B-5014
[1-7]

RESEARCH ARTICLE Indian Journal of Animal Research

Clinico-Haemato-Biochemical Changes and Therapeutic Efficacy of Diminazene Aceturate and Artesunate against Bovine Babesiosis in Kashmir Valley

T. Ahmad[2], N.A. Tufani[1], A.U. Haq[3], S.R. Tramboo[4], I.M. Allaie[4], H.U. Malik[1], N.A. Dar[2], A.A. Dar[1]

10.18805/IJAR.B-5014

ABSTRACT

Background: The purpose of the study trial was to assess therapeutic efficacy of diminazene aceturate and artesunate with respect to clinical, haematological and biochemical changes in the cattle affected by babesiosis.

Methods: For the analysis, a total of 16 clinically affected cattle were selected, with eight animals in each group. Eight healthy cattle were also selected under the control group. Clinical symptoms, blood smear microscopy and PCR-based molecular tools were used to confirm babesiosis. For the therapeutic trial, Groups I and II received treatment with diminazene aceturate and artesunate, respectively and the efficacy was estimated on the basis of survival rate, and improvement in the clinico-haemato-biochemical parameters.

Result: The major clinical signs recorded were persistent high fever, pale mucous membranes, presence of ticks, decreased ruminal motility and haemoglobinuria. In diseased cattle (n=16) Hb, PCV, and TEC levels were significantly ($P<0.05$) low but with a significant ($P<0.05$) increase in TLC as compared to the control group (n=8). Significantly ($P<0.05$) neutrophilia, lymphopenia, hypoglycaemia, hypoproteinemia, hypoalbuminaemia, was recorded in the diseased group. Moreover, significantly ($P<0.05$) increased levels of BUN, creatinine, AST, LDH and iron were recorded in the infected animals. Group I treated with diminazene aceturate was the most effective and cost- efficient treatment for bovine babesiosis compared to Group II treated with Artesunate.

Key words: Artesunate, *Babesia*, Diminazene aceturate, Haemato-biochemical.

INTRODUCTION

Among various tick-borne diseases, babesiosis in cattle is an important haemo-protozoan disease that causes significant morbidity and mortality in cattle (Sharma *et al.,* 2016). The disease has a seasonal influence when tick activity is high. Due to climatic changes in the Kashmir valley, the vector population has been steadily extending in recent years to previously unanticipated places like high altitude or temperate zones (Haq *et al*., 2017; Farooq *et al*., 2018; Haq *et al*., 2021). Imidocarb dipropionate and Diminazene acceturate, which are the preferred medications for minimizing the economic damage caused by *Babesia* parasites, are used to successfully treat bovine babesiosis (Radostitis *et al*., 2007). Unfortunately, some studies have suggested the possible development of Diminazene acceturate resistance in *Babesia* parasites (Hwang *et al*., 2010; Yamasaki *et al*., 2017). Moreover, Imidocarb dipropionate and Diminazene acceturate have been found to have persistent effects in the edible tissues and milk of sheep and cattle for up to 21 days and 6 months, respectively (Traynor *et al*., 2013). Therefore, to control bovine babesiosis, other anti-babesia medications such as Artemisinin derivatives (Artesunate) with low toxicity to the host are utilised (Mosqueda *et al*., 2012). Only scarce studies on blood protozoal diseases such as *Babesia*, *Theileria* and

[1]Division of Clinical Veterinary Medicine Ethics and Jurisprudence, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar-190 006, Jammu and Kashmir, India.

[2]Department of Animal Husbandry, Kashmir-190 001, Jammu and Kashmir, India.

[3]Department of Veterinary Medicine, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004, Punjab, India.

[4]Division of Veterinary Parasitology, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama Campus-190 006, Jammu and Kashmir, India.

Corresponding Author: I.M. Allaie, Division of Veterinary Parasitology, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama Campus- 190 006, Jammu and Kashmir, India. Email: dr.idreesallaie@gmail.com

How to cite this article: Ahmad, T., Tufani, N.A., Haq, A.U., Tramboo, S.R., Allaie, I.M., Malik, H.U., Dar, N.A. and Dar, A.A. (2023). Clinico-Haemato-Biochemical Changes and Therapeutic Efficacy of Diminazene Aceturate and Artesunate against Bovine Babesiosis in Kashmir Valley. Indian Journal of Animal Research. 10.18805/IJAR.B-5014.

