# Molecular identification and phylogenetic analysis of *Babesia ovis* in sheep in Siirt, Türkiye: relationship with some oxidant/antioxidant parameters

Burçak Aslan Çelik[1a], Murat Kara[1b], Özgür Yaşar Çelik[2c], Kıvanç İrak[3d], Muhammed Ahmed Selçuk[1e], Kerem Ercan[2f], Adnan Ayan[4g]

[1]Siirt University, Faculty of Veterinary Medicine, Department of Parasitology, Siirt, Türkiye
[2]Siirt University, Faculty of Veterinary Medicine, Department of Internal Medicine, Siirt, Türkiye
[3]Siirt University, Faculty of Veterinary Medicine, Department of Biochemistry, Siirt, Türkiye
[4]Aksaray University, Faculty of Veterinary Medicine, Department of Parasitology, Aksaray, Türkiye

ORCIDs: [a]0000-0002-0130-970X, [b]0000-0002-4144-2409, [c]0000-0001-6365-2688, [d]0000-0001-9765-0330, [e]0000-0003-1769-4558, [f]0000-0003-4914-8578, [g]0000-0002-6564-3416

*Received October 12, 2023*
*Accepted December 12, 2024*

**Abstract**

The *Babesia* genus includes tick-borne haemoprotozoan parasites that infect a wide variety of vertebrate hosts, both domestic and wild, around the world. *Babesia* spp. cause oxidative stress by increasing the number of free radicals in erythrocytes. Among this genus, *Babesia ovis* causes babesiosis in sheep and goats, especially in tropical and subtropical regions, causing significant economic losses. In this study, we aimed to determine the presence and prevalence of *B. ovis* in sheep in Siirt province, Türkiye, using molecular method and to evaluate some oxidant/antioxidant parameters in infected sheep. The animal material used in this study consisted of a total of 500 sheep. DNA extraction, Polymerase Chain Reaction (PCR) amplification and sequence analysis of blood samples and Enzyme-Linked ImmunoSorbent Assay (ELISA) analysis of serum samples were performed. As a result of the study, 84 of the 500 samples (16.80%) examined were PCR positive. Advanced oxidation protein products (AOPP) and nitric oxide (NO) concentrations were found to be higher in sheep with babesiosis compared to the control group. Superoxide dismutase (SOD) activity was higher in the control group and catalase (CAT) activity was higher in infected sheep. As a result of this study, the presence of *Babesia ovis* was detected in all districts of Siirt province. It has been determined that infection caused by *Babesia ovis* in sheep causes oxidative stress as a result of increased nitric oxide and oxidized protein levels and this process may participate in the pathology of the disease.

*Ovine, haemoprotozoa, parasites, oxidative stress*

The *Babesia* genus includes tick-borne haemoprotozoan parasites that infect a wide variety of vertebrate hosts, both domestic and wild, around the world (Aktas et al. 2007; Hosein et al. 2007; Esmaeilnejad et al. 2015; Arwa and Kawan 2022; Ulucesme et al. 2023). Among this genus, *Babesia ovis* causes babesiosis in sheep and goats, especially in tropical and subtropical regions, causing significant economic losses (Emre et al. 2001; Razmi et al. 2002; Aktas et al. 2007; Hosein et al. 2007; Kose et al. 2022).

Babesiosis in sheep and goats is caused by at least three parasites, *Babesia ovis*, *Babesia motasi* and *Babesia crassa* (Ferrer et al. 1998; Altay et al. 2008; Tumwebaze et al. 2020).

*Babesia* sp. are transmitted both transovarially and transstadially by ixodid ticks (Saraylı et al. 2006; Gökpınar et al. 2021). The primary vectors for *Babesia ovis* and *B. motasi* include *Rhipicephalus bursa*, while additional transmission has been reported through *Rhipicephalus turanicus*, *Hyalomma anatolicum excavatum*, *Haemaphysalis punctata* as well as *Ixodes ricinus*. Notably, while several *Babesia* species and their vectors have been well-documented, the specific vector responsible for transmitting *Babesia crassa* remains

**Address for correspondence:**
Burçak Aslan Çelik
Department of Parasitology
Faculty of Veterinary Medicine
Siirt University, Siirt, Türkiye,

