Ankara Üniversitesi Veteriner Fakültesi Dergisi/Ankara Univ Vet Fak Derg; 2026:73(1): 77-82
DOI: 10.33988/auvfd.1780265
Research Article

Microscopic examination and molecular characterization of tick-borne blood protozoa in domestic cats in shelters in Ankara

Mert ARSLANBAŞ[1,2,a,✉], Anıl YILMAZ[2,b], Ömer ORKUN[3,c], Ayşe Serpil NALBANTOĞLU[3,d]

[1]Eville & Jones Ltd, Official Veterinarian, Leeds, United Kingdom; [2]Ankara University, Faculty of Veterinary Medicine, The Graduate School of Health Sciences, Ankara, Türkiye; [3]Ankara University, Faculty of Veterinary Medicine, Department of Parasitology, Ankara, Türkiye.

[a]ORCID: 0000-0002-9307-4441; [b]ORCID: 0000-0003-4903-3527; [c]ORCID: 0000-0002-4478-2521; [d]ORCID: 0000-0002-9670-7566

ABSTRACT

Ticks are important vectors that transmit viral, bacterial, and protozoan pathogens and cause diseases in vertebrates. The epidemiology of tick-borne pathogens in cats has been less well investigated than in dogs, and the role of cats in this epidemiology is only partially understood. The main tick-borne protozoa reported in cats include species of *Babesia*, *Hepatozoon*, and *Cytauxzoon*. The aim of this study was to investigate tick-borne blood protozoa in 200 domestic cats in Ankara province of Turkey. Blood and tick samples were collected from each animal. Of 200 cats examined, 6 (3%) were found positive for *Hepatozoon* spp., and two different haplotypes were identified. *Hepatozoon* gamonts were observed in 1% of blood smears. In conclusion, the species and haplotypes present in the region were identified and compared with those found worldwide. These data provided molecular characterization of the relevant pathogens and revealed potential risks to domestic cats.

Keywords: Blood protozoa, Domestic cat, *Hepatozoon felis*, Molecular characterization, Tick-borne disease

✉**Corresponding author:** dvm.mertarslanbas@gmail.com

Received: 08.09.2025, **Accepted:** 05.11.2025, **Publication Date:** 01.01.2026

How to cite this article: Arslanbaş M, Yılmaz A, Orkun Ö, Nalbantoğlu AS (2026): Microscopic examination and molecular characterization of tick-borne blood protozoa in domestic cats in shelters in Ankara. Ankara Univ Vet Fak Derg, 73 (1), 77-82. DOI: 10.33988/auvfd.1780265.

Introduction

Tick-borne haemoprotozoa of cats include *Babesia*, *Cytauxzoon*, and *Hepatozoon*. *Babesia* spp. are obligate intraerythrocytic apicomplexans transmitted by ixodid ticks; sporozoites that invade erythrocytes undergo merogony, and gametocytes are taken up by ticks. Transmission is mainly transstadial and can be transovarial in some species. Clinical presentations range from subclinical infection to haemolytic anaemia, and diagnosis relies on blood smears and PCR, alongside strict tick control (2, 12, 30, 31).

In *Cytauxzoon felis* infection, sporozoites firstly proliferate as schizonts within mononuclear phagocytes, and released merozoites parasitize erythrocytes as piroplasms. Domestic cats typically develop an acute febrile illness characterized by lethargy, anorexia, pallor or icterus, and respiratory compromise; laboratory abnormalities often include anaemia and thrombocytopenia. Case fatality can be high without prompt therapy; current recommendations combine atovaquone and azithromycin, with supportive care, while prevention hinges on effective tick control (5, 7, 14, 20, 29).

Feline *Hepatozoon* infections, most commonly *H. felis*, are acquired chiefly by ingestion of infected ticks or a paratenic host; merogony occurs in tissues, and leukocytic gamonts circulate in the blood. Many infections are subclinical, but clinical disease may present with fever, lethargy, weight loss, anaemia, and occasional myositis, with severity influenced by comorbidities or immunosuppression (3, 4, 18, 21). Although light microscopy can reveal gamonts, it has limited analytical sensitivity (8).