Submitted: 07-09-2022 **Accepted:** 27-01-2023 **Online:**

Anaplasma have been carried out in Kashmir valley in recent years in bovines, sheep and goats (Haq *et al*., 2017; Farooq *et al*., 2018; Haq *et al*., 2021), therefore, the present paper describes the clinico- haemato- biochemical and therapeutic studies on bovine babesiosis in Kashmir, India.

MATERIALS AND METHODS

Study area and animals

The study was carried out in central Kashmir *viz;* District Ganderbal, Srinagar and Budgam. In central Kashmir summers are usually mild with little rain, but with higher relative humidity and cool nights. The hottest month is July (mean minimum temperature 6°C, mean maximum temperature 32°C) and the coldest is January (mean minimum temperature -15°C, mean maximum temperature 0°C). In the present study, a total of 16 bovines clinically affected with babesiosis were sampled and 8 apparently healthy bovines were selected under the control group for the comparative therapeutic study.

Samples and diagnosis

Blood samples were collected from the jugular vein of animals and the disease was confirmed in animals exhibiting clinical signs of babesiosis on Giemsa-stained thin blood smears and additionally blood from these animals was subjected to PCR test for further confirmation of the disease. In PCR test, initially, genomic DNA was isolated from blood samples using DNeasy® DNA blood mini kit (QIAGEN, GmbH, Germany). The eluted DNA was labeled and stored at -20°C till further use. The nucleotide sequence of 18S rRNA gene of *Babesia* spp. was amplified in PCR reaction as per the protocol of Olmeda *et al*. (1997) using F:5′-AATACCCAATCCTGACACAGGG-3′ and R:5′-TTAAATACGAATGCCCCCAAC-3′ as forward and reverse primers, respectively at 55°C annealing temperature. Similarly, the status of apparently healthy animals was determined on thin blood smear examination stained with Giemsa stain followed by PCR test to verify the absence of latent or chronic infection of babesiosis.

Clinico-haemato-biochemical analysis

The animals were clinically evaluated for vital parameters like rectal temperature, heart rate, respiration rate, color of mucous membranes and rumen motility. Other signs like presence or absence of haemoglobinuria and tick infestation were also recorded. Haematological parameters (like Hb, PCV, TEC, TLC, DLC, MCV, MCH and MCHC) were evaluated as per the method of Schalm *et al*. (1986) and for biochemical parameters (like glucose, total protein, albumin, globulin, BUN, creatinine, AST, LDH and iron) spectro photometer-based estimation was conducted and blood glucose levels were measured using glucometer ACCU-CHEK® (Zulfiqar *et al*., 2012).

Therapeutic trial

A total of 16 cattle infected with babesiosis were selected, and they were then split into two equal groups (Group I and II) with eight animals each. For the comparative treatment trial, eight healthy cattle were also included in the control group (Group III). Diminazene aceturate was administered intramuscularly (IM) in single dose of 3.5 mg/kg body weight to animals in Group I. Animals in Group II received three 12 hourly IM doses of artesunate at a rate of 1 mg/kg body weight. The clinical recovery, survival rate, microscopy, and restoration of clinico-haemato-biochemical indicators were used to assess the therapeutic efficiency of the medications.

Statistical analysis

The differences of means of estimated parameters between infected and healthy control groups were compared using student t-test and one-way analysis of variance (ANOVA) using SPSS, 20.0. The difference was considered significant when $P<0.05$.