Phone: +905428277616,
E-mail: burcakaslan@siirt.edu.tr
http://actavet.vfu.cz/

unknown (Çakmak et al. 1991; Çiçek et al. 2004; Ulucesme et al. 2023). The disease caused by *B. motasi* can be acute and/or chronic (Razmi et al. 2002). The pathogenicity of this agent appears to be moderately virulent (Aktas et al. 2007; Altay et al. 2008). *Babesia crassa* is considered non-pathogenic for small ruminants (Razmi et al. 2002; Aktas et al. 2007; Altay et al. 2008). *Babesia ovis* is highly pathogenic especially in sheep and mortality rates vary between 30%–50% in the field (Aktas et al. 2007; Tumwebaze et al. 2020). Clinical signs of the disease include fever, anaemia, icterus, haemoglobinuria (Esmaeilnejad et al. 2015; Tumwebaze et al. 2020; Stevanović et al. 2022; Ulucesme et al. 2023) and death occurs in some cases (Esmaeilnejad et al. 2015; Arwa and Kawan 2022; Stevanović et al. 2022).

Oxidative stress occurs when the balance between oxidant and antioxidant compounds is disrupted due to overproduction of free radicals (Halliwell and Whiteman 2004). *Babesia* species cause oxidative stress by increasing the number of free radicals in erythrocytes. As a result of lipid peroxidation in the erythrocyte membrane due to oxidative stress, erythrocytes become haemolysed and haemolytic anaemia occurs (Crnogaj et al. 2010; Solano-Gallego and Baneth 2011). Measurement of nitric oxide (NO) and advanced oxidation protein products (AOPP) levels can be used to assess oxidative stress status (Witko-Sarsat et al. 1998; Da Silva et al. 2012). Antioxidant mechanisms develop a defence system against free radicals that have harmful effects on body tissues. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) form the main line of defence against free radicals in the cell (Aslankoç et al. 2019).

Although there are studies investigating the prevalence of *B. ovis* in sheep in Türkiye, the number of studies using molecular method is very limited. In this study, we aimed to determine the presence and prevalence of *B. ovis* in sheep in Siirt province located in the Southeastern Anatolia Region of Türkiye using molecular method and to evaluate some oxidant/antioxidant parameters (NO, AOPP, SOD, CAT) in infected sheep.

## Materials and Methods

### Ethical approval

Ethical clearance for the present study was obtained from Siirt University Animal Experiments Local Ethics Committee with an ethical approval number of 20230215.

### The study area and sample collection

The animal material of this study consisted of a total of 500 sheep in Siirt province located in the Southeastern Anatolia region of Türkiye. Blood samples were collected from the jugular vein of the sheep in anticoagulant and non-anticoagulant tubes. Breed, age, and sex information were recorded. Blood samples taken into non-anticoagulant tubes were centrifuged at 3,000 *g* for 10 min, then sera were transferred into 1.5 ml tubes and stored at −20 °C for NO, AOPP, SOD, CAT analysis.

### DNA extraction

After the DNA extraction processes were completed for all blood samples, the A260/A280 values of the resulting gDNA extracts were measured using a nanodrop (Qubit® Fluorometric Quantitation System, USA), and were determined to be between 1.7 and 2.0 for each sample. Following these measurements, the extracts were stored at −20 °C until analysis.

### PCR amplification

During the Polymerase Chain Reaction (PCR) amplification process, the 18S ribosomal RNA (rRNA) gene region, 549 base pairs in length, was targeted using forward (Bbo-F: 5'-TGGGCAGGACCTTGGTTCTTCT-3') and reverse (Bbo-R: 5'-CCGCGTAGCGCCGGCTAAATA-3') primers, as defined by Aktaş et al. (2005). The PCR reaction mixture was prepared with a total volume of 22.5 µl, consisting of 12.5 µl of 2X Taq Master Mix (ready-to-load), which includes PCR buffer (KCl, 0.25 U/µl Taq polymerase, 0.4 mM dNTP, 3.2 mM $MgCl_2$, and Orange G dye), 1.5 µl (10 pmol) of each primer, and 7 µl of sterile distilled water. Subsequently, 2.5 µl of template DNA was added to the mixture. The PCR protocol began with an initial denaturation step at 95 °C for 15 min, followed by a total of 38 cycles with each cycle comprising 30 s of denaturation at 95 °C, 30 s of annealing at 62 °C, and 1 min of elongation at 72 °C. A final elongation step was then performed at 72 °C for 5 min to ensure

complete strand synthesis. To verify the PCR products obtained following amplification, electrophoresis was conducted. For this purpose, the PCR products were run in a 1.5% agarose gel at a constant current of 90 volts for approximately 60 min in an electrophoresis tank. After electrophoresis, the DNA bands were visualized under UV light using a gel imaging system (GEN-BOX imagER FX, Ankara, Türkiye).