In Türkiye, *Hepatozoon canis* infection is commonly detected in dogs, with several studies reporting high prevalence rates across different regions, whereas *H. felis* infection in cats appears to be rare and underreported (17, 27).

Among these protozoa, early diagnosis is aided by molecular testing: surveys commonly target the 18S rRNA

gene with species-specific or nested PCR, and sequencing supports species confirmation and phylogenetic context (8, 13, 22). Region-specific data for Türkiye remain limited; given the conservation of 18S, low intraspecific divergence is expected among local *H. felis* isolates, motivating the present targeted molecular investigation (23, 24, 25, 26, 32).

This study was carried out to investigate the occurrence of tick-borne hemoprotozoa in domestic cats housed in shelters in Ankara.

Materials and Methods

Under sterile conditions, 1 mL of venous blood was collected from the cephalic or saphenous veins of domestic cats into EDTA tubes, and each sample was coded KdA1 to KdA200. Each cat was examined for ectoparasites. All the collected ectoparasites were stored in 70% ethanol, subsequently cleared in 10% KOH and lactic acid, and mounted in Canada balsam. Ectoparasites were identified according to Lewis (19). Parasites were imaged with a light microscope equipped with a digital camera, and morphometrics were recorded in micrometers using standard imaging software. Whole-blood aliquots were stored at −20 °C until DNA extraction and downstream analyses.

Genomic DNA was extracted from whole blood using a commercial silica-membrane kit following the manufacturer's instructions. Species-specific PCR assays targeted the 18S rRNA gene for *Babesia* spp. (primers BJ1/BN2) (6), *Hepatozoon* spp. (HepF/HepR) (13), and *Cytauxzoon* spp. (a nested assay using 7549F/7548R followed by Cyt-SSU-F2/Cyt-SSU-R4) (9, 22, 28). Amplicons were purified and Sanger sequenced bidirectionally; consensus sequences were assembled after chromatogram inspection.

After purification of the amplicons, bidirectional Sanger sequencing was performed. Raw sequence data obtained in both directions were checked against the chromatogram, edited, and merged. Subsequently, these sequences were subjected to nucleotide sequence similarity analyses (BLASTn) against the GenBank database (National Center for Biotechnology Information, https://www.ncbi.nlm.nih.gov/genbank; accessed 12/12/2023) to determine their identities.

A phylogenetic tree was constructed using the maximum-likelihood method via the online platform available at http://www.phylogeny.fr The 18S rRNA sequences obtained in this study (GenBank accession numbers OQ076291–OQ076296) were analyzed alongside reference sequences retrieved from the NCBI GenBank database (https://www.ncbi.nlm.nih.gov), including *Hepatozoon felis* isolates from Türkiye (e.g., MN905024, MN905025, MZ895464, OP941801–OP941805), Portugal (e.g., MZ476769, MZ475990, MZ475977, MZ475978), Croatia (MH656727), and other regions. Additional *Hepatozoon* spp. sequences were included as outgroups. The relationships between the study isolates and previously deposited sequences were evaluated, and bootstrap values were calculated to assess the robustness of the branches.

Results

Fourteen of 200 shelter cats were infested with a total of 21 fleas, all identified as Ctenocephalides felis. No ticks were observed on any cats. Microscopic examination of 200 Giemsa-stained smears revealed leukocytic gamonts compatible with *Hepatozoon* in two cats (KdA59 and KdA65), whereas no intraerythrocytic piroplasms compatible with *Babesia* or *Cytauxzoon* were observed. Species-specific PCRs yielded no positive amplicons for *Cytauxzoon* spp. or *Babesia* spp., while six cats (KdA10, KdA54, KdA59, KdA65, KdA130, KdA182) were positive for *Hepatozoon* spp.. Among these PCR-positive cats, gamonts were detected microscopically in two of six, indicating greater analytical sensitivity of PCR at low parasitaemia.