RESULTS AND DISCUSSION

Clinical examination and diagnosis

The major clinical signs and symptoms recorded were persistent high fever, pale mucous membranes (Fig 1), presence of ticks on body surface (Fig 2), haemolysed plasma/serum (Fig 3), elevated heart and respiration rates, decreased ruminal motility, and coffee coloured urine (Fig 4). Microscopic examination of thin blood smears (n=16) of animals having clinical symptoms revealed different intra-erythrocytic forms of haemoparasites which were morphologically compatible with *Babesia* piroplasms (Fig 5). The samples (n=16) that were found microscopically positive for babesiosis showed 408bp amplification on PCR employing *Babesia* 18S rRNA gene specific primers (Fig 6). The samples of apparently healthy animals (n=8) were negative on microscopic examination of thin blood smears as well as on the PCR test, indicating that the animals are free from babesiosis infection of any type.

Clinical parameters

The mean rectal temperature, heart rate and respiration rate were increased in both the diseased groups (Table 1). However, Post treatment rectal temperature, heart rate and respiration rate decreased significantly ($P<0.05$) (Table 1).

Fig 1: Pale conjunctival mucous membrane in babesiosis affected cattle.

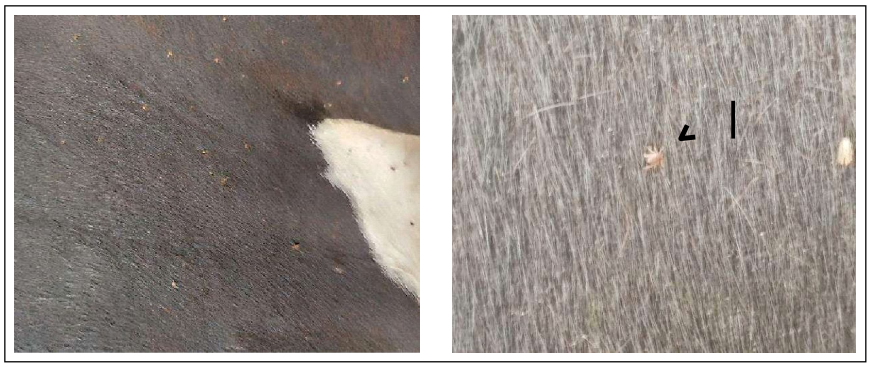

Fig 2: Tick infestation on body surface of cattle.

The pre-treatment mean rumen motility was significantly (P<0.05) lower in diseased groups compared to control group. After treatment significant (P<0.05) increase in rumen motility was recorded (Table 1). Similar results were recorded by Tufani *et al*. (2009), Talkhan *et al*. (2010), Sevinc *et al*. (2013), Ajith *et al*. (2017) and Haq *et al*. (2021). Pyrexia results from the release of endogenous pyrogens, which activate the hypothalamus and raise body temperature (Constable *et al*., 2017). Tachycardia, tachypnea, and decreased haemoglobin concentration may be caused by anoxia, erythrocytopenia, and anaemia (Radostits *et al*., 2007). Decreased rumen motility may be brought on by histamine release and reduced muscular tone brought on by anaemia.

Fig 3: Haemolysis of blood in babesiosis affected cattle.

Haematological parameters

Haematological parameters recorded before start of treatment (day 0) and after treatment (day 7) in *Babesia* affected (n=16) and healthy cattle (n=8) are depicted in Table 2. The pre-treatment mean Hb, PCV and TEC levels were significantly (P<0.05) lower in diseased cattle due to erythrophagocytosis and erythropoietic suppression. Jyothisree *et al*. (2013), and Mahmoud *et al*. (2015) reported similar results. Post treatment results showed significant increase in haematological values (Table 2). Pre-treatment TLC count was significantly high compared to the healthy cattle and it was in close agreement with the findings of Jyothisree *et al*. (2013). Post treatment TLC values decreased significantly (P<0.05) but remained higher compared to the control group. Increased TLC count is attributed to stress induced by the infection (Bhikane *et al*., 2001). DLC showed significantly (P<0.05) higher neutrophils and decreased lymphocytes in both the groups before treatment compared to the healthy group (Table 2). Post treatment neutrophil values decreased and lymphocyte values increased significantly (P<0.05) compared to pre-treatment values (Table 2). The pre-treatment monocyte values were significantly (P<0.05) higher in the diseased group as compared to healthy group (Table 2) however, post treatment monocyte values significantly decreased (P<0.05) in both the treated groups. Both pre and post treatment mean values of eosinophil and basophil showed no significant (P<0.05) difference among diseased and healthy group

Fig 4: Coffee coloured urine in babesiosis affected cattle.

