Research article

Evaluating serum electrolyte and trace element variations between Babesia ovis-infected and uninfected Lohi sheep

Muhammad Sajid1*, Syed A. H. Naqvi2, Muhammad W. R. Marral3 and Mourad Ben Said4,5

1 Zoology Division, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan 60800, Pakistan
2 Department of Plant Pathology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan
3 Department of Soil Sciences, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan
4 Department of Basic Sciences, Higher Institute of Biotechnology of Sidi Thabet, University of Manouba, Manouba 2010, Tunisia
5 Laboratory of Microbiology, National School of Veterinary Medicine of Sidi Thabet, University of Manouba, Manouba 2010, Tunisia

Abstract

Babesiosis caused by Babesia ovis is a major threat to the livestock industry worldwide. In Pakistan, the Lohi sheep breed is an important economic resource, but limited information is available on the impact of B. ovis infection on this breed. This study aimed to investigate the serum electrolyte and trace element variations between B. ovis-infected and uninfected Lohi sheep from Pakistan. A total of 97 Lohi sheep was stratified based on the geographical distribution of Multan district, employing a multistage cluster sampling method. Blood and serum samples were collected from randomly selected sheep, and DNA extraction and PCR amplification were performed to detect B. ovis infection. Serum electrolyte and trace element levels were analyzed in infected (n=67) and uninfected (n=30) groups, stratified by age and gender. The study revealed a 69.07% overall infection rate of B. ovis in Lohi sheep. Infected sheep showed significantly elevated serum copper levels (p<0.0001), with no substantial differences observed in serum iron, sodium, potassium, and chloride levels. However, age-wise variance analysis revealed statistically significant variations in sodium and potassium levels (p<0.0001 and p<0.05, respectively). Notably, serum chloride levels differed between infected and uninfected females in individuals younger than two years (p<0.05). Serum iron levels remained consistent across different age groups. Comparative analysis indicated a higher prevalence of electrolyte imbalances, such as hyponatremia, hyperkalemia, hyperchloremia, and hypoferremia, in infected sheep compared to normal reference ranges, while instances of hypernatremia, hypokalemia, hypochloremia, and hyperferremia were less frequent. In conclusion, our study suggests that B. ovis infections could lead to alterations in serum electrolyte and trace element levels in Lohi sheep, emphasizing the importance of further research into the specific mechanisms driving these alterations to enhance disease management strategies tailored to this breed.

Keywords: Babesia ovis, Molecular identification, Serum electrolytes, Trace elements, Sheep infection, Pakistan


Introduction

Electrolytes, including Sodium (Na+), Potassium (K+), and Chloride (Cl-), are vital for upholding the body’s normal physiological functions and are routinely assessed to gauge an individual’s clinical status (Tella, 2005). Understanding the variations in the levels of these electrolytes in sheep affected by babesiosis is crucial for the management of this disease. Indeed, these variations can have important implications for the development of targeted diagnostic and therapeutic strategies. These electrolytes play essential physiological roles (Berend et al., 2012). Na+ is indispensable for maintaining the membrane potential required for nerve impulse transmission and muscle function. K+ is the primary cytoplasmic cation, influencing acid-base balance and water homeostasis. Cl- is a key anion responsible for preserving electroneutrality in bodily fluids (Berend et al., 2012).
Copper, an indispensable mineral distributed across all body tissues, serves dual roles as an antioxidant or pro-oxidant, playing a crucial role in the optimal functioning of numerous vital enzymes (Osredkar and Sustar, 2011). Iron, a key structural component of hemoglobin, directly contributes to erythropoiesis, while other elements play indirect yet essential roles in the interaction, metabolism, and utilization of iron (Mullally et al., 2004). Zinc, copper, and selenium are integral components of the antioxidant defense system, guarding against free radical-induced tissue damage (Evans and Halliwell, 2001). Copper, functioning within pivotal enzymes, is ubiquitously present in nearly all body cells, emphasizing the necessity of assessing its status for proactive health measures and targeted disease treatments (Angelova et al., 2011).

Babesiosis, caused by Babesia protozoa, is a widespread and economically significant disease affecting various regions worldwide, including Pakistan. It is a tick-borne parasitic infection that can have severe consequences for animal health and productivity. In small ruminants, babesiosis can lead to anemia, fever, hemoglobinuria, and even death if left untreated (Khan et al., 2022). However, the current understanding of the epidemiology and impact of babesiosis in small ruminant populations in Pakistan remains limited. Relatively few studies are available in the literature regarding babesiosis in small ruminants from this region. The reported range of prevalence of babesiosis is from 7 to 58.5% in sheep and 7 to 23% in goats (Khan et al., 2022).

The severity of babesiosis is contingent upon the specific Babesia species involved (Schoeman, 2009). Babesia ovis stands out as the primary culprit behind ovine babesiosis (Ranjar-Bahadori et al., 2012). Acid-base disturbances and fluctuations in monovalent ions, such as Na⁺, K⁺, and Cl⁻, have been highlighted by Leisewitz et al. (2001) as significant factors in the serum of Babesia-affected dogs. Micronutrients, including iron and copper, play crucial roles in inflammatory and immune responses, both of which are activated by infection, and changes in their levels may contribute to the diverse presentations of the disease (Evans and Halliwell, 2001).