### Sequence analysis and phylogeny

Among the PCR positive samples, 6 samples suitable for sequencing were sent to a private company (Hibrigen, Van, Türkiye) for DNA sequence analysis. The DNA sequences obtained were checked and aligned in the MAFFT Version 7 program and made available for analysis (Katoh and Standley 2013). The edited sequences were then registered with NCBI for BLASTn search, sequence alignment and analysis (Altschul et al. 1990). To construct the phylogenetic tree, the data sets were aligned in MAFFT version 7 and model testing was performed in MEGA X using the Maximum Likelihood statistical method. According to the Bayesian Information Criterion, the most appropriate model was determined to be K2+G and phylogenetic trees were constructed according to this model with 1000 bootstraps (Katoh and Standley 2013; Kumar et al. 2018).

### Biochemical analysis

According to the PCR results, NO (Cat No: E0037Sh, BT LAB), AOPP (Cat No: EA0026Sh, BT LAB), SOD (Cat No: E0119Sh, BT LAB) and CAT (Cat No: E0128Sh, BT LAB) assays were performed by Enzyme-Linked ImmunoSorbent Assay (ELISA) method using commercial kits in 20 positive and 20 negative serum samples.

### Statistical analysis

Statistical processing of the obtained results was performed using parametric (independent sample *t*-test) and non-parametric tests (chi-square). Statistical analysis was done in the SPSS V16.0 (IBM, Chicago, IL, USA) program. Differences were considered significant at $P < 0.05$.

## Results

As a result of the study, 84 of the 500 samples (16.80%) examined were PCR positive. The highest prevalence was found in females according to sex, in Hamdani according to animal breeds, in 1–3 age groups among age groups, and in Pervari district among locations (Table 1). While there was no significant difference between sex, location and age groups ($P > 0.05$), there was a significant difference between animal breeds ($P < 0.05$). As a result of this study, 6 sequences obtained from PCR positive samples were deposited in GenBank. When the DNA sequences obtained in the study were compared with the database in NCBI Basic Local Alignment Search Tool, we observed that all samples overlapped 100% with *B. ovis* (Table 2). Phylogenetic analysis of 18s rRNA gene sequences confirmed *B. ovis* in this study (Fig. 1).

Serum NO, AOPP, SOD and CAT levels of control and infected groups are presented in Table 3. AOPP and NO levels were found to be higher in sheep with babesiosis compared to the control group ($P < 0.05$). SOD activity was higher in the control group and CAT activity was higher in infected sheep, but the difference between the groups in terms of both parameters was not significant ($P > 0.05$).

## Discussion

*Babesia ovis* is a tick-borne disease with high morbidity and mortality in small ruminants and causes high economic losses in many tropical and subtropical regions (Shahbazi et al. 2013; Rjeibi et al. 2014).

In studies carried out to determine the prevalence of *B. ovis* in the world; 6.1% positivity was reported in Spain (Ferrer et al. 1998), 24.6% in Iran (Razmi et al. 2002), 71.3% in Egypt (Hosein et al. 2007), 34% in Pakistan (Shahzad et al. 2013), 7.8% in Tunisia (Rjeibi et al. 2016), 5.5% in Uganda (Tumwebaze et al. 2020) and 61.1% in Nigeria (Adewumi et al. 2022). Türkiye's natural conditions lead to the occurrence of many tick-borne infections (Gökpınar et al. 2021). Studies conducted in various regions of Türkiye have reported a prevalence of 0%–72% (Sevinç and Dik 1996; Emre et al. 2001; Çiçek et al. 2004; Gökpınar et al. 2021; Kose et al. 2022; Ulucesme et al. 2023).

Table 1. Distribution of *Babesia ovis* prevalence by sex, breed, location and age.

| Parameter | No of examined sheep (n) | Positive (n) | % | *P* |
|---|---|---|---|---|
| **Sex** | | | | |
| Female | 423 | 75 | 17.73 | NS |
| Male | 77 | 9 | 11.69 | |
| **Breed** | | | | |
| Hamdani | 368 | 73[a] | 19.84 | ** |
| Romanov | 97 | 7[b] | 7.22 | |
| Assaf | 35 | 4[a,b] | 11.43 | |
| **Location** | | | | |
| Center | 300 | 49 | 16.33 | NS |
| Kurtalan | 34 | 7 | 20.59 | |
| Aydınlar | 14 | 4 | 28.57 | |
| Baykan | 34 | 2 | 5.88 | |
| Şirvan | 55 | 9 | 16.36 | |
| Pervari | 19 | 7 | 36.84 | |
| Eruh | 44 | 6 | 13.64 | |
| **Age** (Year) | | | | |
| < 1 | 67 | 9 | 13.43 | NS |
| 1–3 | 215 | 42 | 19.53 | |
| > 3 | 218 | 33 | 15.14 | |
| Overall | 500 | 84 | 16.80 | |

NS: Non-significant, **: $P < 0.05$, [a,b,c]: Different superscripts in the same column indicate significant differences ($P < 0.05$).

