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Molecular detection of *Babesia ovis* and blood parameters' investigation reveal hematological and biochemical alterations in babesiosis-infected Lohi sheep in Multan, Pakistan

Muhammad Sajid[1*], Syed Atif Hasan Naqvi[2], Muhammad Riaz[1], Ummad Ud Din Umar[2], Nasreen Nasreen[3], Adil Khan[4,5] and Mourad Ben Said[6,7]

[1]*Zoology Division, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan*
[2]*Department of Plant Pathology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan*
[3]*Department of Zoology, Khyber Pakhtunkhwa, Abdul Wali Khan University, Mardan, Pakistan*
[4]*Department of Zoology, Bacha Khan University, Charsadda, Pakistan*
[5]*Department of Biology, Mount Allison University, Sackville, Canada*
[6]*Laboratory of Microbiology, National School of Veterinary Medicine of Sidi Thabet, University of Manouba, Manouba, Tunisia*
[7]*Department of Basic Sciences, Higher Institute of Biotechnology of Sidi Thabet, University of Manouba, Manouba, Tunisia*

Abstract

Background: *Babesia* infections in sheep can cause a wide range of clinical and laboratory presentations. Changes in blood parameters are a meaningful manifestation of physiological and pathological changes in an organism.

Aim: Therefore, the present study was conducted to analyze and compare hematological and biochemical parameters between blood profiles of Lohi sheep naturally infected and uninfected with *Babesia ovis*, the main causative agent of ovine babesiosis.

Methods: Initially, blood and serum samples from 67 Lohi sheep were collected, DNA was extracted and babesial infection was detected through polymerase chain reaction. The overall infection rate of *B. ovis* was 37% (25/67). Sixteen infected (experiment group) and 16 uninfected (control group) sheep that were apparently healthy with no history of previous treatment for babesiosis, were selected for hemato-biochemical analysis. Blood samples were analyzed through an automatic CBC analyzer, while serum collected from gel vacutainers was analyzed for blood urea, blood urea nitrogen (BUN), creatinine, and total bilirubin. Each parameter was compared between infected and uninfected animals using a paired *t*-test in Minitab Express™ software for statistical analyses.

Results: Erythron comparison showed a highly significant ($p < 0.0001$) decrease in RBC, hemoglobin, and Hct. A nonsignificant increase in mean corpuscular volume (MCV), red cell distribution width (RDW), and RDW–standard deviation (RDW-SD), while a nonsignificant decrease in mean corpuscular haemoglobin (MCH) and MCH concentration (MCHC) values was recorded in infected sheep. Leukon comparison showed a significantly low level of total leukocyte ($p < 0.001$) in infected sheep. Platelet (Plt) along with platelet crit (Pct) and platelet distribution width (PDW) were nonsignificantly higher, whereas a nonsignificant decrease in mean Plt volume was recorded in infected sheep as compared to uninfected animals. Among biochemical parameters, blood urea, BUN, and total bilirubin showed significant differences ($p < 0.05$), while creatinine showed a nonsignificant difference.

Conclusion: To the best of our knowledge, this is the first report on hemato-biochemical changes associated with babesiosis in the Lohi breed. Consistent with hemolytic anemia, these data would justify physical examination and, together with the medical history, would provide an excellent basis for the diagnosis of babesiosis.

Keywords: Ovine babesiosis, Molecular detection, Blood parameters, Lohi sheep breed, Pakistan.

Introduction

"*Blood urination*" currently known as "babesiosis" in "*Ovine Hosts*" was first described in 1880 by Focsa who observed similarities with cattle blood urination in a sheep flock, near Constanţa, Romania (Mihalca, 2010). The disease spectrum ranges from a deceptively quiescent infection to fulminant, malaria-like episodes that are often life-threatening (Aguilar-Delfin *et al.*, 2001). Clinical symptoms of acute disease include pyrexia, hemolytic anemia, pale mucous membrane, and

***Corresponding Author:** Muhammad Sajid. Zoology Division, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan. Email: *sajidmultani@hotmail.com*

hemoglobinuria. Naturally infected sheep with an intact spleen show more placid symptoms of babesial infection than splenectomized sheep (Yeruham *et al.*, 1998; Sevinc *et al.*, 2007). However, they may have a significant impact on sheep's productive and reproductive capabilities (Islam *et al.*, 2018).