Numerous investigations have documented possible hematological and biochemical aberrations associated with B. ovis infection in sheep (Yeruham et al., 1998; Marco et al., 2000; Rahbari et al., 2008; Esmaeilnejad et al., 2012; Sevinc et al., 2013). These studies have provided valuable insights into the impact of B. ovis on various physiological parameters in different sheep breeds. Despite the wealth of existing research, a critical gap persists in the literature concerning the specific blood electrolyte changes and serum copper chemistry associated with babesial infection, particularly in the context of Lohi sheep. This breed, prevalent in Pakistan, represents a distinct population with unique physiological characteristics. Understanding the electrolyte dynamics and trace element alterations in Lohi sheep infected with B. ovis is imperative for a better comprehension of the pathophysiological consequences of babesiosis in this specific breed.

Against this backdrop, the primary objective of our study was to fill this gap by systematically evaluating the possible alterations in key electrolytes (Na⁺, K⁺, and Cl⁻) as well as the levels of Fe²⁺ and Cu²⁺ in the serum of Lohi sheep infected with B. ovis. By addressing this specific knowledge gap, our research aims to contribute not only to the broader understanding of babesial infections in sheep but also to provide breed-specific insights that can inform targeted diagnostic and therapeutic strategies for Lohi sheep, thereby enhancing the overall management and health outcomes in the context of babesiosis.

Materials and methods

Study area

The study was conducted in the district Multan, situated in the southern region of the Punjab province (Figure 1). District Multan is characterized by an arid climate with temperatures ranging from 18.5 to 31.4 °C and minimal precipitation, averaging between 75 and 200 mm (Syed et al., 2021). It comprises six distinct towns: Shah Rukn-e-Alam, Sher Shah, Jalalpur Pirwala, and Mousa Pak town (Figure 1).

With approximately 2.28% of the country’s population, district Multan is predominantly rural, with 56.62% of its residents engaged in agriculture. Livestock plays a pivotal role in sustaining the local population, providing essential resources such as milk, food, and cash income (Sheikh et al., 2020). In alignment with crop husbandry practices, sheep rearing in the
region primarily relies on grazing and the utilization of crop residues. Supplementary feed, including pods, dry leaves, grass, and shrubs, is provided as available, often managed by employed shepherds overseeing the flocks (Ahmad et al., 2001).

The natural flora in the study area serves as a significant source of nutrition for the animals, encompassing perennial grasses such as Deb (Desmostachya spp.) and Indian doab (Cynodon spp.), as well as trees like Jand (Prosopis spp.), Frash (Tamarix spp.), and Kikar (Acacia arabica). Additionally, cultivated trees like Shisham (Dilbergia spp.), Mango (Mangifera sp.), and Sirin (Albicia spp.), along with seasonal crops like wheat, rice, and cotton, contribute to the diverse natural flora of the study area (Lashari and Tasawar, 2013). The agro-mineral resources in the region include alum, rock phosphate, gypsum, and anhydrite deposits; however, as of the latest information, active mining activities have yet to be reported in the study area (Malkani et al., 2017). This detailed overview of the study area sets the contextual stage for understanding the local environment and its relevance to the investigation of B. ovis infection in Lohi sheep.

Animal sampling

To ensure comprehensive representation, the study population was stratified based on the geographical distribution of Multan district, categorizing the six towns as distinct strata or clusters. Within these clusters, herds were identified as secondary units, and from these herds, individual sheep constituted the final units. For employing a simple random sampling technique at the second and third stages, a multistage cluster sampling method was adopted to eliminate bias and ensure a representative sample, thereby avoiding a specific farm-centric study (Thrufield, 2005).

A total of 97 Lohi sheep (85 females and 12 males) were randomly selected for blood and serum sample collection from four towns within Multan district: Shah Rukn-e-Alam (28 females and five males), Sher Shah (18 females and three males), Jalalpur Pirwala (20 females and 0 males), and Mousa Pak (19 females and four males). This selection of locations aimed to ensure a diverse geographic representation of the Lohi sheep population within the Multan district.

The selected animals were further categorized into three age groups: 39 individuals younger than one year (age group-1), 30 individuals younger than two years (age group-2), and 28 individuals older than two years (age group-3). This age-based stratification was chosen to reflect the different age classes encountered in the local ovine population and allow for the potential evaluation of age-related influences on the studied parameters. The overrepresentation of females in the sample is reflective of the typical demographic structure of sheep herds in this region, where females generally constitute the majority of the livestock.

Sample collection

Each time, 2 ml out of 4 ml of blood drawn from the jugular vein of the sheep was collected in EDTA-containing vacutainers (blood collection tubes), while the remaining 2 ml in “serum separator tubes BD SST™ gel tubes, commonly called “gel vials” specially made for serum collection. Vacutainers were inverted gently 5-6 times to thoroughly mix the blood with anticoagulant. while The gel vials after inverting 5-6 times to activate "clot activator" were kept standing for 30 minutes in order to give time for clotting of blood and separation of serum. The serum was kept in an ice box. All samples (whole blood and sera) were transferred to the well-equipped Parasitology Laboratory at the Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan.