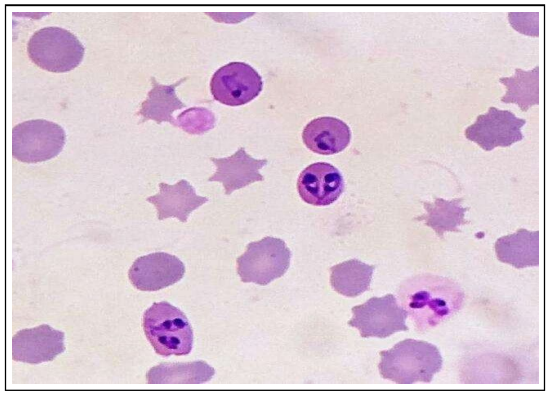

Fig 5: *Babesia* piroplasms in cattle RBCs (Giemsa stained 1000X).

(Table 2). The pre and post treatment values of erythrocyte indices like MCV, MCH and MCHC are presented in Table 2. Significant decrease (P<0.05) was observed in MCHC in *Babesia* affected cattle compared to healthy cattle (Table 2).

Biochemical parameters

The mean values of biochemical parameters are presented in Table 3. Pre-treatment glucose, total protein, and albumin levels were significantly (P<0.05) lower in Group I and II which is attributed to inappetence and metabolic disturbances (Pandey and Mishra, 1987). Hypoglycaemia, hypoproteinemia and hypoalbuminaemia in babesiosis corroborates with the findings of Tufani *et al*. (2009), Talkhan *et al*. (2010) and Singh *et al.* (2014). Hypoproteinaemia and hypoalbuminaemia is attributed to liver dysfunction, kidney dysfunction and anorexia (Esmaeilnejad *et al*., 2012). After

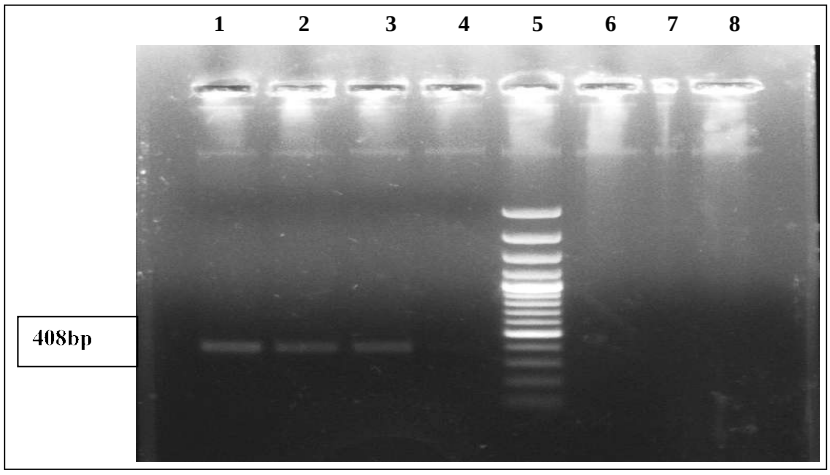

Fig 6: Amplified *Babesia* genus 18S rDNA gene.
Lane 1: *Babesia* Positive control; Lane 2 and 3: *Babesia* Positive samples; Lane 4, 6 and 8: *Babesia* Negative samples; Lane 5: 100-bp plus DNA Marker; Lane 7: *Babesia* Negative control.

Table 1: Pre and post treatment clinical parameters (Mean±S.E) in cattle affected with babesiosis.

