











Research article

Evidence of tick-borne pathogens in *Albanian equids* detected by molecular and serological methods

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Abstract

Equine piroplasmiasis (EP) is a tick-borne, non-contagious protozoal disease primarily caused by *Theileria equi* and *Babesia caballi*. This disease affects horses, mules, and donkeys, resulting in hemolytic anemia and systemic illness. The present study determined the seroprevalence of *T. equi* in equids from three regions of Albania (Gjirokastrë, Elbasan, and Durrës) using the Indirect Fluorescent Antibody Test (IFAT), and provided preliminary molecular data from Korça. A total of 139 serum samples were collected from clinically healthy equids, comprising 32 horses, 33 mules, and 74 donkeys, between April and September 2023. Samples were tested for *T. equi* antibodies using a commercial IFAT kit, with positive and negative controls included in all assays. The overall seroprevalence was 42.5% (59/139), with higher rates in mules (54.5%) and horses (53.1%) compared to donkeys (32.4%). Regionally, seroprevalence ranged from 37.8% in Durrës to 46.6% in Gjirokastrë. No statistically significant differences were detected among species, sex, or regions ($\chi^2 = 14.27$, $p = 0.113$). However, female mules exhibited the highest positivity rate (58.8%) and female donkeys the lowest (26.5%). The relatively high prevalence in mules and horses may be linked to increased exposure to tick vectors resulting from management practices. In Korça, molecular analysis of 50 equids identified 11 cases of *T. equi*, as well as single detections of *Babesia vulpes*, *Ehrlichia* sp., and *Mitochondria mitochondrii*. These findings confirm the circulation of these pathogens in the region. In conclusion, the combined serological and molecular findings demonstrate the presence of *T. equi* among equids in Albania and provide baseline epidemiological data. These results underscore the need for larger-scale studies to better characterize the prevalence, risk factors, and impact of EP on equine health nationwide.

Keywords: Albania, IFAT, Molecular method, Seroprevalence, *Theileria equi*, Tick-borne diseases

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Introduction

Equine piroplasmiasis (EP) represents a globally distributed tick-borne infection of equids, associated with intraerythrocytic apicomplexan parasites, mainly *Theileria equi* and *Babesia caballi*, while the recently described *Theileria haneyi* has further expanded the known etiological spectrum of the disease (Mendoza et al., 2024; Cabete et al., 2025; Cardillo et al.,

2026). These parasites can be transmitted by several tick species, including *Dermacentor*, *Hyalomma*, and *Rhipicephalus*, but iatrogenic and vertical transmission are also common (Mendoza et al., 2024). Clinical signs are generally nonspecific and may include lethargy, anorexia, fever, icterus, and peripheral edema, with severe cases potentially resulting in death

(Cabete et al., 2025).

T. equi establishes persistent infection in equids, with infected animals often remaining lifelong carriers and maintaining low-level parasitemia, whereas *Babesia caballi* is usually cleared after a few years (Onyiche et al., 2019). Equine piroplasmosis can compromise animal welfare and impose economic burdens due to restrictions on horse transport between endemic and non-endemic regions, reduced performance in sport horses, and treatment costs (Tirosh-Levy et al., 2020). EP shows variable prevalence, with *T. equi* typically more common than *B. caballi* in the Balkans, including neighboring regions of Albania (Vougiouka et al., 2013).

Ehrlichia is a genus of Gram-negative, obligate intracellular bacteria transmitted by ticks, including six recognized species (*E. canis*, *E. chaffeensis*, *E. ewingii*, *E. muris*, *E. ruminantium*, and *E. minasensis*) (Aziz et al., 2022; Molazadeh et al., 2023). Equine ehrlichiosis is primarily associated with *Neorickettsia* and *Anaplasma* species, and recent reports show that *Ehrlichia* can also infect horses in the United States and Central America (Molazadeh et al., 2023). These pathogens cause ehrlichiosis, a disease complex of growing veterinary and public health importance, by infecting leukocytes and endothelial cells of a variety of vertebrate hosts. *Ehrlichia* infections in domestic animals are frequently associated with fever, hematological abnormalities, and immunosuppression; in endemic areas, subclinical and persistent infections may lead to underdiagnosis and pathogen persistence (Ristic et al., 1991). Ehrlichiosis affects leukocytes and endothelial cells, producing a spectrum of outcomes from subclinical to acute disease, often associated with fever, hematologic abnormalities, immunosuppression, and persistent infection in endemic areas, highlighting its growing veterinary and public health importance (Aziz et al., 2022).

The intracellular bacterium *Midichloria mitochondrii*, a member of the Rickettsiales order, is unique because it is found only in the mitochondria of tick cells. It has been found in vertebrate hosts, including humans and animals, and is quite common in several tick species, especially *Ixodes* spp., suggesting potential transmission during blood feeding. *M. mitochondrii* is becoming more widely acknowledged as a possible indicator of tick exposure and may influence host–tick–pathogen

relationships, even if its pathogenic function is yet unknown. This requires additional research in veterinary epidemiology (Sassera et al., 2006).

Ehrlichia spp. *Ehrlichia* spp. infections in horses have occasionally been documented, with many infected animals exhibiting unclear clinical signs or remaining asymptomatic, and the epidemiological significance of these infections in equids is increasingly recognized as molecular and serological evidence accumulates in diverse geographical regions (Muraro et al., 2021; Tsachev et al., 2019). The molecular detection of *Ehrlichia* DNA in horse hosts underscores the need for focused surveillance to better understand their incidence, clinical importance, and potential involvement in equine tick-borne disease complexes (Tyrrell et al., 2019).