Determination of infected and uninfected animals

The process of DNA extraction and PCR amplification was carried out as follows: First, 200μl of whole blood samples were extracted for DNA using the Favor Prep Blood Genomic DNA Extraction Mini Kit from Favorgen Biotech Corp. based on the manufacturer’s instructions. The extracted DNA was eluted in a final volume of 100μL and stored at -20°C until use. B. ovis infection was screened in all samples with polymerase chain reaction (PCR) amplification of fragment (549 bp) of the ssu rRNA sequence specific for B. ovis by using specific primers BboF (5’-TGGGCAGGACCCTGTTCTTCTT-3′) and BboR (5’-CCCGTATGCGCCGTAAATA-3′), as described by Aktas et al. (2005). The PCR reaction was performed in a final volume of 25μL containing 12.5μL of Green Master Mix® (Promega, Madison, WI, USA), 1.0μL of 10μM
primer mix (BboF + BboR), 0.5µL of 1M Betaine and 5µL of template DNA solution. Green Master Mix® is a premixed ready-to-use solution containing DNA polymerase along with dNTPs and MgCl2 in a 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 100bp DNA Ladder (Fermentas).

Serology for metals

Both infection-positive and negative samples were analyzed for Sodium, Potassium, Chloride, Copper, and Iron in order to compare them. Serum sodium concentration was measured with a colorimetric method using “vitro Sodium reagent” intended for both automatic and manual systems from Vitro-Scient™, Cairo, Egypt, following the manufacturer’s protocol. Serum K⁺ concentration was measured using the Tetraphenylborate method (without deproteinization) with “vitro potassium reagent” intended for both automatic and manual systems from Vitro-Scient™, Cairo, Egypt, according to the manufacturer's protocol. Serum chloride concentration was measured with a colorimetric method using “Chloride fluid Monoreagent” Order # CF07R05050 from Centronic GmbH, Wartenberg/Germany, according to the manufacturer's protocol. Serum copper concentration was measured manually using a Copper kit (cat. # CU-2340) from Randox, UK, that is intended for in vitro quantitative determination of Cu²⁺ in Serum and plasma. Serum iron concentration was measured manually using an Iron liquid color kit (Ref. # 10229) from HUMAN, Wiesbaden, Germany, that uses the CAB method involving a photometric, colorimetric test for iron with LCF (lipid clearing factor).

Statistical analysis

The statistical analysis was carried out via paired t-test by comparing the mean values among different groups of sheep, utilizing Minitab® version 15.1.30.0 for the analysis. The groups were segregated based on gender and age categories, making a distinction between those infected and those uninfected. The following groups underwent comparative analysis. Firstly, a comparison was made between all infected animals, encompassing both males and females and all uninfected animals of both genders. Moving forward, specific evaluations involved comparing all infected males to all infected females within the age group 1 category, considering that all the male subjects within this study were one year old or younger and infected. Additionally, an assessment was conducted by contrasting all infected females with all uninfected females without regard to age. To delve deeper, further distinctions were drawn within the infected female group: a comparison between infected females and uninfected females within the age group 1 (equal to or less than one year), another comparison within the age group 2 (more than one year but less than or equal to two years), and finally, a comparison within the age group 3 (more than two years).

Ethical approval

All procedures involving animal handling, blood and serum collection, and analysis were carried out according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) at Bahauddin Zakariya University in Multan, Pakistan. The protocol was approved by the Ethical Committee of the Institute of Pure and Applied Biology at Bahauddin Zakariya University in Multan, Pakistan.

Results

In this study, 97 Lohi sheep were tested for B. ovis infection through PCR, with an overall infection rate of 69.07% (67/97). Babesia-infected (n=67) and uninfected (n=30) sheep, grouped by age and gender, were selected for comparison of serum electrolytes and trace elements. No significant differences were found in the levels of sodium, potassium, chloride, and iron between B. ovis-infected and uninfected sheep groups, as demonstrated by Table 1. However, the difference in serum copper levels was highly significant (p<0.0001). Further subgroup analyses were conducted by dividing the infected and uninfected groups based on age and gender to compare their differences. However, Fisher’s LSD test indicates some significant differences in different groups, demonstrated in Tables 2 and 3.

Comparison of serum sodium concentration

The comparison of serum Na⁺ concentrations between B. ovis-infected and uninfected sheep groups did not show a significant difference. The
Table 1: Comparison of serum electrolytes and trace elements between *Babesia ovis*-infected and uninfected sheep.