Ovine babesiosis, caused by *Babesia ovis*, has a significant impact on national sheep production, as it ranks as the third most epidemiologically important sheep disease in Pakistan (Rashid *et al.*, 2010). Pathological changes can only be better assessed when normal blood values are available for comparison (Njidda *et al.*, 2014). The current study focused on "Lohi," which is the largest and most productive sheep breed in Pakistan (Nouman and Abrar, 2014); however, published references for frequently measured hematological and biochemical parameters for this breed are limited. Although considerable information is available on normal blood parameters of domestic animals, it is difficult to be convinced that the use of such reference intervals is consistent across breeds of sheep (Lepherd *et al.*, 2009) given that many genetic and nongenetic parameters, including breed, genotype, management system, drugs, nutrition, and climate, influence the hematology and serum biochemistry (Onasanya *et al.*, 2015). Very limited data are available on hemato-biochemical indices of sheep breeds indigenous to the Indian subcontinent (Rahman *et al.*, 2018). Although ovine babesiosis is an old disease, the literature provides little information on the hematological and biochemical profiles of victims (Sevinc *et al.*, 2013).

To the best of our knowledge, there are no published reports on the complete hemato-biochemical changes associated with ovine babesiosis in the Lohi breed. Therefore, the present study involved uninfected sheep, from the same herd, as a control group; these animals had been reared under similar feeding, management, and environmental conditions throughout the study period. The data generated in this study would justify the physical examination and that when combined with medical history would provide an excellent basis for diagnosis. It would also help in determining the measure of tissue/organ damage, immune status, and the type of anemia, which is a logical outcome of the intra-erythrocytic nature of *B. ovis* infection.

Materials and Methods

Study area

District Multan has an arid climate with very hot summers and mild winters. The city witnesses some of the most extreme weather in the country. The highest recorded temperature is approximately 54°C (129°F), and the lowest recorded temperature is approximately −1°C (30°F). The average rainfall is roughly 127 mm (5.0 in). Dust storms are a common occurrence within the city.

Animal selection

The study included 67 Lohi sheep that resided in a private sector breeding farm located in Qasba Marral, district Multan. All sheep appeared to be healthy and did not show any signs of babesiosis, nor had they been previously treated for it. To eliminate the potential influence of age, gender, and pregnancy on the results, only nonpregnant females between 1 and 3 years old were selected for the final comparison, with a total of 32 sheep included, and equally divided between 16 infected and 16 uninfected animals.

Blood and serum sampling

Each time, 2 ml out of 4 ml of sheep jugular vein blood was collected in EDTA-containing vacutainers (blood collection tubes), while the remaining 2 ml into serum separator tubes-BD SST™ gel tubes commonly referred to as "gel vials" specifically designed for serum collection. Vacutainers were gently inverted 5–6 times to thoroughly mix the blood with the anticoagulant, while the gel vials after inverting 5–6 times to activate the "clot activator" were kept standing for 30 minutes to allow time for blood to clot and serum to separate that was then collected in serum cups. Kept in the ice box, all samples (whole blood and sera) were transferred to a well-equipped Laboratory.