Although several seroprevalence studies have been conducted in neighboring countries, such as Greece (Garcia-Bocanegra et al., 2013) and other Balkan regions (Davitkov et al., 2017), data from Albania remain limited. Considering the importance of equids in local agriculture and transportation, understanding the distribution of EP in the country is essential.

The aim of this study was to investigate the occurrence of major equine tick-borne pathogens in Albania by assessing the seroprevalence of *Theileria equi* in equids using Indirect Fluorescent Antibody Test (IFAT), and by conducting a complementary molecular screening for selected pathogens, including *T. equi*, *Ehrlichia* spp., *Babesia* spp., and *Midichloria mitochondrii*, in equids from the Korça region. The study aims to provide baseline epidemiological data to better understand the emergence of equine tick-borne diseases in Albania and to support future surveillance and control efforts.

Materials and methods

Sampling

A total of 139 equids (32 horses, 33 mules, and 74 donkeys) were randomly sampled from the districts of Gjirokastrë, Elbasan, and Durrës from April to September 2023. The sample size was calculated assuming an expected prevalence of 10%, a 95% confidence level, and a precision of 5%. The standard formula for sample size estimation for proportions was applied: $n = (z^2 \times p \times q) / d^2$, where z is the z -value for 95% confidence (1.96), p is the assumed prevalence (0.10), $q = 1 - p$, and d is the desired precision (0.05). This calculation provided the minimum

number of animals required to reliably estimate *Theileria equi* prevalence in the study population. Blood samples were collected by jugular venipuncture from clinically healthy adult animals, and sera were separated and stored at -20°C until analysis. Serological testing for *T. equi* antibodies was performed using a commercial IFAT kit following [Farkas et al. \(2013\)](#), including positive and negative controls. Data were analyzed statistically using chi-square tests, with significance set at $p < 0.05$. A total of 50 blood samples were collected from equines, including horses, mules, and donkeys, across various administrative units in the Korça region (Tren, Grabovicë, Vercun, Sheqeras). Whole blood was drawn from the jugular vein using sterile EDTA-coated vacutainer tubes and was used for molecular analysis. In parallel, 50 blood samples were collected in plain vacutainer tubes for serological analyses. After 30-60 minutes, the samples were transported to the laboratory using a cold box, and equine serum was harvested. All samples were stored at -20°C until further testing.

The collected blood samples were pre-treated with Proteinase K (Roche, Penzberg, Germany) at 56 °C to inactivate potential contaminants and facilitate enzymatic cell lysis. This step prepared the samples for subsequent enzymatic digestion, enhancing DNA extraction efficiency. Genomic DNA was extracted at the Laboratory of Food Safety and Veterinary Institute, Tirana, using the Zeesan Lab-Aid 824 automatic extractor (Xiamen Zeesan Biotech Co., Xiamen City, Fujian Province, P.R. China) in combination with the Lab-Aid 824s DNA Extraction Kit (Xiamen Zeesan Biotech Co., Xiamen City, Fujian Province, P.R. China), following the manufacturer's protocol. This automated system ensures high-throughput, contamination-free DNA isolation with optimized yield and purity, providing high-quality nucleic acids suitable for downstream molecular analyses.

IFAT

The IFAT was used to assess the seroprevalence of *Theileria equi* in equines. Harvested equine sera, which were kept at -20 °C until analysis. IFAT was carried out using *T. equi*-infected erythrocytes on antigen-coated slides. Serum was titrated using serial two-fold dilutions after screening at a predetermined cut-off dilution. Slides were reacted with a FITC-conjugated anti-equine IgG, mounted, and assessed by

fluorescence microscopy following incubation and PBS washing (MegaScreen® Fluotheileria equi, Megacor, GmbH, Austria). Each test contained both positive and negative control sera, and samples exhibiting specific intraerythrocytic parasite fluorescence were considered seropositive ([Figure 1](#)).

Molecular analyses

PCR amplification for the detection of *Anaplasma* spp., *Babesia* spp., *Theileria* spp., and *Ehrlichia/Anaplasma* (*Anaplasmataceae*) was performed at the Croatian Veterinary Institute / Croatian Veterinary Laboratory (Zagreb, Croatia). *Babesia/Theileria* were amplified using a nested PCR targeting the 18S rRNA gene. Multiple PCR protocols were used for *Theileria* and *Anaplasma/Ehrlichia* to accommodate different diagnostic purposes. For each genus, one protocol employed genus-specific primers to detect the presence of the entire genus (*Theileria*: P1/P2, 18S rRNA; *Anaplasmataceae*: EC9/EC12A, 16S rRNA), providing a broad screening tool. A second protocol used species- or clinically relevant-specific primers (*Theileria*: Bab F/Bab R; *Anaplasma/Ehrlichia*: EHR16SD/EHR16SR) to identify particular species or generate PCR products suitable for sequencing and phylogenetic analysis. Differences in fragment size, annealing temperature, and number of cycles reflect optimization for specificity, amplification efficiency, and intended downstream applications ([Hacilarlioglu et al, 2025](#); [Elsawy et al, 2021](#); [WAOH, 2021](#)).