<table>
<thead>
<tr>
<th>Electrolytes and trace elements</th>
<th>Infection status</th>
<th>Means ± SD.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (Na⁺)</td>
<td>Infected</td>
<td>127.30±6.865 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>129.80±5.552 mmol/L</td>
</tr>
<tr>
<td></td>
<td><em>p</em>-value</td>
<td>0.093</td>
</tr>
<tr>
<td>Potassium (K⁺)</td>
<td>Infected</td>
<td>5.21±0.717 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>5.17±0.662 mmol/L</td>
</tr>
<tr>
<td></td>
<td><em>p</em>-value</td>
<td>0.795</td>
</tr>
<tr>
<td>Chloride (Cl⁻)</td>
<td>Infected</td>
<td>100.38±6.83 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>101.83±4.98 mmol/L</td>
</tr>
<tr>
<td></td>
<td><em>p</em>-value</td>
<td>0.227</td>
</tr>
<tr>
<td>Copper (Cu²⁺)</td>
<td>Infected</td>
<td>26.70±8.604 µmol/L</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>50.60±13.329 µmol/L</td>
</tr>
<tr>
<td></td>
<td><em>p</em>-value</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Iron (Fe²⁺)</td>
<td>Infected</td>
<td>32.68±9.21 µmol/L</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>34.80±9.21 µmol/L</td>
</tr>
<tr>
<td></td>
<td><em>p</em>-value</td>
<td>0.299</td>
</tr>
</tbody>
</table>

mean values of both groups were lower than the normal reference range described in the literature. In *Babesia*-infected sheep, there was no significant variation in mean serum Na⁺ concentration compared to uninfected sheep (Table 1). However, there was a higher incidence of hyponatremia (as low as 112.74 mmol/L) and hypernatremia (as high as 146.34 mmol/L) in *B. ovis*-infected sheep, with a statistically significant (*p*<0.004) incidence of hyponatremia observed in infected females when compared with uninfected females (Table 2).

Comparison of serum potassium concentration

Comparison of serum K⁺ concentrations between *Babesia*-infected and uninfected (normal) sheep groups varied non-significantly (Table 1). The means of both groups (5.21±0.717 mmol/L in *Babesia* infected and 5.17±0.662 mmol/L in normal) lie within the reference range as described by Radostits et al. (2007). Nevertheless, the number of infected sheep showing hyperkalemia 24/67 was significantly higher than sheep showing hypokalaemia, i.e., 2/67. Among subgroup comparisons, significant (*p*<0.05) hypokalaemia was shown by infected and uninfected females of age group 1 (Table 2).

Comparison of serum chloride concentration

Comparison of serum Cl⁻ concentrations between *B. ovis*-infected and uninfected (normal) sheep groups varied non-significantly (Table 1). The means of both groups (100.3±6.829 mmol/L in *B. ovis*-infected and 101.8±4.975 mmol/L in normal) lie within the reference range as described by Radostits et al. (2007). Still, the number of infected sheep showing hyperchloraemia 21/67 was higher than sheep showing hypochloraemia, i.e. 15/67. It is noteworthy that if present values are compared to those described as reference values by Merck (2012), the number of sheep showing hypochloraemia becomes significantly higher (40/67) than hyperchloraemic sheep (3/67). Among subgroup comparisons, significant (*p*<0.05) hypochloraemia was shown by infected and uninfected females of age group 2 (Table 2).

Comparison of serum copper concentration

The serum Cu²⁺ concentrations were significantly lower (*p*<0.0001) in the *B. ovis*-infected sheep group (26.7±8.6 µmol/L) compared to the normal group (50.6±13.3 µmol/L), indicating a remarkable hypocupremia in the infected group (Table 1). All subgroup comparisons also indicate significant hypocupremia in infected vs uninfected animals (Table 3).

Comparison of serum iron concentration

The comparison of serum iron (Fe) concentrations between *B. ovis*-infected and uninfected (normal) sheep groups varied non-significantly with a *p*-value of 0.299 (Table 1). The mean±SD of both groups was 32.68±9.21 µmol/L in *B. ovis* infected and 34.80±9.20 µmol/L in normal. Analysis of variance between different groups (based on gender and age) was also statistically non-significant, with a *p*-value of 0.583 (Table 3). The means of both infected and uninfected groups align within the normal range of serum iron levels as described by Radostits et al. (2007) and Kaneko et al. (2012). However, 40.3% (27/67) of
Table 2: Comparison of serum electrolytes Sodium (Na⁺), Potassium (K⁺), and Chloride (Cl⁻) between different *Babesia ovis*-infected and uninfected sheep groups.

<table>
<thead>
<tr>
<th>Groups based on gender/age</th>
<th>Infection status (number)</th>
<th>Na⁺ (mmol/L)</th>
<th>K⁺ mmol/L</th>
<th>Cl⁻ mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group 1</td>
<td>Infected (29)</td>
<td>131.317±6.25bc</td>
<td>5.006±0.739bc</td>
<td>100.10±5.93bc</td>
</tr>
<tr>
<td></td>
<td>Uninfected (10)</td>
<td>133.707±8.49ab</td>
<td>5.263±0.670ab</td>
<td>100.70±4.76ab</td>
</tr>
<tr>
<td>All males</td>
<td>Infected (12)</td>
<td>135.69±5.353a</td>
<td>5.36±0.636a</td>
<td>98.65±6.00a</td>
</tr>
<tr>
<td></td>
<td>Uninfected (00)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All females</td>
<td>Infected (55)</td>
<td>125.478±5.720ef</td>
<td>5.144±0.720ab</td>
<td>100.76±6.99ab</td>
</tr>
<tr>
<td></td>
<td>Uninfected (30)</td>
<td>129.805±6.552bc</td>
<td>5.263±0.670ab</td>
<td>101.83±4.98ab</td>
</tr>
</tbody>
</table>