Table 2. The DNA sequences deposited in GenBank as a result of this study.

| Obtained sequences | | | | | Reference sequences from GenBank | |
|---|---|---|---|---|---|---|
| Pathogen | Host | Target Gene | Accession number | Length (bp) | Identity (%) | Accession number |
| *B. ovis* | Sheep | 18S rRNA | OR374039 | 479 | 100 | EF194112, AY533146 |
| *B. ovis* | Sheep | 18S rRNA | OR374040 | 479 | 100 | AY533146, EF194112 |
| *B. ovis* | Sheep | 18S rRNA | OR374041 | 479 | 100 | KJ829366, EF194112 |
| *B. ovis* | Sheep | 18S rRNA | OR374042 | 479 | 100 | KJ829366, AY533146 |
| *B. ovis* | Sheep | 18S rRNA | OR374043 | 479 | 100 | EF194112, AY533146 |
| *B. ovis* | Sheep | 18S rRNA | OR374044 | 479 | 100 | AY533146, KJ829366 |

*B. ovis*: *Babesia ovis*

Table 3. Mean and standard error mean of the levels of AOPP, CAT, and SOD in the serum of seronegative and seropositive for *Babesia ovis* in sheep.

| Parameter | Group | Mean ± SEM | *P* |
|---|---|---|---|
| AOPP | Control | 6.97 ± 0.42 | * |
| | Infected | 8.77 ± 0.54 | |
| CAT | Control | 2.96 ± 0.65 | NS |
| | Infected | 4.17 ± 0.54 | |
| NO | Control | 30.02 ± 4 | * |
| | Infected | 44.81 ± 6 | |
| SOD | Control | 13.46 ± 3.13 | NS |
| | Infected | 11.72 ± 1.36 | |

NS: Non-significant, *: $P < 0.05$, AOPP: advanced oxidation protein products; CAT: catalase; NO: nitric oxide; SOD: superoxide dismutase