All six amplicons were identified as *Hepatozoon felis* and had read lengths of 614–624 bp (KdA10: 624 bp; KdA54: 614 bp; KdA59: 618 bp; KdA65: 617 bp; KdA130: 620 bp; KdA182: 614 bp); they were deposited under GenBank accession numbers OQ076291–OQ076296. Sequence alignment resolved two haplotypes: Hp-KdA10, Hp-KdA54, Hp-KdA59, and Hp-KdA65 (Haplotype 1) were 100% identical, whereas Hp-KdA130 and Hp-KdA182 (Haplotype 2) were identical to each other but differed from Haplotype 1 by a single A↔T transition (0.16%) in the 18S rRNA fragment. For BLASTn comparisons, the longest representative of each haplotype was used (Hp-KdA10 for Haplotype 1; Hp-KdA130 for Haplotype 2). Haplotype 1 was identical to previously deposited *H. felis* sequences from Türkiye and abroad, whereas Haplotype 2 matched Portuguese tick-derived sequences.

In BLAST comparisons, Haplotype 1 (Hp-KdA10, Hp-KdA54, Hp-KdA59, Hp-KdA65) was identical to multiple *H. felis* records: Tr/Hp1127 (MN905024) and Tr/Hp1129 (MN905025) from *Haemaphysalis parva* in Ankara (25); Ork/Hp-Va2 (MZ895464) from Eurasian lynx blood in Yozgat (24); MH656727 from a Croatian cat (also reported as “wild1” from *Rhipicephalus turanicus*) (25, 32); and Turkish cat-blood isolates TR-B311 (OP941801), TR-B3112 (OP941802), TR-B515 (OP941803), TR-B517 (OP941804) and TR-B623 (OP941805). In addition, the Portuguese tick sequence PTTiHep_Mel_2021 (OL604173) showed 100% identity with 98% query coverage.

Haplotype 2 (Hp-KdA130, Hp-KdA182) matched Portuguese tick-derived sequences H14 (MZ476769), H10 (MZ475990), H4 (MZ475977) and H3 (MZ475978) (97% query coverage, 100% identity), and aligned with

DKK29 (JQ867388) from *Rhipicephalus sanguineus* in Diyarbakır (1).

A maximum-likelihood tree based on 18S rRNA (Figure 1) clustered all six study isolates within the *H. felis* clade, together with previously deposited sequences from Türkiye and other regions worldwide. The two haplotypes formed closely related subclusters, consistent with the single-nucleotide difference observed in the alignment.