Female groups based on age

| Age group 1               | Infected (17)             | 128.228±4.921de | 5.36±0.636a  | 101.13±8.66ab |
|                           | Uninfected (10)           | 133.707±8.49ab | 5.263±0.670ab | 100.70±4.76ab |
| Age group 2               | Infected (20)             | 122.939±4.646c | 5.294±0.713ab | 101.11±6.50ab |
|                           | Uninfected (10)           | 126.10±3.73def | 4.94±0.720bc  | 104.80±3.89a  |
| Age group 3               | Infected (18)             | 125.700±6.468f | 5.459±0.619a  | 101.13±8.66ab |
|                           | Uninfected (10)           | 129.609±4.484cde | 5.366±0.555ab | 99.99±5.20a  |

Abbreviations: * significant value, † value calculated using Age group 1 (only females) uninfected. Notes: Means (in the same column) that do not share a superscript are significantly different according to Fisher’s LSD test.

Table 3: Comparison of serum trace elements Copper (Cu++) and Iron (Fe++) between different *Babesia ovis*-infected and uninfected sheep groups.

<table>
<thead>
<tr>
<th>Groups based on Gender/age</th>
<th>Infection status (number)</th>
<th>Copper (Cu++)</th>
<th>p-value</th>
<th>Iron (Fe++)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group 1</td>
<td>Infected (29)</td>
<td>28.908±10.80</td>
<td>&lt;0.0001*</td>
<td>33.702±10.70</td>
<td>0.209</td>
</tr>
<tr>
<td></td>
<td>Uninfected (10)</td>
<td>45.055±6.34a</td>
<td></td>
<td>39.180±11.56</td>
<td></td>
</tr>
<tr>
<td>All males</td>
<td>Infected (12)</td>
<td>32.122±10.223b</td>
<td>0.002†</td>
<td>35.832±13.94</td>
<td>0.545†</td>
</tr>
<tr>
<td></td>
<td>Uninfected (00)</td>
<td>50.604±13.329a</td>
<td></td>
<td>34.809±9.21</td>
<td></td>
</tr>
<tr>
<td>All females</td>
<td>Infected (55)</td>
<td>25.527±7.826d</td>
<td>&lt;0.0001*</td>
<td>32.004±7.84</td>
<td>0.164</td>
</tr>
<tr>
<td></td>
<td>Uninfected (30)</td>
<td>50.804±13.329a</td>
<td></td>
<td>34.809±9.21</td>
<td></td>
</tr>
<tr>
<td>Female groups based on age</td>
<td>Infected (17)</td>
<td>26.639±10.906bc</td>
<td>&lt;0.0001*</td>
<td>32.198±7.80</td>
<td>0.114</td>
</tr>
<tr>
<td></td>
<td>Uninfected (10)</td>
<td>45.055±6.339a</td>
<td></td>
<td>39.180±11.56</td>
<td></td>
</tr>
<tr>
<td>Age group 2</td>
<td>Infected (20)</td>
<td>23.033±4.165d</td>
<td>&lt;0.0001*</td>
<td>32.055±7.08</td>
<td>0.841</td>
</tr>
<tr>
<td></td>
<td>Uninfected (10)</td>
<td>53.882±15.159a</td>
<td></td>
<td>32.577±6.42</td>
<td></td>
</tr>
<tr>
<td>Age group 3</td>
<td>Infected (18)</td>
<td>27.249±7.178bcd</td>
<td>&lt;0.001*</td>
<td>31.763±9.04</td>
<td>0.789</td>
</tr>
<tr>
<td></td>
<td>Uninfected (10)</td>
<td>52.875±15.830a</td>
<td></td>
<td>32.671±8.15</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: * significant value, † value calculated using Age group -1 (only females) uninfected. Notes: Means (in the same column) that do not share a superscript are significantly different according to Fisher’s LSD test. Conc. units are expressed in mmole/L.
the infected group showed low serum Fe with a minimum of 12.21µmol/L than the normal range, while 20.9% (14/67) showed high serum Fe with a maximum of 68.24µmol/L.

**Discussion**

Babesiosis is a tick-borne hemolytic disease caused by protozoan parasites of the genus *Babesia*. The disease affects a wide range of domestic and wild animals, including sheep, cattle, horses, dogs, and cats (Hunfeld et al., 2008). The clinical manifestations of babesiosis are variable and can range from mild to severe, depending on the host's immune status, the *Babesia* species involved, and other factors (Sevinc et al., 2013). One of the common laboratory findings in babesiosis is hyponatremia, a condition characterized by low serum sodium concentrations (Sharma et al., 2016; Narukar et al., 2017). This study aims to investigate, in part, the serum sodium concentrations in *B. ovis*-infected and uninfected sheep and evaluate the potential role of hyponatremia in the pathogenesis of babesiosis. In this study, the comparison of serum Na⁺ concentrations between *B. ovis*-infected and uninfected sheep groups did not show any significant variation. However, the mean of both groups showed hyponatremia when compared with the normal reference range described by Radostits et al. (2007). Nonetheless, it is important to consider that there may be great variations in the hematological and biochemical parameters between different breeds of small ruminants, and the formulation of a universal metabolic profile can be difficult.