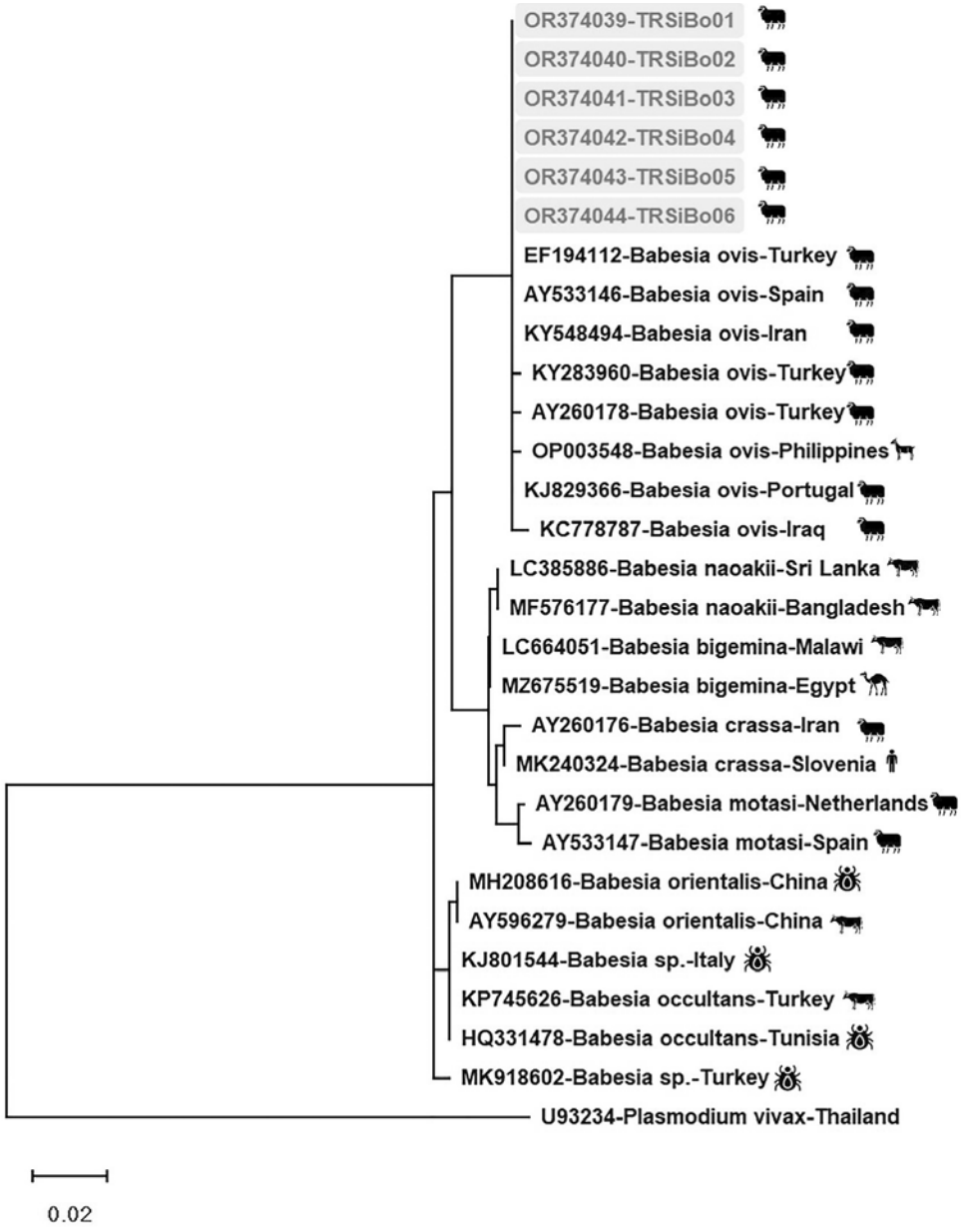


Fig. 1. Phylogenetic relationships of *Babesia ovis* isolates using Maximum Likelihood method analysis based on 18S rRNA gene region (1000 bootstrap). *Plasmodium vivax* was used as an outgroup.

Babesiosis can be diagnosed by microscopic examination of Giemsa-stained blood smears and clinical signs in the acute stage of the disease (Aktaş et al. 2005; Arwa and Kawan 2022). The disadvantage of this method is that it is not sufficient for the diagnosis of latent and chronic infections (Sevinç and Dik 1996). To overcome this situation, diagnostic tests based on serology have been used, but false positive and false negative results have been observed in serological methods for the identification of carrier animals (Aktaş et al. 2005; Gökpınar et al. 2021). Recently, DNA amplification methods have been developed and used for the detection of some *Babesia* species, including *B. ovis*, which are more sensitive and specific than other conventional methods (Altay et al. 2008). Therefore, molecular techniques are more sensitive and specific than other traditional diagnostic methods (Aktas et al. 2007; Esmaeilnejad et al. 2015; Gökpınar et al. 2021).

In this study, PCR method was used in accordance with the reports of previous researchers (Aktas et al. 2007; Esmaeilnejad et al. 2015; Gökpınar et al. 2021) and a positivity rate of 16.80% (84/500) was determined. The 18S rRNA gene of *B. ovis* was used for sequencing to confirm the PCR results. BLAST analysis of *B. ovis* showed 100% similarity with *B. ovis* strains reported in Türkiye (EF194112), Spain (AY533146), and Portugal (KJ829366) and 99.79% similarity with *B. ovis* strains reported in Iran (KY548494), Germany (AY260178), and Japan (OP003548).

The prevalence obtained in this study was higher than the findings of some previous studies (Ferrer et al. 1998; Rjeibi et al. 2016; Tumwebaze et al. 2020; Gökpınar et al. 2021; Ulucesme et al. 2023), compatible with the results of some studies (Altay et al. 2008; Shahzad et al. 2013) and lower than the results of others (Sevinç and Dik 1996; Emre et al. 2001; Razmi et al. 2002; Çiçek et al. 2004; Hosein et al. 2007; Adewumi et al. 2022). The reasons for the differences between the studies include geographical conditions, sample size, number of samples, method used, presence/prevalence of vector ticks and sampling period. The sheep sampled in this study graze on pasture. These sheep may be in more contact with ticks. This may explain the prevalence in this study.