Primers targeting the 18S rRNA gene of *Babesia* and *Theileria* were used (Bab F: 5'-GTTTCTGMCCCATCAGCTTGAC-3'; Bab R: 5'-CAAGACAAAAGTCTGCTTGAAAC-3'), generating an expected ~660 bp amplicon under the conditions described. PCR reactions were prepared in a total volume of 50 µl, consisting of 25 µl 2X master mix, 1 µl of each primer, 18 µl nuclease-free water, and 5 µl DNA template. Thermal cycling conditions included an initial denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, annealing at 53 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 7 min, and storage at 4 °C until analysis. Amplified products were visualized using agarose gel electrophoresis: 1.5% agarose gel for fragments ≤660 bp (*Babesia*, *Theileria*, *Anaplasma/Ehrlichia* 345 bp) and compared with an appropriate DNA size marker to confirm the expected fragment lengths ([WAOH,](#)

2021).

PCR amplification and gel electrophoresis for *Theileria* detection: Genus-specific primers targeting the hypervariable region of the 18S rRNA gene were used to detect *Theileria* spp.

The forward primer P1 (5'-CACAGGGAGGTAGTGACAAG-3') and reverse primer P2 (5'-AAGAATTTACCTATGACAG-3') amplify a fragment of approximately 426–430 bp. PCR reactions were performed in a total volume of 50 µl, containing 25 µl 2X master mix, 1 µl of each primer, 18 µl nuclease-free water, and 5 µl DNA template. Thermal cycling conditions consisted of an initial denaturation at 94 °C for 2 min, followed by 40 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 7 min, and storage at 4 °C until analysis. Amplified products were visualized using 1.5% agarose gel electrophoresis and compared with a DNA size marker to confirm the expected fragment length (Hacilarlioglu et al, 2025; Elsayw et al, 2021).

PCR amplification and gel electrophoresis for Anaplasmatataceae (*Ehrlichia/Anaplasma*) detection: To detect *Anaplasmatataceae* spp., genus-specific primers targeting the 16S rRNA gene (EC9: 5'-TACCTTGTTACGACTT-3'; EC12A: 5'-TGATCCTGGCTCAGAACGAACG-3') were used to amplify a fragment of approximately 1460 bp. PCR reactions were prepared in a total volume of

50 µl, containing 25 µl 2X master mix (Promega), 1 µl of each primer, 18 µl nuclease-free water, and 5 µl DNA template. Thermal cycling included an initial denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 7 min. Amplified products were visualized using 0.8–1% agarose gel electrophoresis and compared to a DNA size marker to confirm the expected fragment length (Hacilarlioglu et al, 2025; Elsayw et al, 2021).

Statistical analysis

The Epi Toolbox was used for basic epidemiological summaries and for initial data curation, cleansing, and structuring. In addition to basic descriptive outputs, prevalence estimates and corresponding 95% confidence intervals were computed using the Toolbox. R (version 4.2) was used for subsequent statistical analysis and visualization, with the tidyverse for data processing and ggplot2 for graphics. We used R to revise prevalence estimates, apply binomial confidence intervals, offer summaries at the species and district levels, and create line, bar, and point-interval graphs to illustrate trends, regional patterns, and uncertainty. All analyses were designed to ensure full reproducibility and unambiguous workflow documentation.

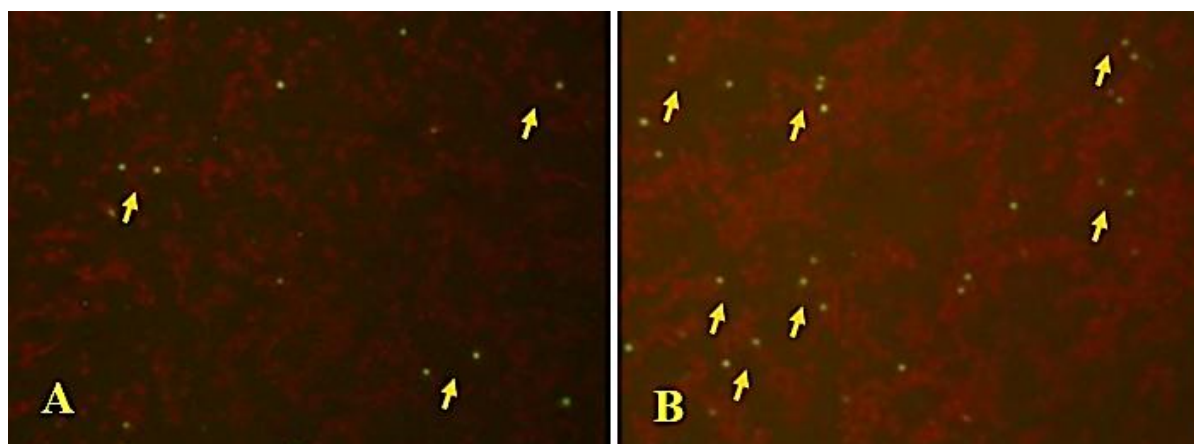


Figure 1: The Indirect Fluorescent Antibody Test (IFAT). Fluorescent signals are indicated by arrows. Panel A shows few scattered signals, indicating a low level of the target. Panel B shows more numerous signals, suggesting increased presence or activity.

Results

Serological test results.