Groups	Temperature (°F)		Heart rate (beats/min.)		Respiration rate (breaths/min.)		Rumen motility (per 2 min.)	
	Pre-T	Post-T	Pre-T	Post-T-	Pre-T	Post-T	Pre-T	Post-T
I	104.20±0.76^{a}	101.82±0.27*	87.88±2.45^{a}	74.50±1.29*	42.62±3.20^{a}	33.75±2.22*	1.50±0.32^{a}	3.10±0.22*
II	105.15±0.27^{a}	101.87±0.40*	81.50±2.87^{a}	70.34±0.87*	42.00±6.04^{a}	31.00±1.29*	1.75±0.32^{a}	3.25±0.25*
III	101.67±0.27^{b}	101.68±0.29	69.38±0.82^{b}	70.88±0.95	28.25±1.16^{b}	29.50±1.45	3.12±0.22^{b}	3.00±0.26

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

Table 2: Pre and post treatment hematological parameters (Mean±S.E) in cattle affected with babesiosis.

Parameters	Group I		Group II		Group III	
	Pre-T	Post-T	Pre-T	Post-T	Pre-T	Post-T
Hb (g/dl)	5.85±0.26^{a}	7.04±0.28*^{a}	6.49±0.31^{a}	7.40±0.26*^{a}	10.75±0.52^{b}	10.54±0.46^{b}
PCV (%)	22.25±1.10^{a}	25.75±0.70*	24.50±1.93^{a}	29.00±0.96*	32.00±1.69^{b}	28.75±1.31
TEC (106/µl)	5.16±0.17^{a}	6.78±0.21*^{a}	6.16±0.53^{a}	8.18±0.28*^{b}	7.94±0.30^{b}	8.00±0.28^{b}
TLC (103/µl)	11.65±0.43^{a}	9.54±0.30*^{a}	11.22±0.64^{a}	8.95±0.44*^{a}	7.76±0.18^{b}	7.55±0.18^{b}
N (%)	51.13±1.90^{a}	35.50±1.35*^{a}	49.75±1.55^{a}	35.50±1.26*^{a}	30.00±1.81^{b}	29.50±1.75^{b}
L (%)	42.13±1.59^{a}	60.38±1.24*^{a}	44.25±1.23^{a}	60.75±1.65*^{a}	69.25±0.94^{b}	67.75±1.52^{ab}
E (%)	1.63±0.26	2.12±0.47	1.50±0.50	1.75±0.48	1.12±0.39	1.62±0.32
B (%)	0.75±0.41	0.13±0.12	0.00±0.00	0.25±0.25	0.00±0.00	0.38±0.18
M (%)	5.38±1.17^{a}	1.88±0.47*	4.50±0.65a	1.75±0.25*	0.88±0.35^{b}	1.00±0.26
MCV (fl)	41.21±1.96	37.07±0.60^{a}	42.01±1.55	39.46±1.00^{a}	40.00±1.83	39.24±1.56^{b}
MCH (pg)	11.05±0.02	12.42±0.43	10.61±0.41	11.08±0.45	13.55±0.44	13.49±0.56
MCHC (%)	26.36±0.48^{a}	31.39±1.11*^{a}	27.39±0.54^{a}	32.60±0.94*^{a}	33.71±0.68^{b}	34.54±0.77^{b}

Mean values bearing different superscripts and * between rows differ significantly.

Table 3: Pre and post treatment biochemical parameters (Mean±S.E) in cattle affected with babesiosis.

Parameters	Group I		Group II		Group III	
	Pre-T	Post-T	Pre-T	Post-T	Pre-T	Post-T
Glucose (mg/dl)	34.50±1.91[a]	49.80±1.27*	42.50±1.71[a]	54.06±2.91*	54.75±1.68[b]	53.67±1.01
T. protein (g/dl)	6.05±0.06[a]	7.51±0.11*	6.99±0.08[a]	7.40±0.14*	8.19±0.12[b]	7.92±0.19
Albumin (g/dl)	2.78±0.05[a]	3.09±0.02*	2.94±0.06[a]	3.17±0.07*	3.20±0.04[b]	3.22±0.04
Globulin (g/dl)	3.28a±0.08	4.43±0.12*	3.05a±0.06	4.03±0.16*	4.99b±0.13	4.69±0.21
BUN (mg/dl)	36.72±1.62[a]	26.72±0.88*[a]	34.20±0.99[b]	26.78±0.47*[a]	23.71±0.48[b]	22.38±0.69
Creatinine (mg/dl)	1.91±0.09[a]	1.07±0.03*	1.58±0.17[a]	1.09±0.02*	1.06±0.03[b]	1.07±0.02
AST (IU/L)	186.63±13.58[a]	85.31±4.71*	150.45±13.89[a]	75.83±1.94*	64.32±1.28[b]	63.42±1.61
LDH (IU/L)	2159.63±136.42[a]	1319.40±20.86*	1825.53±175.77[a]	1246.86±26.80*	1292.12±45.61[b]	1291.29±49.46
Iron (µg/ dl)	148.45±1.77[a]	94.12±1.34*[a]	144.31±2.02[a]	95.73±1.91*[a]	88.97±2.01[b]	89.16±2.13b