While some researchers (Iqbal et al. 2011; Esmaeilnejad et al. 2015; Gökpınar et al. 2021; Adewumi et al. 2022; Arwa and Kawan 2022) reported a higher prevalence in males, other studies (Emre et al. 2001; Rjeibi et al. 2014; Rjeibi et al. 2016; Stevanović et al. 2022) reported a higher prevalence in females. In this study, higher prevalence was found in females, but the difference was not statistically significant. The difference between sexes may be due to the number of samples collected, environmental exposures, genetic or hormonal effects (Mellanby et al. 2011).

Some studies (Iqbal et al. 2011; Esmaeilnejad et al. 2015; Rjeibi et al. 2016; Gökpınar et al. 2021) reported a higher prevalence in those younger than one year of age, whereas other studies found a higher prevalence in the 1–3 age group (Adewumi et al. 2022; Stevanović et al. 2022). In the study conducted by Razmi et al. (2003), a higher prevalence was reported in those older than three years. In this study, similar to the studies conducted by Adewumi et al. (2022) and Stevanović et al. (2022), a higher prevalence was found in the 1–3 age group. This may be due to the fact that adult sheep go out to pasture for longer periods of time and thus have a higher risk of exposure to the vector causing *B. ovis* infection (Adewumi et al. 2022). Among the three sheep breeds, the highest prevalence was found in Hamdani sheep and the lowest prevalence was found in Romanov sheep, and the difference was statistically significant. This may be due to the fact that Romanov breeding is generally intensive, whereas Hamdani and Asaf sheep are regularly pastured and therefore more exposed to vectors.

An imbalance between oxidants and antioxidant compounds leads to an increase in free radicals. Highly reactive oxygen free radicals are involved in the pathogenesis of various parasitic infections such as *Babesia*, *Theileria*, *Hepatozoon*, *Plasmodium*, *Leishmania*, and *Ehrlichia* (Crnogaj et al. 2010).

In a study evaluating nitric oxide levels, protein oxidation and total antioxidant status in the serum of dogs infected with *Leishmania infantum*, *Ehrlichia canis*, and *Babesia vogeli*, NO and AOPP levels were increased in serum samples of infected dogs, and it was concluded that infections caused by haemoparasites cause oxidative stress and this process may participate in disease pathology (Baldissera et al. 2015). Similarly, in this study, AOPP and NO levels were significantly higher in sheep with babesiosis compared to control group. The increase in nitric oxide may be considered to be involved in the pathogenesis of babesiosis as a marker of oxidative stress and an important mediator of inflammatory response (Baldissera et al. 2015). Keita et al. (2000) suggested that NO cytotoxicity may be due to its ability to generate peroxynitrite, which initiates various oxidative reactions, including modifications of nucleic acids, lipids and proteins leading to tissue damage.

In this study, AOPP levels were higher in the infected group compared to the control group, consistent with the findings of Baldissera et al. (2015). As observed in canine rangeliosis (França et al. 2012) and ehrlichiosis (Da Silva et al. 2013) infections, high AOPP levels are indicative of protein oxidation caused by cellular lesions.

In conclusion of this study, the presence of *Babesia ovis* was detected in all districts of Siirt province. Taking control measures such as tick control for this disease, which also causes death in sheep, and conducting more extensive blood and vector tick surveys including the surrounding provinces will be useful to determine the epidemiology and distribution of infection in this region, and control strategies can be determined. It has been determined that infection caused by *Babesia ovis* in sheep causes oxidative stress as a result of increased NO and oxidized protein levels, and this process may participate in the pathology of the disease.

**Conflict of interest**

The authors state no conflict of interest.

**Acknowledgements**

This study was supported by the Scientific Research Project Directory of the Siirt University (grant number 2022-SİÜVET-048).

The study has been orally presented at the 3rd International Veterinary Biochemistry and Clinical Biochemistry Congress May 23–25, 2024 in Van, Türkiye.

**References**

Adewumi TS, Takeet MI, Akande FA, Sonibare AO, Okpeku M 2022: Prevalence and molecular characterization of *Babesia ovis* infecting sheep in Nigeria. Sustainability **14**: 16974

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

Aktaş M, Altay K, Dumanlı N 2005: Development of a polymerase chain reaction method for diagnosis of *Babesia ovis* infection in sheep and goats. Vet Parasitol **133**: 277-281

Altay K, Aktas M, Dumanli N 2008: Detection of *Babesia ovis* by PCR in *Rhipicephalus bursa* collected from naturally infested sheep and goats. Res Vet Sci **85**: 116-119

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ 1990: Basic local alignment search tool. J Mol Biol **215**: 403-410