DNA extraction and polymerase chain reaction (PCR) amplification

A total of 200 µl of whole blood samples was used for DNA extraction with the Favor Prep Blood Genomic DNA Extraction Mini Kit from Favorgen Biotech Corp., according to the manufacturer's instructions. DNA was eluted in a final volume of 100 µl. DNA extracts were then stored at −20°C until use. *Babesia ovis* infection was screened in all samples with PCR amplification of fragment (549 bp) of the ssu rRNA sequence specific for *B. ovis* by using specific primers BboF (5'- TGGGCAGGACCTTGGTTCTTCT-3') and BboR (5'-CCGCGTAGCGCCGGCTAAATA-3'), as described by Aktas *et al.* (2005). The PCR reaction was performed in a final volume of 25 µl containing 12.5 µl of Green Master Mix® (Promega, Madison, WI), 1.0 µl of 10 µM primer mix (BboF + BboR), and 0.5 µl of 1 M betaine and 5 µl of template DNA solution. Green Master Mix® is a premixed ready-to-use solution containing DNA polymerase along with dNTPs and $MgCl_2$ in reaction buffer. The thermal cycling profile was described by Aktas *et al.* (2005). Distilled water and DNA extracted from *B. ovis* were used as negative and positive controls, respectively. PCR products were electrophoresed in 1.5% agarose gel and sized with 100 bp DNA ladder (Fermentas).

Hemato-biochemical analysis

All infected and uninfected samples were analyzed by using the automatic CBC analyzer Mythic™-18, Orphee SA, Switzerland, while sera collected from gel vacutainers were analyzed for blood urea, blood urea nitrogen (BUN), creatinine, and total bilirubin through Rayto-9200 semi-auto chemistry analyzer.

Statistical analysis

As all parameters had a co-variant effect, we therefore used adjusted means. Each parameter was statistically compared between *B. ovis* infected and uninfected animals by using a paired *t*-test in Minitab Express™ software (Minitab LLC, Pennsylvania, USA) with a threshold value of 0.05.

Ethical approval

All animal experiments were approved by the State Committee on Animal Ethics, Bahauddin Zakariya University, Pakistan. The recommendations of the European Council Directive (86/609/EC) of November 24, 1986, regarding the standards in the protection of animals used for experimental purposes were also followed.

Results

The overall infection rate of *B. ovis* was 37% (25/67). Thirty-two animals (half infected and half uninfected with *B. ovis*) selected for hemato-biochemical comparison revealed the following results.

Leukon comparison

Leukon comparison showed a significantly low level of total leukocyte (TLC) ($p < 0.001$) in infected than uninfected. Similarly, lymphocyte (LYM) was found to be significantly lower ($p < 0.001$) than uninfected sheep. Monocyte (MON) and granulocyte (GRA) showed a nonsignificant ($p > 0.05$) difference between infected and uninfected. However, MON% and GRA% were found to be significantly decreased in infected sheep with *p*-value of <0.001 and <0.05, respectively (Table 1).

Erythron comparison

Erythron comparison showed a highly significant ($p < 0.0001$) decrease in RBC, hemoglobin (Hb), and Hct. in infected than uninfected. Mean corpuscular volume (MCV), red cell distribution width (RDW), and RDW–standard deviation (RDW-SD) showed a minor, nonsignificant ($p > 0.05$) increase in infected sheep while mean corpuscular volume (MCH) and MCH concentration (MCHC) showed a minor, nonsignificant ($p > 0.05$) decrease in infected sheep (Table 2).

Platelet (Plt) comparison

Plt count was found nonsignificantly ($p > 0.05$) higher in infected sheep. Similarly, platelet crit (Pct) and platelet distribution width (PDW) were nonsignificantly ($p > 0.05$) higher in infected animals than uninfected.

Table 1. Means (±standard error) of TLC, LYM, MON, GRA, LYM%, MON%, and GRA% in *B. ovis*-infected Lohi sheep compared to uninfected.

Parameters	Unit	Ref. range[a]	Uninfected	Infected	*p*-value
TLC	$\times 10^3/\mu l$	4.0–12.0	53.05 ± 2.56	38.18 ± 2.86	<0.001
LYM	$\times 10^3/\mu l$	2.0–9.0	46.46 ± 2.29	31.73 ± 2.86	<0.001
MON	$\times 10^3/\mu l$	0–0.8	2.78 ± 0.24	3.17 ± 0.27	–
GRA	$\times 10^3/\mu l$	NA	3.76 ± 0.42	3.26 ± 0.33	–
LYM%	%	40–75	87.59 ± 0.99	81.86 ± 1.76	<0.01
MON%	%	0–06	5.26 ± 0.36	9.01 ± 0.96	<0.001
GRA%	%	NA	7.13 ± 0.75	9.12 ± 0.98	<0.05

NA: Not available.
[a]Ref. range according to Radostits *et al.* (2007).