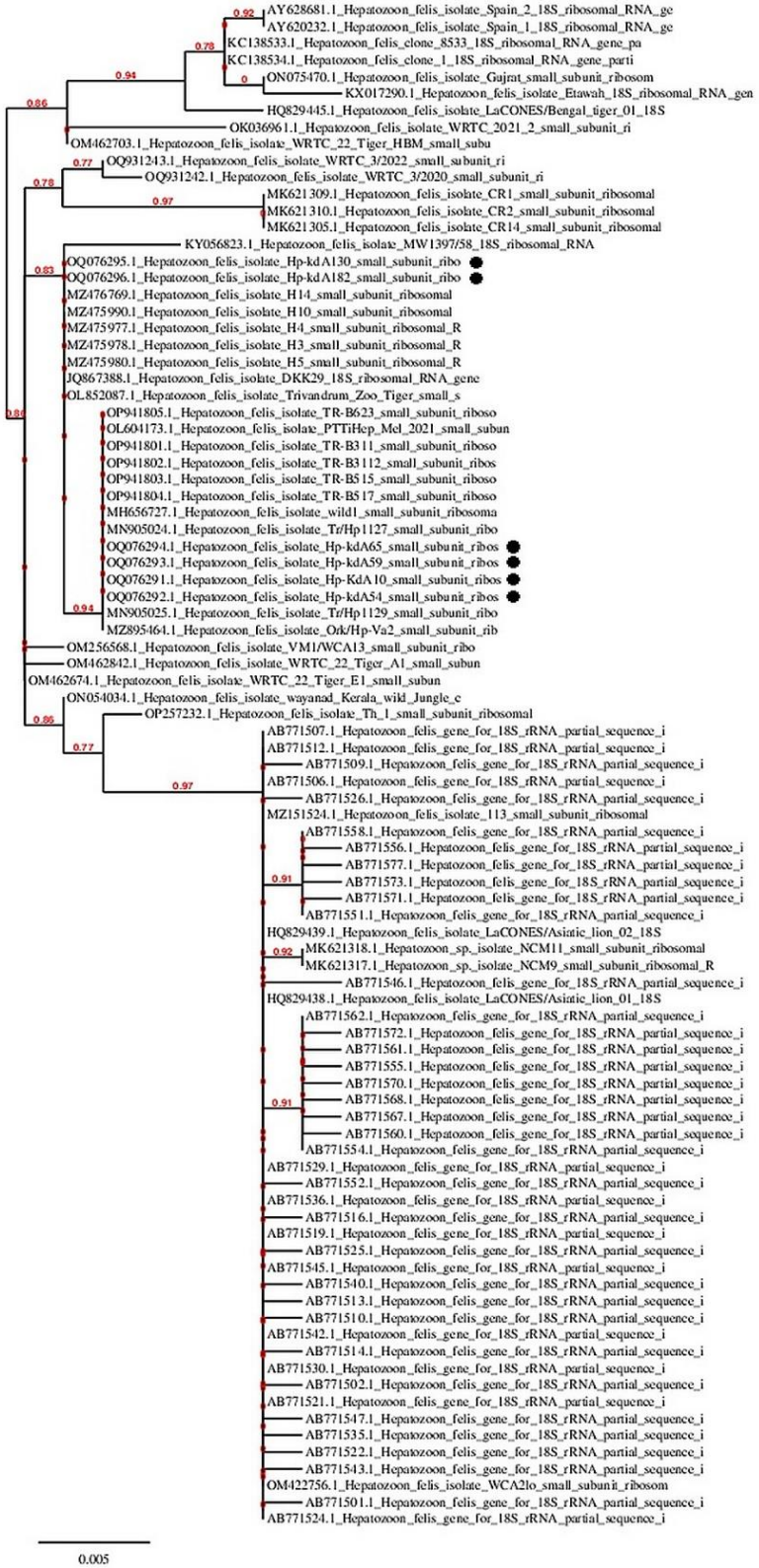

Figure 1. Phylogenetic placement of study isolates of *Hepatozoon felis* (OQ076291–OQ076296) among selected reference sequences based on 18S rRNA; tree constructed using the https://www.phylogeny.fr online platform (accessed 4 September 2025). Bootstrap values are shown at key nodes.

Discussion and Conclusion

This study documents *Hepatozoon felis* in shelter cats from Ankara and resolves two closely related 18S rRNA haplotypes that differ by a single A↔T substitution (0.16%). Given the high conservation of 18S in apicomplexans, such shallow divergence is expected and limits phylogeographic resolution at this locus (12, 23, 31).

Haplotype 1 showed 100% identity to multiple H. felis sequences reported from Türkiye and other countries worldwide, including records from domestic and wild felids and ticks, consistent with broad host and vector ranges (24, 25, 32). Perfect matches across distant localities are biologically plausible when a conserved marker is used and when vectors or hosts move across regions through natural dispersal or human-mediated translocation (11, 23). In Türkiye, reports of *Hepatozoon* DNA from wild felids and ticks collected from wildlife and domestic animals, together with sequences identical to our Haplotype 1, support regional connectivity rather than local endemism at 18S (24, 25).

