sheep affected with babesiosis before and after treatment.

Group	Neutrophil (%)		Lymphocyte (%)		Monocyte (%)		Eosinophil(%)		Basophil (%)	
N=6	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
I	59.67 ± 4.67^{bc}	43.33 ± 2.29*^{b}	37.50 ± 5.13b	53.17 ± 2.12*^{b}	2.17 ± 0.79	2.50 ± 0.43	1.00 ± 0.52	0.33 ± 0.21	0.17 ± 0.16	0.67 ± 0.21
II	65.50 ± 5.27^{bc}	44.33 ± 2.74*^{b}	30.83 ± 4.94^{ab}	52.67 ± 2.79*^{b}	2.50 ± 0.43	1.83 ± 0.17	0.83 ± 0.40	0.67 ± 0.21	0.33 ± 0.21	0.50 ± 0.22
III	70.83 ± 4.68^{c}	53.83 ± 4.17*^{c}	25.00 ± 4.60^{a}	43.00 ± 3.90*^{a}	1.83 ± 0.31	2.00 ± 0.26	1.50 ± 0.43	0.83 ± 0.31	0.83 ± 0.40	0.33 ± 0.21
IV	57.00 ± 2.19^{b}	39.00 ± 3.71*^{ab}	40.00 ± 2.65^{b}	57.83 ± 3.33*^{b}	1.83 ± 0.31	2.50 ± 0.56	0.83 ± 0.54	0.33 ± 0.21	0.33 ± 0.21	0.33 ± 0.21
V	31.33 ± 1.48^{a}	30.00 ± 2.03^{a}	66.00 ± 1.75^{c}	67.67 ± 2.08^{c}	1.67 ± 0.33	1.50 ± 0.22	0.33 ± 0.21	0.33 ± 0.21	0.67 ± 0.21	0.50 ± 0.22

Mean values bearing different superscripts in rows and * between columns differ significantly (P<0.05).

Table 3a: Biochemical profile of sheep affected with babesiosis before and after treatment.

Group	Glucose(mg/dl)		Total protein(g/dl)		Albumin (g/dl)		Globulin (g/dl)		A/G ratio	
N=6	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
I	33.50 ± 6.09^{a}	59.50 ± 2.58*^{a}	6.44 ± 0.32^{a}	6.45 ± 0.19^{bc}	2.47 ± 0.39^{a}	3.41 ±0.49*^{a}	3.97 ±0.14^{b}	3.04 ±0.39*^{bc}	0.63 ±0.14^{a}	1.12 ±0.02*^{a}
II	36.83 ± 5.51^{a}	59.17 ± 2.80*^{a}	6.56 ± 0.29^{a}	6.80 ± 0.18^{c}	2.89 ± 0.43^{a}	4.10 ±0.36*^{a}	3.67 ±0.27^{b}	2.70 ±0.15*^{ab}	0.78 ±0.09^{ab}	1.51 ±0.06*^{a}
III	32.50 ± 5.01^{a}	57.33 ± 2.14*^{a}	5.85 ± 0.45 ^{a}	5.88 ± 0.16^{a}	2.40 ± 0.40^{a}	3.43 ±0.39*^{a}	3.65 ±0.33^{b}	2.45 ±0.27*^{ab}	0.60 ±0.08^{a}	1.40 ±0.07*^{a}
IV	43.17 ± 2.74^{a}	60.33 ± 2.75*^{a}	6.59 ± 0.38 ^{a}	6.67 ± 0.29^{bc}	2.86 ± 0.25^{a}	4.02 ±0.34*^{a}	3.73 ±0.28^{b}	2.65 ±0.21*^{a}	0.76 ±0.10^{b}	1.43 ±0.05*^{a}
V	71.50 ± 3.07^{b}	72.50 ± 2.28^{b}	7.06 ± 0.15^{b}	7.15 ± 0.18^{d}	3.56 ± 0.17^{b}	3.66 ±0.25^{b}	3.50 ±0.19^{a}	3.39 ±0.12^{c}	1.01 ±0.07^{ab}	1.07 ±0.09^{b}

Mean values bearing different superscripts in rows and * between columns differ significantly (P<0.05).