Table 2. Means (±standard error) of RBC, Hb, Hct., MCV, MCH, MCHC, RDW, and RDW-SD in *B. ovis*-infected Lohi sheep compared to uninfected.

Parameters	Unit	Ref. range[a]	Uninfected	Infected	*p*-value
RBC	$\times 10^6/\mu l$	9–15	9.04 ± 0.17	7.06 ± 0.23	<0.0001
Hb	g/dl	9–15	8.93 ± 0.12	6.79 ± 0.20	<0.0001
Hct	%	27–45	25.20 ± 0.77	19.93 ± 0.75	<0.0001
MCV	μM^3	28–40	27.84 ± 0.55	28.20 ± 0.33	–
MCH	pg	8–12	9.91 ± 0.15	9.62 ± 0.09	–
MCHC	g/dl	31–34	35.83 ± 0.96	34.25 ± 0.56	–
RDW	%	18–24.6	16.16 ± 0.54	17.00 ± 0.27	–
RDW-SD	μM^3	NA	17.21 ± 0.60	17.78 ± 0.40	–

NA: Not available.
[a]Ref. range according to Radostits *et al.* (2007).

Whereas, a nonsignificant ($p > 0.05$) decrease was observed in mean Plt volume (MPV) (Table 3).

Biochemical comparison

Among biochemical parameters, blood urea, BUN, and total bilirubin showed significant differences ($p < 0.05$), while creatinine showed a nonsignificant difference (Table 4).

Discussion

Babesia infections in sheep can cause a wide range of clinical and laboratory presentations. Blood parameters are a meaningful manifestation of physiological and pathological changes in an organism. Therefore, the present study was conducted to analyze and compare hematological and biochemical parameters between blood profiles of Lohi sheep naturally infected and uninfected with *B. ovis*.

Leukopenia, a significant decrease in total TLC with a marked decrease in GRAs and LYMs, but an increase in MONs observed in infected animals are in partial agreement with Sevinc *et al.* (2013) who found *leukopenia* with decreased GRA and LYM in infected sheep with very high parasitemia in all infected sheep. An increase in MON was found in sheep with low and moderate parasitemia that gradually declined in sheep with a high parasitemic load. The difference probably resulted from infection of animals with different subspecies of *B. ovis* (Esmaeilnejad *et al.*, 2012a). *Monocytosis*, i.e., increase in MON is also consistent with the findings of Esmaeilnejad *et al.* (2012a) who observed *monocytosis* in ovine babesiosis, and Wright *et al.* (1998) who reported similar results in bovine babesiosis. El-Sifi *et al.* (1990) suggested a response of *monocytosis* as the body's defense against infection. The marked elevation in the number of MON in *Babesia* infection is due to their role as active mediators in the natural, innate, nonspecific immune response that involves the activation of macrophages (Court *et al.*, 2001). A protective role is known for macrophages during infection by several *Babesia* species (Homer *et al.*, 2000). Adejinmi *et al.* (2004) found a similar reduced TLC response in West African Dwarf sheep infected with *Babesia* sp. Rahbari *et al.* (2008) also observed a significant decrease in the TLCs; however, the LYM count was higher than normal.