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

treatment a significant rise in glucose, total protein and albumin (P<0.05) was recorded but it was non-significant compared to the control group (Table 3). The pre-treatment globulin levels in Group I and II were low, however after treatment significant increase in globulin levels were recorded but were having non-significant difference compared to the control group (Table 3). Similar findings of decreased á globulin were recorded in babesia affected dogs by Lobetti *et al*. (2000). BUN, creatinine, AST, LDH and iron levels were significantly (P<0.05) higher in Group I and II as compared to the healthy cattle (Table 3). After treatment, the BUN, creatinine, AST, LDH and iron levels decreased significantly (P<0.05) in Groups I and II. Babesiosis causes elevated levels of creatinine and BUN because the degraded byproducts of damaged RBCs are deposited in the kidneys during glomerular filtration, which reduces the excretion of BUN and creatinine. Talkhan *et al*. (2010) also observed similar increase in BUN and creatinine. Increase in AST level occurs due to massive haemolysis in conjunction with hypoxia. Similar findings of increased AST were reported by El-Hamed *et al*. (2016) and Mohanapriya *et al*. (2017). LDH is a marker of erythrocyte death and hepatocellular injury since it is present in large quantities in erythrocytes and hepatocytes (Latimer, 2011). The parasite causes leakage of this enzyme into the bloodstream, which raises the serum level of LDH. Similarly, Hashem *et al*. (2018) noted increased LDH concentrations in *Babesia* affected cattle. The higher blood iron levels are the consequence of intravascular hemolysis, which releases free haemoglobin, which breaks down to become globin, haeme, and iron. Talkhan *et al*. (2010) found similar results.

Therapeutic efficacy

The efficacy of Artesunate was evaluated with Diminazene aceturate in terms of survival rate, clinical recovery, improvements in clinico-haemato-biochemical parameters and clearance of *Babesia* parasites. Group I animals treated with Diminazene aceturate recovered uneventfully after 7 days of treatment with normal body temperatures. Microscopy revealed significantly reduced *Babesia* piroplasms. The clinical signs gradually disappeared and the levels of haemato- biochemical parameters returned to almost normal. Hence, Diminazene aceturate was 100% effective to cure babesiosis in cattle with no mortality, which is in close agreement with the observations of Tufani *et al*. (2009). In Group II animals treated with Artesunate, only four cattle recovered uneventfully and remaining four cattle died during the treatment with 50% recovery rate. The 50% survival of animals in this group could be attributed to the low level of parasitemia, moderate level of anaemia and prompt and early treatment. Animals that survived showed normal clinico-haemato-biochemical parameters after 7 days of treatment. No death was observed in healthy control Group III during the study period. According to Patel *et al*. (2001), Artesunate and oxytetracycline together have a 66.7% efficiency against bovine tropical theileriosis. Research is still going on to understand the potential of artesunate in treating *Babesia* species infection either alone or in combination with other drugs. *In vitro* growth of *B. bovis* and *B. gibsoni* was suppressed by use of artesunate, which was also successful in treating *B. microti*-infected mice (Goo *et al*., 2010). *Babesia* species share a similar life cycle, as well as clinical symptoms, with *Plasmodium* species, and Artesunate is quite efficient against the malarial parasite (Reddy *et al*., 2010) as well as in several non-malarial parasites (Goo *et al*., 2010). Mild parasitemia remained present after 7 days of treatment in both the groups. It could be explained by the fact that the cattle that have recovered from acute infection may have low parasitemia, often microscopically undetectable and turn out to be carriers of the disease.