Arwa R, Kawan M 2022: Microscopic examination of ovine babesiosis at Baghdad city/Iraq. Iraqi J Agric Sci **53**: 798-801

Aslankoç R, Demirci D, Ümmahan İ, Yildiz M, Öztürk A, Çetin M, Savran EŞ, Yilmaz B 2019: The role of antıoxidant enzymes in oxidative stress - superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Med J SDU **26**: 362-369

Baldissera MD, Sousa KCMd, André MR, Guarda NdS, Moresco RN, Herrera HM, Machado RZ, Jaques JAdS, Tinucci-Costa M, Silva ASd 2015: Nitric oxide, protein oxidation and total antioxidant levels in serum of dogs naturally infected by *Ehrlichia canis*, *Leishmania infantum* and *Babesia vogeli*. Acta Sci Vet **43**: 1320

Crnogaj M, Petlevski R, Mrljak V, Kis I, Torti M, Kucer N, Matijatko V, Sacer I, Stokovic I 2010: Malondialdehyde levels in serum of dogs infected with *Babesia canis*. Vet Med **55**: 163-171

Çakmak A, Dinçer Ş, Karaer Z 1991: Samsun Yöresindenki Koyunlarda *Babesia ovis*'in Serodiagnozu Üzerine Araştırmalar (in Turkish, Studies on serodiagnosis of *Babesia ovis* in sheep in Samsun region). Ankara Üniv Vet Fak Derg **38**: 242-251

Çiçek H, Düzgün A, Emre Z, Karaer Z 2004: Seroprevalence of *Babesia ovis* in sheep around Afyon. Turk J Vet Anim Sci **28**: 683-686

Da Silva AS, Munhoz TD, Faria JL, Vargas-Hérnandez G, Machado RZ, Almeida TC, Moresco RN, Stefani LM, Tinucci-Costa M 2013: Increase nitric oxide and oxidative stress in dogs experimentally infected by *Ehrlichia canis*: effect on the pathogenesis of the disease. Vet Microbiol **164**: 366-369

Da Silva AS, Paim FC, Santos RC, Sangoi MB, Moresco RN, Lopes ST, Jaques JA, Baldissarelli J, Morsch VM, Monteiro SG 2012: Nitric oxide level, protein oxidation and antioxidant enzymes in rats infected by *Trypanosoma evansi*. Exp Parasitol **132**: 166-170

Emre Z, Düzgün A, İriadam M, Sert H 2001: Seroprevalence of *Babesia ovis* in Awassi sheep in Urfa, Turkey. Turk J Vet Anim Sci **25**: 759-762

Esmaeilnejad B, Tavassoli M, Asri-Rezaei S, Dalir-Naghadeh B, Mardani K, Golabi M, Arjmand J, Kazemnia A, Jalilzadeh G 2015: Determination of prevalence and risk factors of infection with *Babesia ovis* in small ruminants from West Azerbaijan Province, Iran by polymerase chain reaction. J Arthropod Borne Dis **9**: 246

Ferrer D, Castellà J, Gutierrez J 1998: Seroprevalence of *Babesia ovis* in sheep in Catalonia, northeastern Spain. Vet Parasitol **79**: 275-281

França RT, Da Silva AS, Costa MM, Paim FC, Paim CB, Thomé GR, Wolkmer P, Pereira ME, Schetinger MRC, Moresco RN 2012: Relationship between oxidative stress and clinical–pathological aspects in dogs experimentally infected with *Rangelia vitalii*. Res Vet Sci **93**: 1309-1313

Gökpınar S, Gazyağcı AN, Aydenizöz M, Kaya U 2021: A research on Babesia and Theileria species in sheep and goats of Kırıkkale provincethrough molecular methods. Turk J Vet Anim Sci **45**: 912-919

Halliwell B, Whiteman M 2004: Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br J Pharmacol **142**: 231-255

Hosein H, Ahmed SA, Ibrahim F, Abou-Elnaga T, Gebely M, Mahmoud MA 2007: Seroprevalence of *Babesia ovis* in small ruminants in Siwa Oasis, Egypt. J Vet Med Res **17**: 19-24

Iqbal F, Fatima M, Shahnawaz S, Naeem M, Shaikh R, Ali M, Shaikh A, Aktas M 2011: A study on the determination of risk factors associated with babesiosis and prevalence of *Babesia* sp., by PCR amplification, in small ruminants from Southern Punjab (Pakistan). Parasite **18**: 229-234