Table 3b: Biochemical profile of sheep affected with babesiosis before and after treatment.

Group	Calcium (mg/dl)		Phosphorus (mg/dl)		Ca/P ratio		Iron (µg/dl)	
N=6	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
I	7.03 ± 0.51^{a}	9.41 ± 0.27*^{a}	3.56 ± 0.27^{a}	4.76 ± 0.16*^{a}	1.97 ± 0.01^{a}	1.98 ± 0.01	230.16 ± 5.77^{b}	220.26 ± 4.07*^{b}
II	7.28 ± 0.45^{ab}	10.07 ± 0.31*^{a}	3.64 ± 0.23^{a}	5.12 ± 0.15*^{a}	2.00 ± 0.00^{b}	1.96 ± 0.00*	233.25 ± 4.48^{b}	222.95 ± 3.55*^{b}
III	8.67 ± 0.62^{b}	9.30 ± 0.50*^{a}	4.33 ± 0.31^{a}	4.69 ± 0.24*^{a}	2.00 ± 0.01^{b}	1.98 ± 0.00	241.46 ± 2.28^{b}	233.10 ± 2.20*^{b}
IV	8.26 ± 0.62^{ab}	9.36 ± 0.46*^{a}	4.15 ± 0.31^{a}	4.71 ± 0.23*^{a}	1.99 ± 0.00^{ab}	1.98 ± 0.00	236.59 ± 2.42^{b}	229.10 ± 4.95*^{b}
V	12.14 ± 0.22^{c}	12.18 ± 0.25^{b}	6.15 ± 0.12^{b}	6.15 ± 0.13^{b}	1.97 ± 0.00^{a}	1.98 ± 0.00	191.20 ± 7.06^{a}	191.91 ± 6.487^{a}

Mean values bearing different superscripts in rows and * between columns differ significantly (P<0.05).

Table 4: Effect of treatment on clinical parameters in sheep treated for Babesiosis.

Groups	Rectal temperature (°F)		Heart rate (beats/min)		Respiratory rate (breaths/ min)		Rumen motility (per 2 min)	
N=6	Pre-treatment	Post-treatment	Pre-treatment	Post-Treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
I	105.10 ± 0.35^{a}	102.37 ± 0.21^{*ab}	98.00 ± 5.20^{ab}	75.00 ± 1.52^{*ab}	55.00 ± 6.84^{a}	28.50 ± 1.73^{*ab}	1.33 ± 0.21^{a}	2.33 ± 0.21^{*a}
II	104.72 ± 0.33^{a}	102.40 ± 0.18^{*ab}	122.83 ± 16.46^{b}	74.00 ± 1.52^{*a}	55.50 ± 7.37^{a}	24.83 ± 2.27^{*a}	1.33 ± 0.21^{a}	3.17 ± 0.17^{*b}
III	105.20 ± 0.32^{a}	102.88 ± 0.34^{*bc}	127.50 ± 11.47^{b}	75.50 ± 1.40^{*ab}	52.33 ± 7.18^{a}	32.16 ± 2.10^{*b}	1.17 ± 0.31^{a}	2.83 ± 0.17^{*b}
IV	105.15 ±0.33^{a}	103.33 ± 0.34^{*c}	132.17 ± 12.82^{b}	82.33 ± 4.65^{*b}	48.00 ± 7.84^{a}	29.83 ± 1.25^{*ab}	1.17 ± 0.31^{a}	2.83 ± 0.17^{*b}
V	101.95 ± 0.18^{b}	101.93 ± 0.20^{a}	73.67 ± 1.12^{a}	74.33 ± 1.74^{a}	26.17 ± 2.20^{b}	28.50 ± 2.11^{ab}	3.00 ± 0.00^{b}	3.00 ± 0.00^{b}