A total of 139 equids from Gjirokaštër, Elbasan, and Durrës districts were tested for *Theileria equi* antibodies using IFAT, revealing an overall seroprevalence of 42.44%. Table 1 shows the

seroprevalence by species or hybrid and gender: mules had the highest positivity rate (54.54%), followed by horses (53%) and donkeys (32.4%). Female mules had the highest seroprevalence within gender groups (58.8%), while female donkeys had the lowest (26.47%). In addition, PCR of 50 equids from Korçë detected *T. equi* in

10 animals, along with sporadic detection of *Babesia vulpes* (1), *Ehrlichia* sp. (1), and *Midichloria mitochondrii* (1), confirming the presence of multiple hemoparasites and tick-borne bacteria in the study population. Positive samples for *Theileria equi* were found in horses and donkeys across different locations.

Horses were the most common species in the dataset; mules and Donkeys represent a smaller portion of the samples. Most samples come from Tren, Grabovicë, Vercun, Sheqeras, and Shqitas. Grabovicë has the highest number of donkeys sampled. *Theileria equi* (most frequently detected parasite), found in multiple locations, affects both horses and donkeys, with the most affected location: Sheqeras (5 positive cases), *Babesia vulpes* detected in 1 donkey from Grabovicë, *Ehrlichia* sp. detected in 1 horse from Vercun, and *Midichloria mitochondrii* detected in 1 mule from Tren. Table 2 presents seroprevalence by region and species. Gjirokastër had the highest overall positivity (46.6%), followed by Elbasan (40.9%) and Durrës (37.8%). Across all three regions, mules and horses consistently showed higher seroprevalence than donkeys.

The IFAT seroprevalence in equids is consistently high across all three geographical regions, with levels often ranging from one-third to half of the tested animals. In every district, mules had the highest seroprevalence (50–53.8%), followed by horses (40–50%) and donkeys (33.3–40.7%) (Figure 2). Durrës has somewhat lower rates, especially for donkeys, but Gjirokastër exhibits the greatest total levels for all three species. These results indicate significant and extensive exposure of working equids to the targeted pathogens, with mules appearing to be most affected, and Gjirokastër emerging as a priority region for additional research and management.

Point estimates and 95% confidence intervals for IFAT seroprevalence across equid species and districts are shown in Figure 2. The overlapping confidence intervals reflect limited sample sizes but underscore the broad and widespread exposure of equids to the targeted pathogens across regions. The modest range across districts indicates persistently elevated regional risk, which requires sustained monitoring and tailored vector-control actions.

Molecular test results

A total of 50 animals, 37 horses, 8 donkeys, and 5 mules, were tested for certain tick-borne

infections, and the results are shown in Table 3.

The most common parasite found was *Theileria equi*, which had an overall prevalence of 20.0% (10/50; 95% CI: 11.2–33.0). Mules (20.0%, 1/5), donkeys (12.5%, 1/8), and horses (21.6%, 8/37) were among the positive animals. One donkey (12.5%; 95% CI: 2.2–47.1) had DNA of *Babesia vulpes*, indicating an overall prevalence of 2.0% (1/50; 95% CI: 0.4–10.5). One horse (2.7%; 95% CI: 0.5–13.8) had *Ehrlichia* spp., indicating an overall prevalence of 2.0% (1/50; 95% CI: 0.4–10.5). One mule (20.0%; 95% CI: 3.6–62.4) had *Midichloria mitochondrii*, while the total prevalence was 2.0% (1/50; 95% CI: 0.4–10.5).

Theileria equi is the most common pathogen found in horses, according to the plot, with an overall prevalence of roughly 20% and large confidence intervals (95% CI 11.2–33.0) due to the small sample size. On the other hand, *Midichloria mitochondrii*, *Babesia vulpes*, and *Ehrlichia* spp. were all found at very low prevalence (~2%), with wide uncertainty intervals suggesting infrequent occurrence. Overall, the results indicate a modest yet discernible circulation of several tick-borne pathogens, with *T. equi* predominating.

Discussion

These findings demonstrate a widespread presence of *Theileria equi* in Albanian equids, with some variation across species and geographic locations. The seroprevalence of *T. equi* in Albanian equids (42.44%) is comparatively higher than reported in some Balkan and European studies (Vougiouka et al., 2013; García-Bocanegra et al., 2013; Davitkov et al., 2017), as well as in more recent regional and European surveys. For example, a molecular prevalence of 24.74% was reported in equids from the Western Aegean region of Türkiye (Hacilarlioglu et al., 2025), a seroprevalence of 32.7% by cELISA and up to 40.5% by qPCR in Portugal (2024), and a PCR-based prevalence of 36.3% in Italian Standardbred horses (Coluccia et al., 2024).

These elevated prevalence levels suggest that *T. equi* is endemic in Albania, likely maintained by favorable environmental conditions and tick vectors, and underscore the need for ongoing surveillance and tailored control strategies.

The highest seropositivity observed in mules (54.54%) could be explained by their increased exposure to tick habitats and possible

immunological factors related to their hybrid nature (Ikadai et al., 2006; Labruna et al., 2001).

Table 1: IFAT results by species and gender.

Species / Gender	Positive samples/Total tested samples	Percentage (95% CI)
Male horses	11/20	55 (34.2-74.2)
Female horses	6/12	50 (25.4-74.6)
Total horses	17/32	53 (36.4-69.1)
Female mules	10/17	58.8 (36-78.4)
Male mules	8/16	50 (28.0-72.0)
Total mules	18/33	54.54 (38-70.2)
Female donkeys	9/34	26.47 (14.6-43.1)
Male donkeys	15/40	37.5 (24.2-53.0)
Total donkeys	24/74	32.4 (22.9-43.7)
Overall total	59/139	42.4 (34.5-50.8)

Table 2: IFAT results by region and species.