Katoh K, Standley DM 2013: MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol **30**: 772-780

Keita M, Vincendeau P, Buguet A, Cespuglio R, Vallat J-M, Dumas M, Bouteille B 2000: Inducible nitric oxide synthase and nitrotyrosine in the central nervous system of mice chronically infected with *Trypanosoma brucei brucei*. Exp Parasitol **95**: 19-27

Kose O, Bilgic HB, Bakirci S, Karagenc T, Adanir R, Yukari BA, Eren H 2022: Prevalence of Theileria/Babesia species in ruminants in Burdur province of Turkey. Acta Parasitol **67**: 723-731

Kumar S, Stecher G, Li M, Knyaz C, Tamura K 2018: MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol **35**: 1547

Mellanby R, Handel I, Clements D, de C. Bronsvoort B, Lengeling A, Schoeman J 2011: Breed and sex risk factors for canine babesiosis in South Africa. J Vet Intern Med **25**: 1186-1189

Razmi G, Naghibi A, Aslani M, Dastjerdi K, Hossieni H 2003: An epidemiological study on *Babesia* infection in small ruminants in Mashhad suburb, Khorasan province, Iran. Small Rumin Res **50**: 39-44

Razmi G, Naghibi A, Aslani M, Fathivand M, Dastjerdi K 2002: An epidemiological study on ovine babesiosis in the Mashhad suburb area, province of Khorasan, Iran. Vet Parasitol **108**: 109-115

Rjeibi MR, Darghouth MA, Gharbi M 2016: Prevalence of *Theileria* and *Babesia* species in Tunisian sheep. Onderstepoort J Vet Res **83**: 1-6

Rjeibi MR, Gharbi M, Mhadhbi M, Mabrouk W, Ayari B, Nasfi I, Jedidi M, Sassi L, Rekik M, Darghouth MA 2014: Prevalence of piroplasms in small ruminants in North-West Tunisia and the first genetic characterisation of *Babesia ovis* in Africa. Parasite **21**

Sevinç F, Dik b 1996: The diagnosis of *Babesia ovis* in Sheep in Konya Around by ELISA. Vet Bil Derg **12**: 73-79

Saraylı H, İnci A, İça A, Yıldırım A, Düzlü Ö 2006: Investigation of the babesia agents in sheep and goats by the reverse line blotting hybridization method around Yeşilhisar. J Health Sci **15**: 181-188

Shahbazi A, Fallah E, Mamagani AJ, Khanmohammadi M, Nematollahi A, Bazmani A 2013: Identification of *Babesia* species in sheep isolated from villages of East Azerbaijan by Semi-nested PCR. J Pure Appl Microbiol **7**: 201-205

Shahzad W, Haider N, Mansur-ud-Din A, Munir R, Saghar MS, Mushtaq MH, Ahmad N, Akbar G, Mehmood F 2013: Prevalence and molecular diagnosis of *Babesia ovis* and *Theileria ovis* in Lohi sheep at livestock experiment station (LES), Bahadurnagar, Okara, Pakistan. Iran J Parasitol **8**: 570-578

Solano-Gallego L, Baneth G 2011: Babesiosis in dogs and cats—expanding parasitological and clinical spectra. Vet Parasitol **181**: 48-60

Stevanović O, Radalj A, Subić I, Jovanović NM, Sladojević Ž, Amović M, Zuko A, Nedić D, Ilić T 2022: The presence of malignant ovine babesiosis in Bosnia and Herzegovina indicates a possible emerging risk for Balkan region. Comp Immunol Microbiol Infect Dis **90**: 101893

Tumwebaze MA, Byamukama B, Tayebwa DS, Byaruhanga J, Angwe MK, Galon EM, Liu M, Lee S-H, Ringo AE, Adjou Moumouni PF 2020: First molecular detection of *Babesia ovis*, *Theileria* spp., *Anaplasma* spp., and *Ehrlichia ruminantium* in goats from western Uganda. Pathogens **9**: 895

Ulucesme MC, Ozubek S, Karoglu A, Turk ZI, Olmus I, Irehan B, Aktas M 2023: Small ruminant piroplasmosis: high prevalence of *Babesia aktasi* n. sp. in goats in Türkiye. Pathogens **12**: 514

Witko-Sarsat VR, Friedlander M, Khoa TN, Capeillère-Blandin C, Nguyen AT, Canteloup S, Dayer J-M, Jungers P, Drüeke T, Descamps-Latscha Ba 1998: Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure1, 2. J Immunol **161**: 2524-2532