Mean values bearing different superscripts in rows and * between columns differ significantly (P<0.05

motility in all the groups. The post-treatment rumen motility in groups (II-IV) didn't differ significantly (P>0.05) from group V except for group I in which it remained significantly (P<0.05) lower than normal (Table 4). Similar findings for rectal temperature, heart rate, respiration rate and rumen motility were reported bySulaiman *et al*. (2010) and Ajith *et al*. (2017). Fever occurs due to the liberation of endogenous pyrogens that stimulate thermoregulatory centres in the hypothalamus. Tachycardia, tachypnea might be due to the anaemic anoxia and reduction of erythrocyte count and haemoglobin concentration (Constable *et al*., 2017).

Results of rumen motility were in agreement with the findings of Ajith *et al*. (2017). The reduction in rumen motility might be due to decreased muscle tone as a result of anaemia andthe release of histamines. Moreover, hypocalcaemia observed in the present study might have resulted in ruminal atony.

Therapeutic efficacy

The therapeutic efficacy was calculated based on the presence and absence of *Babesia* piroplasms in peripheral blood smears after treatment (day-10) in each animal of the treatment groups. Treatment, given in these groups of animals eliminated 100% piroplasms from blood in group II, followed by group IV (83.33%), group I (66.66%) and treatment employed in group III was least effective as it removed *Babesia* piroplasms only from 50% animals. It was evident that the treatment given in group II was most effective (100%). Comparative efficacy of different therapeutic regimes was also evaluated based on early clinical recovery and improvement in the haemato-biochemical parameters after giving treatment. All the treatment groups were assigned ranks as per the recovery in clinical and haemato-biochemical parameters. Based on rank, it was observed that animals of group II achieved the highest rank, followed by group IV, group I and group III in a respective manner. Group I receiving imidocarb achieved the third rank but with mild parasitaemia recorded in two animals. The combined therapy using oxytetracycline in group II and IV was found to be more efficacious in terms of resolution of clinical signs, reduction in parasitaemia and early improvement in haemato-biochemical parameters and it is in agreement with the findings of Taylor *et al*., (1986); Pipano *et al*., (1987); Ijaz *et al*., (2013) and Tufani *et al*., (2017).

CONCLUSION

Mild to moderate parasitaemia continued to be present in all the animals except in group II animals treated with the combination of imidocarb and oxytetracycline. It appears that most treatments act to minimize parasitaemia and the recovered animals remain carriers of the disease. Treatment with the combination of imidocarb dipropionate and oxytetracycline proved highly effective and also helps to eliminate the parasitaemia, thus it can be recommended for the treatment of ovine babesiosis safely in field conditions.

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REFERENCES

Ajith, Y., Rajeswaria, T.U., Raj Sijia, S. and Dilipa, C. (2017). Case report on babesiosis associated pre-hepatic jaundice in a Malabari goat. Veterinary and Animal Science. 3: 1-3.

Aktas, M., Altay, K. and Dumanli, N. (2005). Development of polymerase chain reaction method for diagnosis of *Babesia ovis* infection in sheep and goats. Veterinary Parasitology. 133: 277-281. doi: 10.1016/j.vetpar.2005.05.057.

Aktas, M., Altay, K. and Dumanli, N. (2007). Determination of prevalence and risk factors for infection with *Babesia ovis* in small ruminants from Turkey by polymerase chain reaction. Parasitology Research. 100: 797-802.

Constable, P.D., Hinchcliff, K.W., Done, S.H. and Grunberg, W. (2017). Veterinary medicine, A textbook of the diseases of cattle, horses, sheep, pigs and goats. 11th ed., Vol. (1), Elsevier; pp. 802-803.

Esmaeilnejad, B., Tavassoli, M., Asri-Rezaei, S. (2012). Investigation of haematological and biochemical parameters in small ruminants naturally infected with *Babesia ovis*. Veterinary Research Forum. 3: 31-36.