Region	Species	Positive samples/Total tested samples	Percentage (CI 95%)
Gjirokastrë	Horses	9/18	50 (29-71)
	Mules	7/13	53.8 (29.1-76.8)
	Donkeys	11/27	40.7 (24.5- 59.3)
	Total	27/58	46.6 (34.3-59.2)
Elbasan	Horses	4/9	44.4 (18.9-73.3)
	Mules	6/12	50 (25.4-74.6)
	Donkeys	8/23	34.8 (18.8-55.1)
	Total	18/44	40.9 (27.7-55.6)
Durrës	Horses	2/5	40 (11.8-76.9)
	Mules	4/8	50 (21.5-78.5)
	Donkeys	8/24	33.3 (18-53.3)
	Total	14/37	37.8 (24.1-53.9)
Overall total		59/139	42.5 (34.5-50.8)

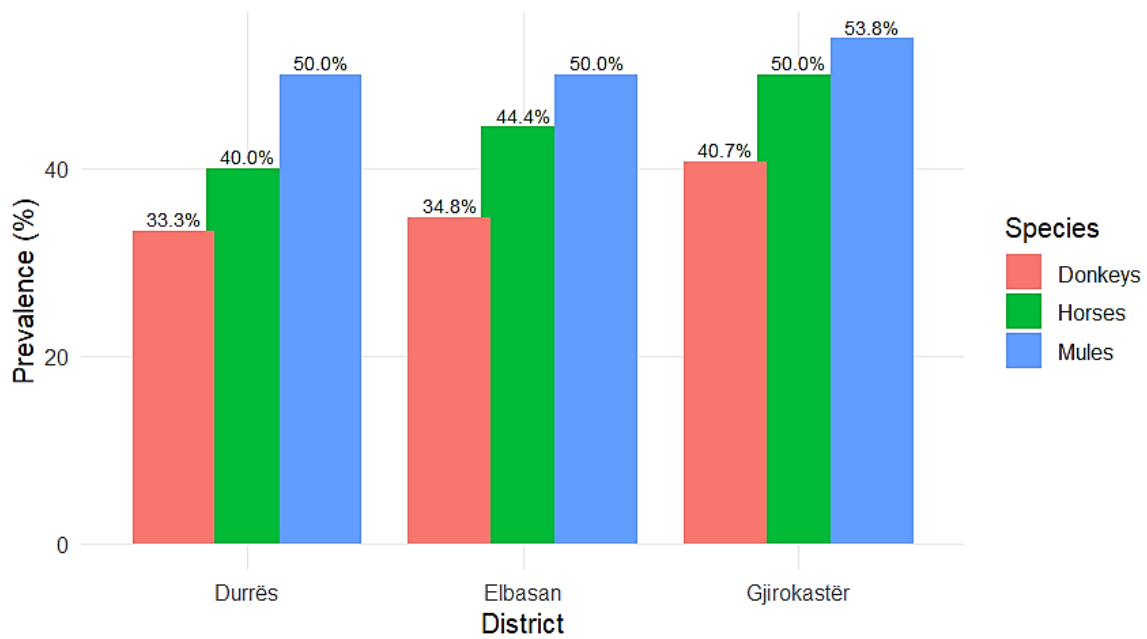


Figure 2: IFAT seroprevalence of equids by district and species in Albania. For every species, the incidence rises steadily from Durrës to Gjirokaštër. In every district, mules are the most frequently seen, followed by horses, while donkeys are the least frequent. Given that Gjirokaštër has the largest overall load, this pattern points to a potential geographic or management-related influence.

Table 3: PCR detection of hemoparasites and tick-borne bacteria in equids from the Korçë district.

Species	Tested (n)	<i>Theileria equi</i>	<i>Babesia vulpes</i>	<i>Ehrlichia spp.</i>	<i>Midichloria mitochondrii</i>
		Positive (n, %, 95%CI)	Positive (n, %, 95%CI)	Positive (n, %, 95%CI)	Positive (n, %, 95%CI)
Horses	37	8 (21.6%/11.4-37.2)	–	1 (2.7%/ 0.5-13.8)	–
Donkeys	8	1 (12.5%/ 2.2-47.1)	1 (12.5%/2.2-47.1)	–	–
Mules	5	1 (20%/ 3.6-62.4)	–	–	1 (20%/3.6-62.4)
Total	50	10 (20%/11.2-33.0)	1 (2%/0.4-10.5)	1 (2%/0.4-10.5)	1 (2%/0.4-10.5)

In several epidemiological surveys of equine piroplasmiasis, mules and other non-horse equids often exhibit higher exposure to parasites than horses, potentially reflecting differences in grazing behavior, management, and vector contact (Onyiche et al., 2020). Furthermore, sex-specific differences in seropositivity, as observed with higher prevalence in female mules (58.8%), may be influenced by physiological states such as pregnancy and lactation that modulate immune responses and susceptibility to tick-borne pathogens, a pattern also observed in recent European surveys (Axt et al., 2024). These findings highlight both classical immunological explanations and contemporary epidemiological evidence of increased risk of parasitemia in mules, underscoring the need for focused surveillance and risk-factor analysis.