Haplotype 2 matched *H. felis* sequences detected in ticks from Portugal (MZ476769, MZ475990, MZ475977, MZ475978) and aligned with a tick-derived Turkish record (1), indicating that the same lineage circulates in both the Iberian Peninsula and Anatolia. The single-site nucleotide difference that separates the two haplotypes likely reflects neutral micro-variation accumulated through mutation and lineage sorting, which is frequently observed in haplotype networks built from conserved loci (11, 30). Geographic proximity or shared vector fauna may facilitate the co-occurrence of closely related haplotypes in different regions, especially where *Rhipicephalus* and other competent ticks overlap in distribution (25).

Comparative sequence analysis of the *Hepatozoon felis* isolates obtained in this study (OQ076291–OQ076296) and the recently deposited sequences from Aydın and İzmir provinces (PV765406–PV765415) revealed high similarity, confirming that all isolates belong to *H. felis*. Although direct alignment of full-length 18S rRNA fragments produced only 82–84% identity, likely because of differences in amplicon length and only partial overlap, comparison of the homologous regions yielded over 99% identity, demonstrating that the Ankara isolates and the *H. felis* Genotype I reported in that study (10) represent the same lineage. This pattern indicates that a single genetically homogeneous *H. felis* population circulates across central and western Türkiye. The minimal sequence divergence observed likely reflects the high conservation of the parasite's 18S rRNA gene and the widespread presence of shared *Rhipicephalus* tick vectors, which facilitate gene flow between feline hosts in different ecological zones. Overall, these results highlight limited regional differentiation and support the maintenance of a conserved 18S rRNA genotype structure of *H. felis* throughout Türkiye (10).

Identical or near-identical 18S sequences in cats and ticks collected from wildlife are consistent with spillover at the wildlife–domestic interface and with regional circulation of parasite lineages through vector ecology. Animal trade, relocation of shelter animals, and natural dispersal of ticks can maintain genetic similarity across administrative borders, while local bottlenecks or founder effects can yield minor differences between co-circulating haplotypes (24, 25).

Neither *Cytauxzoon felis* nor *Babesia felis* was detected in the present study; in contrast, previous microscopic reports from Van Province identified *C. felis* and *B. felis* in 7.5% and 10.8% of examined cats, respectively (15,16). The absence of these protozoa in the current dataset may reflect true regional variation in parasite distribution or differences in the ecology of tick vectors between eastern and central Türkiye. Another possibility is that parasitemia in naturally infected cats may remain below the analytical thresholds of microscopy and single-locus PCR assays, particularly when subclinical or chronic infections predominate. Because both *C. felis* and *B. felis* have been recorded only sporadically and mostly by microscopy, further molecular surveys incorporating multi-locus detection and broader spatial coverage are required to clarify their epidemiological status in domestic cats throughout Türkiye (15,16).

Gamonts were visualized in only 2 of 6 PCR-positive cats, underscoring the limited analytical sensitivity of smear microscopy at low parasitaemia and supporting PCR as the primary tool for detection and surveillance in cats. Key limitations include single-site sampling, use of a single conserved locus for diversity assessment, and absence of clinical covariates and seasonality. Because 18S offers low resolving power, multi-locus panels (including more variable mitochondrial markers) sampled across seasons and sites, and paired with standardized clinical metadata, would allow stronger tests of geographic structure, host factors, and vector associations (11, 23).

Hepatozoon felis circulates among shelter cats in Ankara, exhibiting low 18S diversity represented by two haplotypes: one identical to records from Türkiye and countries worldwide (Haplotype 1: Tr/Hp1127, Tr/Hp1129, Ork/Hp-Va2, MH656727, TR-B311, TR-B3112, TR-B515, TR-B517, TR-B623, PTTiHep_Mel_2021), and the other matching Portuguese tick-derived sequences and a Turkish tick record (Haplotype 2: H14, H10, H4, H3, DKK29). These patterns indicate that *H. felis* lineages exhibit regional connectivity shaped by vector ecology and host movement, rather than pronounced phylogeographic structuring at 18S.