CONCLUSION

Diminazene aceturate was the most effective and cost-efficient drug for the treatment of bovine babesiosis compared to Artesunate with only 50% efficacy. Further validation is warranted for use of Artesunate alone or in combination with other antibabesial drugs.

ACKNOWLEDGEMENT

The authors are highly thankful to Dr. M.R. Fazili, Prof. and Head, Teaching Veterinary Clinical Complex, FVSc. and AH,

SKUAST-Kashmir for providing facilities to carry out this research work. Authors are also highly thankful to Dr. L.D. Singla, Professor and Head, Department of Veterinary Parasitology, GADVASU, Ludhiana, Punjab for providing *Babesia* positive control sample for PCR.

Conflict of interest: None.

REFERENCES

Ajith, Y., Rajeswari, T.U., Siji, S.R., Dilip, C. (2017). Case report on babesiosis associated pre-hepatic jaundice in a Malabari goat. Journal of Veterinary and Animal Science. 3: 1-3.

Bhikane, A.U., Narladkar, B.W., Anantwar, L.G., Bhokre, A.P. (2001). Epidemiology, clinicopathology and treatment of babesiosis in cattle. Indian Veterinary Journal. 78: 726-729.

Constable, P., Hinchcliff, K.W., Done, S., Gruenberg, W. (2017). Veterinary Medicine. A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats. 11th ed., Saunders Ltd. Saunders, London, 1483-97.

El-Hamed, H.A.A., Salem, S.M., Ibrahim, H.N. (2016). Haemato-biochemical alterations in cattle suffering from anaemia and their effect on quality of some meat. Egyptian Journal of Chemistry and Environmental Health. 2(2): 232-249.

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.

Farooq U., Tufani, N.A., Malik, H.U., Mir, M.S. (2018). Clinical and morphomolecular epidemiology of bovine theileriosis in Kashmir. Indian Journal of Animal Research. 53(3): 375-381.

Goo, Y.K., Terkawi, M.A., Jia, H., Aboge, G.O., Ooka, H., Nelson, B., Kim, S., Sunaga, F., Namikawa, K., Igarashi, I., Nishikawa, Y., Xuan, X. (2010). Artesunate, a potential drug for treatment of *Babesia* infection. Parasitology International. 59(3): 481-486.

Haq, A.U., Tufani, N.A, Gugjoo, M.B., Nabi, S.U., 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.

Haq, A.U., Tufani, N.A., Malik, H.U., Najar, T.A. (2021). Haemato-biochemical and Therapeutic Studies on Ovine Babesiosis in Kashmir. Indian Journal of Animal Research. DOI:10.18805/IJAR.B-4524.

Hashem, M., Neamat-Allah, A.N., Gheith, M.A. (2018). A study on bovine babesiosis and treatment with reference to hemato -biochemical and molecular diagnosis. Slovenian Veterinary Research. 55: 165-173.

Hwang, S.J., Yamasaki, M., Nakamura, K., Sasaki, N., Murakami, M., Wickramasekara Rajapakshage, B.K., Ohta, H., Maede, Y., Takiguchi, M. (2010). Development and characterization of a strain of *Babesia gibsoni* resistant to diminazene aceturate *in vitro*. Journal of Veterinary Medical Science. 72: 765-771.

Jyothisree, C.H., Naik, S., Samatha, V. (2013). A study on prevalence and clinicotherapeutic management of babesiosis in H.F. cross bred cattle in Anantapur district of Andhra Pradesh. International Journal of Food Agriculture and Veterinary Science. 3(2): 88-91.

Latimer, K.S. (2011). Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology, 5th ed., Blackwell Publishing. pp. 67-8.

Lobetti, R.G., Möhr, A.J., Dippenaar, T., Myburgh, E. (2000). A preliminary study on the serum protein response in canine babesiosis. Journal of the South African Veterinary Association. 71(1): 38-42.