The lower prevalence of *Theileria equi* in donkeys may reflect reduced exposure to tick vectors and species-specific biology. Donkeys usually move less, graze more selectively, and are

often kept closer to households or stabled more frequently than horses and mules, which can limit contact with tick-infested pastures. In addition, physiological and immunological differences, such as more effective skin barriers and a potentially stronger innate response to tick bites and hemoparasites, may further reduce the likelihood of successful infection and help control parasitemia once exposure occurs. No statistically significant differences were found between species or gender groups ($p = 0.113$), indicating widespread exposure across equid populations. This observation is consistent with meta-analytical evidence showing that equine piroplasmiasis and other tick-borne pathogens circulate broadly among horses, donkeys, and mules, with no clear patterns of prevalence by species or sex in many settings (Onyiche et al., 2020; Nadal et al., 2022). Such widespread transmission reflects the complex tick-host transmission cycle and varied ecological determinants that promote pathogen

dissemination across multiple equid host categories (Axt et al., 2024). Though tick presence was not directly assessed, tick infestation is the primary transmission route for *T. equi* (Labruna et al., 2001; Kerber et al., 2009). Further investigations on tick species distribution and seasonality in Albania are warranted.

Regional variation in seroprevalence was observed, with higher rates in Gjirokastër, which may reflect differences in local climate, tick abundance, and equid management practices. Similar patterns have been documented in other European regions; for example, in Andalusia, Spain, local climatic and ecological differences influenced tick populations and pathogen prevalence among equids (Duaso et al., 2025). These findings highlight the importance of considering environmental and management factors when assessing risk and planning surveillance programs in endemic areas.

Molecular detection in the Korçë equine population revealed multiple hemoparasites and tick-borne bacteria among domestic equids. *Theileria equi* was most frequently detected in horses, confirming its widespread circulation and risk for equine piroplasmiasis (Wise et al., 2013; Mans et al., 2015). *Theileria equi* was most frequently detected in horses, confirming its widespread circulation and risk for equine piroplasmiasis. Recent molecular surveys continue to report high prevalence of *T. equi* in horses, such as in Mexico, where up to 78.8% of horses were positive by PCR or serology (Salinas-Estrella et al., 2022), and in Kyrgyzstan where 24.8% of horses were PCR positive for *T. equi* (Berdikulov et al., 2024). Global meta-analyses also show that *T. equi* prevalence is generally higher compared to *B. caballi* across studies from multiple regions, underscoring its widespread presence (Onyiche et al., 2020).

Most positive cases were observed in horses, suggesting either higher susceptibility or increased exposure to vectors, such as ticks and hematophagous insects, in this species. It is important to note that the number of horse samples analyzed in Korçë was higher than that of mules and donkeys, which may partly explain the higher number of positive cases observed in horses. Therefore, these results suggest a similar exposure trend across all equid species, rather than indicating higher susceptibility of horses. Sporadic detection of *Babesia vulpes* (donkey), *Ehrlichia* sp. (horse), and *Midichloria mitochondrii*

(mule) confirms the presence of multiple pathogens with potential veterinary and zoonotic relevance, although their prevalence remains low.

With an overall prevalence of 20%, the current study confirms the circulation of *Theileria equi* among horses in Albania, supporting earlier findings that equine piroplasmiasis is still prevalent in the area. The wide host susceptibility and potential importance of all equid species in sustaining transmission cycles are highlighted by detection across horses, donkeys, and mules.

Given that *Babesia vulpes* is primarily associated with canids and that its presence in a donkey indicates either accidental exposure or a wider host range than previously thought, the low detection rate of this parasite is notable. However, these results should be interpreted with caution, given the small sample size.

As noted in more comprehensive European and global reviews of equine piroplasmiasis, northern European nations often exhibit far lower prevalence (<5%), which is consistent with colder climates and reduced activity of competent vectors (Onyiche et al., 2019). The sporadic detection of *Babesia vulpes*, *Ehrlichia* spp., and *Midichloria mitochondrii* in Albanian equids mirrors their low frequency in most European surveys. *B. vulpes* is primarily associated with canids and is only occasionally reported outside this host group, while *M. mitochondrii* is a mitochondrial endosymbiont of *Ixodes ricinus* that is sometimes detected as a by-catch signal in vertebrate samples rather than as a primary pathogen. Overall, the Albanian data fit the Mediterranean–Balkan epidemiological profile, characterized by moderate *T. equi* endemicity and sporadic occurrence of other tick-borne agents. Continued surveillance with larger sample sizes will be important to determine whether these patterns remain stable under climate and land-use change (Onyiche et al., 2019).

The sporadic detection of *Babesia vulpes*, *Ehrlichia* spp., and *Midichloria mitochondrii* mirrors the pattern in most European studies, where these agents are typically detected at very low prevalence or in isolated cases. *Babesia vulpes* is better known in canids, and equid infections remain rare, consistent with the single positive identified here. *Midichloria mitochondrii*, a symbiont of *Ixodes ricinus*, is occasionally detected in equids across central and southern Europe, often at <5%, matching the low

frequency observed in Albania (Sassera, 2006). *Mitochondria* *mitochondrii* is a widespread endosymbiont of *Ixodes ricinus*, residing in ovaries and detected in salivary glands, which suggests potential transfer to vertebrate hosts during blood feeding (Sassera et al., 2006; Cafiso et al., 2019). Although detailed prevalence studies in equids are limited, its presence at low levels in vertebrate hosts is consistent with the low frequency observed in Albanian equids.

Taking all consideration, our results highlight the complexity of tick-borne pathogen circulation in horses and stress the significance of combined genetic and serological surveillance to better understand dynamic infection and potential health consequences in both animals and humans.