Present findings of a significant decrease in Hb, Hct., and total RBC in *B. ovis* infected sheep clearly indicated "*hemolytic anemia*" as previously described in ovine babesiosis by Kozat *et al.* (2003), Adejinmi *et al.* (2004), Bicek *et al.* (2005), Rahbari *et al.* (2008), Rashid *et al.* (2010), Sulaiman *et al.* (2010), Esmaeilnejad *et al.* (2012a, 2012b), Ijaz *et al.* (2013), Sevinc *et al.* (2013), Esmaeilnejad *et al.* (2014), and Orunç-Kilinç *et al.* (2015). Statistically significant low values observed Hb, Hct., and total RBC were expected because *Babesia* destroys erythrocytes (Sevinc *et al.*, 2013). Numerous mechanisms link infection and *hemolysis* (Berkowitz, 1991) that may be extravascular mediated by the mononuclear phagocytic system, or intravascular. *Babesia* causes extravascular *hemolysis* by direct invasion of RBCs and membrane alteration. Cellular invasion and metabolic activity of the parasite alter the cell membrane, leading to splenic sequestration (Dhaliwal *et al.*, 2004).

MCV and MCHC are the necessary investigations to codify anemia on a morphological basis (Wiwanitkit, 2007). In this study, the MCV was slightly higher, and the MCHC was lower than in healthy animals, although both were within the reference ranges described by Radostits *et al.* (2007). Increased MCV (*macrocytosis*) and low MCHC (*hypochromia*) are due to *reticulocytosis* (Latimer, 2011) in which reticulocytes are large, young, and anucleate that lack their full concentration of Hb. *Reticulocytosis* causes an increase in red cell distribution

Table 3. Means (±standard error) of Plt, MPV, Pct, and PDW in *B. ovis*-infected Lohi sheep compared to uninfected.

Parameters	Unit	Ref. range[a]	Uninfected	Infected	*p*-value
Plt	$\times 10^3/\mu l$	250–750	1,524.1 ± 254.8	1,700.3 ± 164.9	–
MPV	μM^3	NA	14.88 ± 0.23	14.19 ± 0.36	–
Pct	%	NA	2.29 ± 0.39	2.42 ± 0.25	–
PDW	%	NA	12.80 ± 0.62	13.43 ± 0.91	–

NA: Not available.
[a]Ref. range according to Radostits *et al.* (2007).

Table 4. Means (±standard error) of urea, BUN, creatinine, and bilirubin in *B. ovis*-infected Lohi sheep compared to uninfected.

Parameters	Unit	Ref. range[a]	Uninfected	Infected	*p*-value
Urea	mg/dl	17–42	43.38 ± 1.57	49.59 ± 3.11	<0.05
BUN	mg/dl	8–20	20.2 4 ± 0.7	23.14 ± 1.45	<0.05
Creatinine	mg/dl	1.2–1.9	0.79 ± 0.01	0.81 ± 0.02	–
Bilirubin	mg/dl	0.1–0.5	0.39 ± 0.03	0.53 ± 0.03	<0.01

[a]Ref. range according to Radostits *et al.* (2007).

width-RDW (Brockus, 2011), which is in accordance with present findings and leads to the conclusion that it is "*macrocytic hypochromic anemia.*" Hypochromic macrocytes indicate either *dyserythropoiesis* or an increased percentage of reticulocytes (Bain, 2006). Macrocytic hypochromic *anemia* may be classified as *reticulocytosis* with regenerative *anemia* as the classification systems overlap (Tvedten, 2010). The type of *anemia* may vary in *Babesia* infection (Kaur, 2014). Rahbari *et al.* (2008), Sulaiman *et al.* (2010), and Esmaeilnejad *et al.* (2012a) described ovine babesiosis associated with *microcytic hypochromic anemia*.

Anemia may also be attributed to immune-mediated phenomena by autoantibodies against the membrane component of *Babesia* infected and uninfected red blood corpuscles (Rubino *et al.*, 2006), the generation of toxic hemolytic factors of the parasite (Rafaj *et al.*, 2007), mechanical desolation by trophozoite intra-cellular binary fission (Zobba *et al.*, 2008), or via the release of vasoactive molecules such as kallikrein-a subgroup of serine proteases (Schetters *et al.*, 2009). In addition, oxidative shocks on RBCs may play a dominant role in the pathogenesis of *anemia* (Esmaeilnejad *et al.*, 2014). Succinctly, the measurement of hematological parameters is important in determining the severity of *Babesia* infection in sheep, as no correlation between the level of parasitemia and the degree of *anemia* could be found in the detailed clinical and laboratory observations made by Sevinc *et al.* (2013).