Mahmoud, M.S., Kandil, O.M., Nasr, S.M., Hendawy, S.H.M., Habeeb, S.M., Mabrouk, D. M., Silva, M.G., Suarez, C.E. (2015). Serological and molecular diagnostic surveys combined with examining haematological profiles suggests increased levels of infection and haematological response of cattle to babesiosis infections compared to native buffaloes in Egypt. Parasites and Vectors. 319(8): 1-15.

Mohanapriya, T., Pazhanivel, N., Enbavelan, P.A., Kumar, V. (2017). Concurrent Babesia bigemina and Anaplasma marginale infection in a Jersey cow. Indian Journal of Veterinary Sciences and Biotechnology. 12(3): 143-145.

Mosqueda, J., Olvera-Ramirez, A., Aguilar-Tipacamu, G., Canto, G.J. (2012). Current advances in detection and treatment of babesiosis. Current Medical Chemotheraphy. 19: 1504-1518.

Olmeda, A.S., Armstrong, P.M., Rosenthal, B.M., Valladares, B., Castillo, A.D., Armas, F.D. (1997). A subtropical case of human babesiosis. Acta Tropica. 67: 229-234.

Pandey, N. and Mishra, S. (1987). Haematological and biochemical response to haemolytic anaemia of clinical Babesiosis in cattle and therapy. Indian Veterinary Journal. 64: 882- 86.

Patel, D., Misraulia, K.S., Reddy, A.G., Garg, U.K., Sharma, R.K., Gupta, B.K. (2001). Experimental studies on the efficacy of artesunate and oxytetracycline combination against induced BTT in crossbred calves. The Indian Journal of Animal Sciences. 71(8): 741-744.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W., Constable, P.D. (2007). Veterinary Medicine. A textbook of the Diseases of Cattle, Horse, Sheep, Pigs and Goats. 10th Ed., Saunders Elsevier, Edinburgh.

Reddy, E.J., Rao, P.S., Narasu, M.L. (2010). Cloning and expression of the gene involved in enhanced production of artemisinin, an effective anti-malarial drug by bioconversion. Journal of Biotechnology. 91: 150.

Schalm, O.W., Jain, N.C., Carrol, E.J. (1986). Veterinary Haematology, 4th Ed. Lea and Febiger, Philadelphia.

Sevinc, F., Sevinc, M., Ekici, O.D., Yildiz, R., Isik, N., Aydogdub, 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.

Sharma, A., Singla L.D., Ashuma, Batth, B.K., Kaur, P. (2016). Clinicopatho-biochemical alterations associated with subclinical Babesiosis in dairy animals. Journal of Arthropod Borne Diseases. 10(2): 259-267.

Singh, J., Gupta, S.K., Singh, R., Hussain, S.A. (2014). Etiology and haemato-biochemical alterations in cattle of Jammu suffering from anaemia. Veterinary World. 7(2): 49-51.

Talkhan, O.F.A., Radwan, M.E.I., Ali, M.A. (2010). Cattle babesiosis and associated biochemical alteration in Kalubyia Governorate. Natural Science. 12: 24-27.

Traynor, I.M., Thompson, C.S., Armstrong, L., Fodey, T., Danaher, M., Jordan, K., Kennedy, D.G., Crooks, S.R.H. (2013). Determination of Imidocarb Residues in Bovine and Ovine Liver and Milk by Immunobiosensor. Food Additives and Contaminants: Part A 30(6): 1108-14.

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

Yamasaki, M., Watanabe, N., Idaka, N., Yamamori, T., Otsuguro, K., Uchida, N., Iguchi, A., Ohta, H., Takiguchi, M. (2017). Intracellular diminazene aceturate content and adenosine incorporation in diminazene aceturate-resistant *Babesia gibsoni* isolate *in vitro*. Experimental Parasitology. 183: 92-98.

Zulfiqar, S., Shahnawaz, S., Ali, M., Bhutta, A.M., Iqbal, S., Hayat, S., Qadir, S., Latif, M., Kiran, N., Saeed, A., Ali, M., Iqbal, F. (2012). Detection of *Babesia bovis* in blood samples and its effect on the hematological and serum biochemical profile in large ruminants from Southern Punjab. Asian Pacific Journal of Tropical Biomedicine. 2(2): 104-8.