Overall, the Albanian equid results closely resemble the Mediterranean–Balkan epidemiological profile, characterized by moderate *T. equi* prevalence and sporadic detection of other tick-borne agents. Continued monitoring with larger sample sizes will be essential to determine whether these patterns reflect stable endemicity or evolving tick-borne disease risks associated with climate and land-use change (Nadal et al., 2022; Onyiche et al., 2019).

Although most infections were subclinical, their detection is epidemiologically significant. The presence of these pathogens across multiple locations underlines the importance of regular surveillance, particularly in areas with frequent animal movement, shared pastures, or limited veterinary control. These results emphasize the importance of implementing integrated control strategies that combine tick control, regulation of animal movements, and regular screening (Wise et al., 2013; OIE, 2012).

Limitations

Due to variable and very small sample sizes across species and geographical areas, this study has limitations that lead to wide confidence intervals and reduced precision of estimates. PCR-based prevalence cannot be extrapolated to all Albanian equids because molecular analysis was limited to the Korçë district. The cross-sectional methodology does not capture seasonal variations or differentiate between recent and ongoing diseases, and the fact that the majority of the animals included were working animals provided by their owners raises the possibility of selection bias. Furthermore, comprehensive risk-

factor data (management, grazing, acaricide use) and tick vectors were not collected, which limited the interpretation of the factors underlying the observed trends. Future research should integrate PCR and serology in all areas and extend sampling to other districts with greater, more evenly distributed populations of horses, mules, and donkeys. In addition to concurrent tick collection and pathogen screening, longitudinal surveillance would help identify vectors and hotspots and shed light on transmission dynamics. Quantifying the actual burden of these illnesses will be made easier by including risk-factor data and evaluating clinical and economic effects. Lastly, to promote evidence-based treatment of tick-borne diseases in Albanian equids, molecular characterization of identified pathogens and assessment of focused control approaches (such as optimal tick control) are required.

Conclusions

This study provides the first data on *Theileria equi* seroprevalence in Albanian equids, demonstrating a substantial level of exposure among horses, mules, and donkeys. The combination of serological and molecular findings highlights the widespread presence of *T. equi* across multiple regions, with higher prevalence in mules and some districts, and confirms the occurrence of additional blood parasites in Korça. These results provide a baseline for future epidemiological studies and monitoring. Efforts to raise awareness among veterinarians and equid owners about the disease and its transmission, together with strategies for tick management and equid health monitoring, will support informed interventions and help maintain equid health in Albania