The interpretation of Plt count becomes more difficult for animals in which laboratory-specific intervals are not available. This is because the methods at hand for measuring Plt, err in precision and accuracy, and results from the same animal may differ between methods (Koplitz *et al.*, 2001; Tasker *et al.*, 2001). Thus, veterinary laboratories enact reference intervals specific to their own equipment. In this study, Plt counts in both *B. ovis* infected and uninfected sheep were found to be suggestive of *thrombocytosis* compared to the reference range provided by Radostits *et al.* (2007). Though nonsignificant, a comparison of the Plt values of the infected with the uninfected group suggests *thrombocytosis* in the infected group. Altered Plt trafficking (physiologic *thrombocytosis*) and inflated Plt production (enhanced thrombopoiesis including reactive *thrombocytosis*) could be the main causes (Boudreaux *et al.*, 2011).

Iron deficiency could be another possible cause of *thrombocytosis,* as moderate to marked *thrombocytosis* is a common finding in iron deficiency (Weiss, 2010). Previous studies on babesiosis (Bicek *et al.*, 2005; Col and Uslu, 2007; Askar *et al.*, 2008; Chaudhuri *et al.*, 2008; Lotfollahzadeh *et al.*, 2012; Swelum *et al.*, 2014) described a marked decrease in serum iron as a frequent finding in different animal species. In addition, higher Plt values in both groups could be an analyzer-dependent artifact. When small, shredded, or haemolyzed RBCs, leukocyte slivers, or particulate cellular debris are erroneously counted as Plts, it shows pseudo-*thrombocytosis* and can potentially occur with any counting method (Stokol, 2010).

MPV meditates the average size of Plt in circulation and generally decreases with increasing Plt (Boudreaux *et al.*, 2011). This is consistent with present findings of lower MPV in infected compared with uninfected sheep. MPV and Plt allow the derivation of an equivalent Plt variable, the Pct. As Hct. is an indicator of total erythrocyte mass in the body, Plt mass in the body translates to Pct (Tvedten *et al.*, 2008). Pct is the physiologically most relevant parameter for hemostasis and regulation of thrombopoiesis (Butkiewicz *et al.*, 2006).

The increase in PDW in the present study implies Plt anisocytosis. Analogous to RDW, PDW, i.e., Plt distribution width increases with variability in Plt size and is a measure of Plt anisocytosis (Bain, 2006). Plt findings, including Plt, Pct, MPV, and PDW in ovine babesiosis during the present study, are in contrast to those of Sevinc *et al.* (2013) who observed thrombocytopenia with a decrease in Pct and PDW while increasing MPV. Although used as a diagnostic sign of babesiosis, changes in Plt index values: Pct and MPV in response to *Babesia* parasites and their treatment are even less understood (Zvorc *et al.*, 2010). Babesiosis in dromedary camels was found to induce a nonsignificant increase in Plt and Pct, while a decrease in Hct. and PDW (Swelum *et al.*, 2014) supported the present findings in infected sheep.

Babesia-infected RBCs are reported to congest renal, hepatic, and pancreatic capillaries and reduce blood flow, which can lead to ischemic injury to such vital organs (Mathe *et al.*, 2007). The higher the parasitemia, the greater the likelihood of ischemic injury; therefore, blood levels of urea, creatinine, and bilirubin are reliable parameters for evaluating hepatic and renal function (Camacho *et al.*, 2005). *Azotemia* and increased creatinine suggest decreased glomerular filtration, hence altered renal function in most animal species (Rosenfeld and Dial, 2010). Previous findings of mild to significantly higher levels of BUN have been reported in babesial infection of sheep (Rahbari *et al.*, 2008; Rasheed and Al-Fetly, 2012; Esmaeilnejad *et al.*, 2012a; Sevinc *et al.*, 2013), goats (Sulaiman *et al.*, 2010), camels (Swelum *et al.*, 2014), dogs (Crnogaj *et al.*, 2010), and cattle (Kaur, 2014) confirm the current results. Induced immune complexes in piroplasm infections can cause mechanical damage to glomeruli resulting in higher levels of urea in sheep (Elsadig *et al.*, 2013).