The hyponatremia found in infected cases, with 18/67 cases exhibiting low serum Na⁺ concentrations (as low as 112.74mmol/L), and hypernatremia observed in 6/67 infected cases (as high as 146.34mmol/L), suggests that *B. ovis* infection is three times more likely to cause hyponatremia development. Statistically significant hyponatremia was observed during sub-group comparison in the infected female group (n=55) when compared to uninfected females (n=30). This observation is consistent with findings reported by Schoeman et al. (2001) in feline babesiosis and later by Adaszek et al. (2012) in dogs with babesiosis, which also showed both hyponatremia and hypernatremia. The most probable explanation for the hyponatremia seen in at least infected females in this study is suspected renal failure, a common clinical manifestation in babesiosis. Water retention connected with renal retention of sodium results in increased plasma levels in *Babesia*-infected hosts (Schetters et al., 1998), which in turn poses an added strain on myocardial function, causing cardiac lesions, which are common in canine babesiosis. Esmaeilinejad et al. (2012) reported significant hyperproteinaemia while studying naturally infected small ruminants with *B. ovis*, which can artefactually decrease serum sodium concentrations and lead to
pseudohyponatraemia. Most of the cats with hyponatremia studied by Schoeman et al. (2001) also had concurrent hyperproteinemia, as Na+ is contained in the aqueous fraction of serum, and hyperproteinemia results in a decreased proportion of the aqueous phase with a consequently decreased Na+ concentration. In contrast, Takeet et al. (2009) reported a significant increase, i.e., hypernatremia, in two stallions naturally infected with Theileria equi. Therefore, due to the small sample size and potential for confounding variables, further research is needed to confirm these findings and investigate the underlying mechanisms.

The present study also investigated serum potassium concentrations in Babesia-infected and uninfected sheep groups. Results showed that there was no significant difference in serum K+ concentrations between the two groups, with means lying within the reference range. However, the number of infected sheep showing hyperkalemia was significantly higher than those showing hypokalemia. Erythrolysis may be the major factor leading to hyperkalemia, consistent with findings in human victims of malarial parasites. The hyperkalemic tendency in ovine babesiosis seen in the present study is strongly supported by findings reported by Tella (2005), who observed a higher level of serum potassium in sheep with single and concurrent B. ovis and Trypanosome congolense infections. Moreover, biochemical abnormalities seen in this study, such as a trend toward hyponatremia and hyperkalemia, can be related to both the severity of the disease and the degree of hypoxia.

The comparison of our findings with previous studies showed contrasting results. Schoeman et al. (2001) observed a higher frequency of hyperkalemia in cats with babesiosis, while Lobetti and Jacobson (2001) observed a higher frequency of hypokalemia in dogs with babesiosis. This discrepancy may stem from variations in erythrocyte potassium (K+) levels between species, with canine erythrocytes exhibiting considerably lower K+ concentrations compared to humans. Additionally, signs of mineral disparity depend on the degree and duration of exposure along with animal variables such as species, age, and sex. Among subgroup comparisons, significant hypokalemia was shown by infected females of age group 1 compared with uninfected AG-1. This is consistent with most of the formerly described cases of canine babesiosis showing hypokalemia.

Furthermore, hypokalemia has been associated with reduced K+ intake and respiratory alkalosis in malaria, both of which can take place in babesiosis. In summary, the present study suggests that erythrocytes may be the major factor leading to hyperkalemia in ovine babesiosis. However, further studies are needed to investigate the underlying mechanisms of mineral disparity in babesiosis and its association with the severity of the disease.

The comparison of serum chloride concentrations between Babesia-infected and uninfected (normal) sheep groups showed no significant difference. The means of both groups were within the reference range described by Radostits et al. (2007), with 100.3±6.829 mmol/L in Babesia infected and 101.8±4.975 mmol/L in normal sheep. However, it is noteworthy that the number of infected sheep showing hyperchloreaemia (21/67) was higher than those showing hypochloreaemia (15/67). If these values are compared to those described as reference values by Merck (2012), the number of sheep showing hypochloreaemia becomes significantly higher (40/67) than hyperchloreaemic sheep (3/67). It is interesting to note that other studies have reported different effects of Babesia infections on chloride levels in animals. For example, Reyers et al. (1998), Tella (2005), and Adaszek et al. (2012) concluded that Babesia infections lead to hypochloreaemia in sheep and dogs, respectively. In contrast, Takeet et al. (2009) found no change in Cl− level in equine piroplasmic infections, while Lobetti (2000) and Konto et al. (2014) found hyperchloraemia in dogs infected with Babesia.