Acknowledgements

This article was derived from the doctoral thesis of Mert Arslanbaş.

Financial Support

This research was supported by the Ankara University Scientific Research Projects (TDK-2022-2322).

Ethical Statement

All procedures were approved by the Ankara University Animal Experiments Local Ethics Committee (Decision No. 2020-6-51).

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

MA conceived and designed the study, coordinated field sampling, performed DNA extractions and PCR assays, assembled sequences, and drafted the manuscript. ÖO provided molecular expertise, assisted with primer selection and PCR optimisation, and supervised molecular analyses. ASN served as thesis advisor, providing critical oversight and revisions. AY contributed to molecular analyses and manuscript preparation. All authors reviewed and approved the manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Animal Welfare

The authors confirm adherence to the ARRIVE guidelines for the protection of animals used for scientific purposes.

References

1. **Aktas, M, Özübek S, İpek DNS** (2013): *Molecular investigations of Hepatozoon species in dogs and developmental stages of Rhipicephalus sanguineus*. Parasitol Res, **112**, 2381-2385.
2. **Ayoob AL, Prittie J, Hackner SG** (2010): *Feline babesiosis*. J Vet Emerg Crit Care, **20**, 90-97.
3. **Baneth G** (2003): *Disease risks for the travelling pet: Hepatozoonosis*. Pract, **25**, 272-277.
4. **Baneth G, Sheiner A, Eyal O, et al** (2013): *Redescription of Hepatozoon felis (Apicomplexa: Hepatozoidae) based on phylogenetic analysis, tissue and blood form morphology, and possible transplacental transmission.* Parasit Vectors, **6**, 1-10.
5. **Birkenheuer AJ, Le JA, Valenzisi AM, et al** (2006): *Cytauxzoon felis infection in cats in the mid-Atlantic states: 34 cases (1998–2004)*. J Am Vet Med Assoc, **228**, 568-571.
6. **Casati S, Sager H, Gern L, et al** (2006): *Presence of potentially pathogenic Babesia sp. for human in Ixodes ricinus in Switzerland.* Ann Agric Environ Med, **13**, 65-70.
7. **Cohn LA, Birkenheuer AJ, Brunker JD, et al** (2011): *Efficacy of atovaquone and azithromycin or imidocarb dipropionate in cats with acute cytauxzoonosis.* J Vet Intern Med, **25**, 55-60.
8. **Criado-Fornelio A, Buling A, Cunha-Filho NA, et al** (2007): *Development and evaluation of a quantitative PCR assay for detection of Hepatozoon sp.* Vet Parasitol, **150**, 352-356.
9. **Gallusová M, Jirsová D, Mihalca AD, et al** (2016): *Cytauxzoon infections in wild felids from Carpathian-Danubian-Pontic space: further evidence for a different Cytauxzoon species in European felids.* J Parasitol, **102**, 377-380.
10. **Hacılarlıoğlu, S, Pekağırbaş M** (2025): *Emerging insights into feline hepatozoonosis in Türkiye: Molecular detection and phylogenetic characterization from the Aegean coast*. Türkiye Parazitol Derg, **49**, 52–57.
11. **Harris DJ, Santos J, Rampedi KM, et al** (2019): *Genetic diversity of Hepatozoon (Apicomplexa) from domestic cats in South Africa, with a global reassessment of Hepatozoon felis diversity*. J S Afr Vet Assoc, **90**, 1-6.
12. **Hartmann K, Addie D, Belák S, et al** (2013): *Babesiosis in cats: ABCD guidelines on prevention and management.* J Feline Med Surg, **15**, 643-646.
13. **Inokuma H, Okuda M, Ohno K, et al** (2002): *Analysis of the 18S rRNA gene sequence of a Hepatozoon detected in two Japanese dogs.* Vet Parasitol, **106**, 265-271.
14. **Jalovecka M, Hajdusek O, Sojka D, et al** (2018): *The complexity of piroplasms life cycles.* Front Cell Infect Microbiol, **8**, 248.
15. **Karaca M, Akkan HA, Tütüncü M, et al** (2007): *Cytauxzoonosis in Van cats*. YYU Vet Fak Derg, **18**, 37-39.
16. **Karaca M, Tütüncü M, Akkan HA, et al** (2005): *Babesiosis in Van cats*. YYU Vet Fak Derg, **16**, 87-88.
17. **Kaya F, Baydar E** (2024): *Case report of a dog heavily infected with hepatozoonosis.* MAKU J Health Sci Inst, **12**, 63–68.
18. **Klopfer U, Nobel TA, Neumann F** (1973): *Hepatozoon-like parasite (schizonts) in the myocardium of the domestic cat.* Vet Pathol, **10**, 185-190.
19. **Lewis RE** (1967): *The fleas (Siphonaptera) of Egypt. An illustrated and annotated key*. J Parasitol, **53**, 863–885.
20. **Lloret A, Addie DD, Boucraut-Baralon C, et al** (2015): *Cytauxzoonosis in cats: ABCD guidelines on prevention and management.* J Feline Med Surg, **17**, 637-641.
21. **Lloret A, Addie DD, Boucraut-Baralon C, et al** (2015): *Hepatozoonosis in cats: ABCD guidelines on prevention and management.* J Feline Med Surg, **17**, 642-644.
22. **Millán J, Naranjo V, Rodríguez A, et al** (2007): *Prevalence of infection and 18S rRNA gene sequences of Cytauxzoon species in Iberian lynx (Lynx pardinus) in Spain*. Parasitol, **134**, 995-1001.
23. **Nguyen AH, Tiawsirisup S, Kaewthamasorn M** (2020): *Low level of genetic diversity and high occurrence of vector-borne protozoa in water buffaloes in Thailand based on 18S ribosomal RNA and mitochondrial cytochrome b genes.* Infect Genet Evol, **82**, 104304.
24. **Orkun Ö** (2022): *Description of a novel Babesia sp. genotype from a naturally infected Eurasian lynx (Lynx lynx) in Anatolia, Turkey, with remarks on its morphology and phylogenetic relation to other piroplasmid species.* Ticks Tick Borne Dis, **13**, 102026.