Creatinine is a more accurate measure of glomerular filtration rate (GFR) than BUN, especially in ruminants (Carlson, 2009). Nevertheless, a small increase in creatinine can be attributed to progressively compromised renal function, as 65%–75% of the total nephrons must be nonfunctional before the serum

creatinine level exceeds the normal range (Gibson *et al.*, 2006, 2007). Saliva, gastrointestinal tract, and sweat are routes of urea excretion in addition to its renal clearance, but there is no secondary cause of creatinine elevation unlike BUN (Tripathi, 2011). A slight increase in serum creatinine level was also observed by Rahbari *et al.* (2008) in ovine babesiosis. Significantly higher creatinine concentrations following *Babesia* infection were observed in sheep (Yeruham *et al.*, 1998; Uilenberg, 2006; Esmaeilnejad *et al.*, 2012a), dogs (Furlanello *et al.*, 2005; Crnogaj *et al.*, 2010), camels (Swelum *et al.*, 2014), and in dairy animals (Talkhan *et al.*, 2010; Kaur, 2014). Whereas, a decrease in serum creatinine has been also reported by Sevinc *et al.* (2013) and Konto *et al.* (2014) in sheep and dogs, respectively. The decrease in creatinine values may be the result of the initial blood dilution, as few dogs showed an increase in creatinine levels during this study (Konto *et al.*, 2014).

Unconjugated bilirubin produced in the spleen is eliminated in the bile from the liver after being conjugated with glucuronides (Olver *et al.*, 2010). The retrograde flow of bile pigments in serum and tissue produces a yellow coloration termed as icterus or jaundice which is the prominent clinical sign of ovine babesiosis (Uilenberg, 2006; Sevinc *et al.*, 2013). Other symptoms such as lethargy, anorexia, vomiting, and diarrhea (Krause *et al.*, 1996) are due to increased levels of bilirubin or hyperbilirubinemia (Rosenfeld and Dial, 2010). The total bilirubin concentration (TBIC) increases following intemperate erythrolysis and hepatic injury, but when these oddities occur together, the concentration increases dramatically (Sevinc *et al.*, 2013). If the conversion of Hb to bilirubin exceeds the ability of the liver to conjugate and excrete bilirubin, then the total bilirubin level becomes elevated in the blood (Turgut, 2000). Therefore, in this study, the elevated TBIC was most likely due to the hemolytic breakdown of RBCs. Present findings of hyperbilirubinemia are consistent with those of Rahbari *et al.* (2008) and Sevinc *et al.* (2013) who reported a significant increase in bilirubin associated with ovine babesiosis. Results on babesiosis in goats (Sulaiman *et al.*, 2010), cattle (Yeruham *et al.*, 2003; Alam and Nasr, 2011; Kaur, 2014), and camels (Swelum *et al.*, 2014) are similar to the present study.

Conclusion

Babesia ovis infection induced leukopenia with monocytosis, anemia with hypochromic macrocytosis, thrombocytopenia, and azotemia with hyperbilirubinemia. Although asymptomatic, the altered blood parameters reduce vitality and lead to a decrease in animal production, and therefore, an increase in economic losses. Current data will provide insight into the pathogenicity of subclinical infections.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

MS designed this study, collected samples and epidemiological data, performed the molecular and microscopic diagnosis, and performed statistical analysis. MS and MBS wrote the manuscript and SAHN, MR, UUDU, NN, and AK edited it. MBS finalized the manuscript and all the authors approved the final version.

Data availability

The data that support the findings of this study are available from the corresponding author upon request.

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