Chlorine is a critical component in maintaining acid-base balance in extracellular fluid, as it makes up over 60% of anions in this compartment. However, its concentration is prone to more variation than that of Na+ due to the ability of other anions, particularly bicarbonates, to barter for chloride (Soetan et al., 2010). Anemia and resulting hypoxemia are characteristic features of babesiosis (Leisewitz et al., 2001), which can lead to anaerobic production of lactate (Button, 1976). Loss of hemoglobin severely jeopardizes blood buffering capacity, making it less able to deal with the metabolic acidosis that is the most commonly reported acid–base disturbance in dogs with babesiosis and severe anemia. Respiratory
alkalosis occurs as frequently as metabolic acidosis (Lobetti, 2000). Stewart’s Strong Ion Difference (SID) theory differs from the classical acid-base interpretation and has been utilized in both human and veterinary medicine (de Morais, 1992; Fencle and Leith, 1993; Whitehair et al., 1995; Russell et al., 1996). Increased SID is associated with metabolic alkalosis, and decreased SID is associated with metabolic acidosis (George and Zabolotzky, 2011). Four factors affect SID, including free water (H₂O), chloride ion (Cl⁻), protein concentration (specifically albumins), and strong anions. Because of the relatively high concentrations of Na⁺ and Cl⁻ in serum, these are the primary ions affecting SID. However, protein concentration change must be considered to fully evaluate acid-base abnormalities, which were not determined in this study. The frequent combination of hyponatremia and hyperchloremia is suggestive of non-respiratory (metabolic) acidosis (Leisewitz et al., 2001). A decrease in pH in the case of metabolic acidosis results in an extracellular shift of K⁺ as a result of an intracellular shift of H⁺, causing hyperkalemia (Zull 1989). This finding supports the present findings of high potassium levels in the serum of B. ovis-infected sheep.

The present study aimed to evaluate serum copper concentrations in Babesia-infected sheep and compare them with those of uninfected (normal) sheep groups. The results showed a highly significant difference between the two groups, with a p-value of <0.0001, indicating a remarkable hypocupremia in the infected group. This finding is in line with previous studies by Omer et al. (2003), Bicek et al. (2005), Dua et al. (2012), and Chaudhuri et al. (2008), who also reported a drop in Cu²⁺ levels in animals infected with Babesia or Theileria. The possible causes of hypocupremia in B. ovis-infected sheep were discussed in the present study. One possible reason for hypocupremia is increased renal losses or increased utilization of copper for the synthesis of Zn-Cu superoxide dismutase to fight oxidative stress associated with the disease (Asri Rezaei and Dalir-Naghadeh, 2006). Another reason for hypocupremia is the lack of copper, which leads to anemia, as Cu²⁺ is required for ceruloplasmin, which loads iron onto transferrin (Sukalski et al., 1997). Hypochromic anemia, which was found in the present study, is presumably the result of Cu²⁺ deficiency. Conflicting results have been reported by Kozat et al. (2003) in natural infection of sheep with B. ovis. Voyvoda et al. (1997) found positive reciprocity between Cu²⁺ and body temperature in ovine babesiosis. Likewise, Pandey and Misra (1987) recorded high Cu²⁺ levels in cattle before treatment of babesiosis, which declined after treatment. In addition, Hussein et al. (2007) reported an increased level of serum Cu²⁺ in cattle infected with Babesia. The increased serum levels of Cu²⁺ in babesiosis could be attributed to hemolysis associated with Babesia infection (Kozat et al., 2003; Hussein et al., 2007).

The present study also discussed the role of copper in the body’s antioxidant defense and its essential role in the deterrence of free-radical-induced damage to tissues (Evans and Halliwell, 2001). Babesiosis may influence erythrocyte lipid peroxidation and erythrocytic antioxidant defense, as Babesia produces oxidative stress and lipid peroxidation by destructing erythrocytes, which in turn leads to decreased activity of antioxidant systems (Bicek et al., 2005). Affected animals generally have microcytic hypochromic anemia (Weiss, 2010). Esmaeilnejad et al. (2014) also reported a decline in serum Cu²⁺ concentration in sheep naturally infected with B. ovis, which is in line with the present findings of hypocupremia in naturally infected sheep with B. ovis. Low plasma or serum Cu²⁺ concentrations are always the result of sharp Cu²⁺ deficiency in both humans and animals (Milne, 1994). However, plasma or serum Cu²⁺ concentration, which is the most commonly measured indicator of copper status, is the least reliable except perhaps in cases of severe Cu-deficiency (Milne, 1998).

In conclusion, the present study showed that B. ovis-infected sheep have significantly lower serum copper concentrations than uninfected (normal) sheep groups, indicating a remarkable hypocupremia in the infected group. The possible causes of hypocupremia were discussed, including increased renal losses or increased utilization of copper for the synthesis of Zn-Cu superoxide dismutase. It is important to note that the results of this study on serum copper concentrations in B. ovis-infected sheep are in agreement with previous studies on mineral changes in ovine babesiosis and Theileria-infected cattle. The study also highlights the importance of copper in the body’s antioxidant defense and its essential role in preventing
oxidative damage to tissues. Conflicting results have been reported in previous studies on serum copper levels in *B. ovis*-infected animals, with some studies reporting increased levels of copper and others reporting decreased levels. However, it is speculated that the increase in serum copper levels in some cases may be due to intravascular hemolysis associated with *B. ovis* infection.