Esmaeilnejad, B., Tavassoli, M., Asri-Rezaei, S., Dalir-Naghadeh, B., Mardani, K., Jalilzadeh-Amin, G., Golabi, M. and Arjmand, J. (2014). PCR-Based Detection of *Babesia ovis* in *Rhipicephalus bursa* and Small Ruminants. Journal of Parasitology Research. 1-6.

Haq, A.U., Tufani, N.A, Gugjoo, M.B., Nabi, S.U. and Malik, H.U. (2017). Therapeutic amelioration of severely anaemic local Kashmiri goats affected with babesiosis. Advances in Animal and Veterinary Sciences. 5(11): 463-467.

Ijaz, M., Rehman, A., Ali, M.M., Umair, M., Khalid S, Mehmood, K. and Hanif, A. (2013). Clinico-epidemiology and therapeutical trials on babesiosis in sheep and goats in Lahore, Pakistan. The Journal of Animal and Plant Sciences. 23: 666-669.

Kozat, S., Yuksek, N., Altug, N., Agaoglu, Z.T. and Ercin, F. (2003). Studies on the effect of iron (Fe) preparations in addition to babesiosis treatment on the haematological and some mineral levels in sheep naturally infected with *Babesia ovis.* yuzuncu Yil Universitesi Veteriner Fakultesi Dergisi. 14(2): 18-21.

Kumar, A., Vihan, V.S. and Sharma, H.N. (2010). Haematological and biochemical effects of tick infestation in common Indian goat. Advanced Biomedical Research. 1(1): 164-169.

Moe, S.M. (2008). Disorders involving Calcium, Phosphorus and Magnesium. Primary Care: Clinics in Office Practice. 35: 215-237.

Mohamed, S.M.M. (2017). Studies of naturally infected babesiosis and its effect on some haematological and biochemical parameters in cattle in Qena, Egypt. Journal of Veterinary Science. 1(2): 1-9.

Oliveira-Sequeira, T.C.G., Oliveira, M.C.S., Araujo, J.P. and Amarante, A.F.T. (2005). PCR-based detection of *Babesia bovis* and *Babesia bigemina* in their natural host *Boophilus microplus* and cattle. International Journal for Parasitology. 35: 105-111.

Pipano, E., Markovics, A., Kriegel, Y., Frank, M. and Fish, L. (1987). Use of long-acting oxytetracycline in the immunisation of cattle against Babesia bovis and B bigemina. Research in Veterinary Science. 43(1): 64-6.

Sevinc, F., Sevinc, M., Ekici, O.D., Yildiz, R., Isik, N. and Aydogdu, U. (2013). *Babesia ovis* infections: Detailed clinical and laboratory observations in the pre- and post-treatment periods of 97 field cases. Veterinary Parasitology. 191: 35-43.

Sulaiman, E.G., Arslan, S.H., Al-Obaidi, Q.T. and Daham, E. (2010). Clinical, haematological and biochemical studies of babesiosis in native goats in Mosul. Iraqi Journal of Veterinary Sciences. 24(1): 31-35.

Taylor, M.A., Coop, R.L. and Wall, R.L. (2007). Veterinary Parasitology. 3rd ed., Blackwell Publishers.

Taylor, S.M., Elliott, C.T. and Kenny, J. (1986). Inhibition of Babesia divergens in cattle by oxytetracycline. Veterinary Record. 118(4): 98-102.

Tufani, N.A., Hafiz, A., Malik, H.U., Peer, F.U. and Makhdoomi, D.M. (2009). Clinico-therapeutic management of acute babesiosis in bovine. Intas Polivet. 10(1): 49-50.

Tufani, N.A., Malik, H.U. and Fazili, M.R. (2017). Clinico-haematological Profile and Therapeutic Management of Acute Babesiosis in Sheep and Goats. Journal of Animal Research. 7(5): 857-863.

Zintl, A., Mulcahy, G., Skerrett, H.E., Taylor, S.M. and Gray, J.S. (2003). Babesia divergens, a bovine blood parasite of veterinary and zoonotic importance. Clinical Microbiology Review. 16: 622-636.