25. **Orkun Ö, Emir H** (2020): *Identification of tick-borne pathogens in ticks collected from wild animals in Turkey.* Parasitol Res, **119**, 3083-3091.
26. **Orkun Ö, Karaer Z, Çakmak A, et al** (2014): *Identification of tick-borne pathogens in ticks feeding on humans in Turkey*. PLoS Negl Trop Dis, **8**, e3067.
27. **Pekağırbaş M, Demirbilek MV, Şimşek E, et al** (2025): *Molecular characterization of Hepatozoon spp. in naturally infected dogs in Aydın province*. Ankara Univ Vet Fak Derg, **72**, 237–242.
28. **Panait LC, Mihalca AD, Modrý D, et al** (2021): *Three new species of Cytauxzoon in European wild felids.* Vet Parasitol, **290**, 109344.
29. **Reichard MV, Thomas JE, Arther RG, et al** (2013): *Efficacy of an imidacloprid 10%/flumethrin 4.5% collar (Seresto®, Bayer) for preventing the transmission of Cytauxzoon felis to domestic cats by Amblyomma americanum.* Parasitol Res, **112**, 11-20.
30. **Schnittger L, Rodriguez AE, Florin-Christensen M, et al** (2012): *Babesia: a world emerging.* Infect Genet Evol, **12**, 1788-1809.
31. **Uilenberg G (2006):** *Babesia—a historical overview.* Vet Parasitol, **138**, 3-10.
32. **Uiterwijk M, Vojta L, Šprem N, et al** (2023): *Diversity of Hepatozoon species in wild mammals and ticks in Europe.* Parasit Vectors, **16**, 1-12.