The comparison of serum iron (Fe) concentrations between *B. ovis* infected and uninfected (normal) sheep groups varied non-significantly with a *p*-value of 0.299, with a mean ± SD of 32.68±9.21 µmol/L in *B. ovis* infected and 34.80±9.20 µmol/L in the normal group. Analysis of variance between different groups (based on gender and age) was also statistically non-significant, with a *p*-value of 0.583. The means of both infected and uninfected groups fall into the normal range of serum iron described by Radostits et al. (2007) and Kaneko et al. (2012). Nevertheless, within the infected group, 40.3% (27/67) exhibited below-normal serum iron levels, reaching a minimum of 12.21 µmol/L, while 20.9% (14/67) demonstrated elevated serum iron levels, peaking at 68.24 µmol/L. The status of serum Fe in babesiosis is a matter of argument, and there are different assumptions on this subject. Hypoferremia, or lower levels of serum iron than normal, have been found in sheep (Bicek et al., 2005), dogs (Chaudhuri et al., 2008), cattle (Col and Uslu, 2007; Askar et al., 2008; Lotfollahzadeh et al., 2012), and in dromedary camels (Swelum et al., 2014) infected with different species of *Babesia*. Similar findings have been reported in calves (Kumar and Malik, 1999; Omer et al., 2003; Hussein et al., 2007), horses (Takeet et al., 2009; Salem and El-Sherif, 2015), and camels (Ismael et al., 2014) infected with various *Theileria* species. Opposite to this, elevated serum Fe has been reported in ovine (Kozat et al., 2003; Esmaeilnejad et al., 2014) and bovine babesiosis (Hussein et al., 2007; El-far et al., 2014). Likewise, studies on ovine (Nazifi et al., 2011) and bovine theileriosis (Watanabe et al., 1998; Razavi et al., 2011) revealed higher levels of serum iron in infected animals. Both hypo and hyper types of variations posed by *Babesia divergens* in serum Fe have also been reported by Jerichow and Junigmann (1969).

Numerous mechanisms have been proposed to explain the link between *Babesia* infection and changes in serum iron levels. One possibility is that hemolytic anemia with extravascular hemolysis occurs, in which abnormal red blood cells are detected and destroyed by macrophages in the liver and spleen, increasing serum iron levels (Stockham and Scott, 2002; Razavi et al., 2011). Alternatively, intravascular hemolysis may occur, in which parasitized red blood cells are directly ruptured inside the vasculature, leading to an increase in serum iron (Pandey and Misra, 1987; Kozat et al., 2003; Esmaeilnejad et al., 2014). Lipid peroxidation and reduced antioxidant potential of infected red blood cells may also contribute to an increase in serum iron levels (El-far et al., 2014). On the other hand, critical decreases in serum iron levels reported in other studies may be due to anemia and excess utilization of iron in regulating the hematopoietic process (Bicek et al., 2005; Hussein et al., 2007). Liver damage may also contribute to hypoferremia, as the liver’s inability to synthesize transferrin may lead to decreased serum iron levels (Burtis and Ashwood, 1996). Acute parasitic infections are also associated with decreased serum iron levels (De-Waal et al., 1987). Inflammation can alter serum and liver iron concentrations because the body both tries to limit the availability of iron to growing organisms and increases the availability of iron to the body’s immune cells. Therefore, the interpretation of the iron status should be made in the context of the overall health of the animal (Herdt and Hoff, 2011).

**Conclusion**

Our findings revealed distinct variations in serum electrolytes and trace elements among *B. ovis*-infected sheep, particularly in serum copper levels. While sodium, potassium, and chloride levels did not exhibit significant differences, the occurrence of hyponatremia in a subset of infected sheep warrants further investigation. This underscores the necessity for additional research to unravel the roles of copper and iron in *Babesia* pathogenesis, facilitating targeted strategies for reducing deficiencies in infected animals. Furthermore, our findings offer valuable perspectives for evaluating treatment approaches and enhancing our understanding of the underlying pathogenesis of babesiosis. These contributions align with ongoing efforts to develop more effective interventions against this disease.
Article Information

Funding. This research received no external funding.

Conflicts of Interest. The authors declare no conflict of interest.

Author contributions. Collection of samples and epidemiological data and performed experiments: M.S., statistical analysis: M.W.R.M., initial draft: S.A.H.N., critical review and writing the final version of the manuscript: M.B.S. All authors have read and agreed to the published version of the manuscript.

Acknowledgments. The authors would like to thank all veterinary practitioners present at the sampling sites in the Multan district for helping in the sample collection.

Publisher’s Note. The claims and data contained in this manuscript are solely those of the author(s) and do not represent those of the GMPC publisher, editors, or reviewers. GMPC publisher and the editors disclaim the responsibility for any injury to people or property resulting from the contents of this article.

References


Kozat, S., Yüksek, N., Altuğ, N., Āgaļū, Z.T., Erçin, F., 2003. Studies on the effect of iron (Fe) preparations in addition to Babesiosis treatment on the haematological and some