Article Information

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References

- Axt, C.W., Springer, A., Strube, C., Jung, C., Naucke, T.J., Müller, E. & Schäfer, I., 2024. Molecular and serological detection of vector-borne pathogens responsible for equine piroplasmosis in Europe between 2008 and 2021. *Microorganisms*, 12(4), p.816.
- Aziz, M.U., Hussain, S., Song, B., Ghauri, H.N., Zeb, J., Sparagano, O.A.E. 2022. Ehrlichiosis in dogs: a comprehensive review about the pathogen and its vectors with emphasis on South and East Asian countries. *Veterinary Sciences* 10(1), 21. <https://doi.org/10.3390/vetsci10010021>
- Berdikulov, A., Atambekova, Z., Aitakin, K., Rysbek, N., Jailobek, O., Ahedor, B., Mumbi, N.N.M., Ma, Y., Otgonsuren, D., Perera, W.P.P.S.I., Guswanto, A., Sivakumar, T. & Yokoyama, N., 2024. *Molecular prevalence and genotypic diversity of Theileria equi and Babesia caballi infecting horses in Kyrgyzstan*. *Parasitology International*, 102, p.102915. <https://doi.org/10.1016/j.parint.2024.102915>.
- Cabete, A., Xufre, À., Padre, L., Bettencourt, E., Nunes, T. & Gomes, J., 2025. *Occurrence and risk factors of equine piroplasmosis in Portugal: a five-year retrospective study*. *Veterinary Parasitology*, 334, p.110378. <https://doi.org/10.1016/j.vetpar.2024.110378>.
- Cafiso, A., Sasser, D., Romeo, C.R., Serra, V., Hervet, C., Bandi, C., Plantard, O. & Bazzocchi, C., 2019. *Midichloria mitochondrii*, endosymbiont of *Ixodes ricinus*: evidence for the transmission to the vertebrate host during the tick blood meal. *Ticks and Tick-Borne Diseases*, 10(1), pp.5–12. <https://doi.org/10.1016/j.ttbdis.2018.08.008>.
- Cardillo, N., Villarino, N.F., Kappmeyer, L.S., Chung, C.J., Suarez, C.E., Bastos, R.G. 2026. Tafenoquine succinate inhibits the growth of the equine piroplasmosis hemoparasites *Theileria equi* and *Babesia caballi*. *Parasites & Vectors* 19, 61. <https://doi.org/10.1186/s13071-026-061>
- Coluccia, P., Gizzarelli, M., Scicluna, M.T., Manna, G., Foglia Manzillo, V., Buono, F., Auletta, L., Palumbo, V. & Pasolini, M.P., 2024. *A cross-sectional study on performance evaluation in Italian standardbred horses' real-time PCR-positive for Theileria equi*. *BMC Veterinary Research*, 20(1), p.79. <https://doi.org/10.1186/s12917-024-03908-0>
- Davitkov, D., Vucicevic, M., Stanisic, L., Radakovic, M., Glavinic, U., Stanimirovic, Z. 2017. A molecular and haematological study of *Theileria equi* in Balkan donkeys. *Acta Veterinaria Hungarica* 65, 234–241. <https://doi.org/10.1556/004.2017.023>
- Duaso, J., Pérez-Écija, A., Navarro, A., Martínez, E., De las Heras Sánchez, A. & Mendoza, F.J., 2025. *True prevalence and seroprevalence of piroplasmosis in horses in Southwestern Europe*. *Animals*, 15(14), p.2047. <https://doi.org/10.3390/ani15142047>
- Elsawy BSM, Nassar AM, Alzan HF, Bhoora RV, Ozubek S, Mahmoud MS, Kandil OM, Mahdy OA. 2021. Rapid Detection of Equine Piroplasms Using Multiplex PCR and First Genetic Characterization of *Theileria haneyi* in Egypt. *Pathogens* 10(11):1414. <https://doi.org/10.3390/pathogens10111414>.
- Farkas, R., Tánzos, B., Gyurkovszky, M., Földvári, G., Solymosi, N., et al., 2013. Serological and molecular detection of *Theileria equi* infection in horses in Hungary. *Veterinary Parasitology* 192, 143–148. <https://doi.org/10.1016/j.vetpar.2012.09.035>
- Hacilarlioglu, S., Bilgic, H.B., Karagenc, T., Aydin, H.B., Toker, H., Kanlioglu, H., Pekagirbas, M. & Bakirci, S., 2025. *Molecular detection and prevalence of equine piroplasmosis and other blood parasites in equids of Western Aegean Türkiye*. *Veterinary Sciences*, 12(9), p.826. <https://doi.org/10.3390/vetsci12090826>
- Ikadai, H., Ishida, H., Sasaki, M., Taniguchi, K., Miyata, N., et al., 2006. Molecular cloning and partial characterization of *Babesia equi* EMA-3. *Molecular and Biochemical Parasitology* 150, 371–373. <https://doi.org/10.1016/j.molbiopara.2006.08.004>
- Kerber, C.E., Labruna, M.B., Ferreira, F., De Waal, D.T., Knowles, D.P., et al., 2009. Prevalence of equine piroplasmosis and its association with tick infestation in the State of São Paulo, Brazil. *Revista Brasileira de Parasitologia Veterinária* 18, 1–8. <https://doi.org/10.4322/rbvp.01804001>
- Labruna, M.B., Kerber, C.E., Ferreira, F., Faccini, J.L., De Waal, D.T., et al., 2001. Risk factors to tick infestations and their occurrence on horses in the state of São Paulo, Brazil. *Veterinary Parasitology* 97, 1–14. [https://doi.org/10.1016/S0304-4017\(01\)00387-9](https://doi.org/10.1016/S0304-4017(01)00387-9)
- Mans, B.J., Pienaar, R., Latif, A.A. 2015. A review of *Theileria* diagnostics and epidemiology. *International Journal for Parasitology: Parasites and Wildlife* 4, 104–118. <https://doi.org/10.1016/j.ijppaw.2014.12.006>
- Mendoza, F.J., Pérez-Écija, A., Kappmeyer, L.S., Suarez, C.E., Bastos, R.G. 2024. New insights in the diagnosis and treatment of equine piroplasmosis: pitfalls, idiosyncrasies, and myths. *Frontiers in Veterinary Science* 11, 1459989. <https://doi.org/10.3389/fvets.2024.1459989>
- Molazadeh, S., Tukmechi, A., Hadian, M., Dalir-Naghadeh, B. 2023. Phylogenetic analysis of *Ehrlichia* species isolated from horses and dogs in Iran. *Comparative Immunology, Microbiology and Infectious Diseases* 103, 102080. <https://doi.org/10.1016/j.cimid.2023.102080>
- Muraro, L.S., de O Souza, A., Leite, T.N.S., Cândido, S.L., Melo, A.L.T., Toma, H.S., Carvalho, M.B., Dutra, V., Nakazato, L., Cabezas-Cruz, A., de Aguiar, D.M. 2021. First evidence of *Ehrlichia minasensis* infection in horses from Brazil. *Pathogens* 10(3), 265. <https://doi.org/10.3390/pathogens10030265>
- Nadal, C., Bonnet, S.I., Marsot, M. 2022. Eco-epidemiology of equine piroplasmosis and its associated tick vectors in Europe: a systematic literature review and a meta-analysis of prevalence. *Transboundary and Emerging Diseases* 69, 2474–2498. <https://doi.org/10.1111/tbed.14261>
- Onyiche, T.E., Suganuma, K., Igarashi, I., Yokoyama, N., Xuan, X., Thekisoe, O.M.M. 2019. A review on equine piroplasmosis: epidemiology, vector ecology, risk factors, host immunity, diagnosis and control. *International Journal of Environmental Research and Public Health* 16(10), 1736. <https://doi.org/10.3390/ijerph16101736>
- Onyiche, T.E., Taioe, M.O., Molefe, N.I., Biu, A.A., Luka, J., Omeh, I.J., et al., 2020. Equine piroplasmosis: an insight into global exposure of equids from 1990 to 2019 by systematic review and meta-analysis. *Parasitology* 147, 1411–1424. <https://doi.org/10.1017/S0031182020001407>
- Ristic, M., Holland, C.J., Khondowe, M. 1991. An overview of research on ehrlichiosis. *European Journal of Epidemiology* 7, 246–252. <https://doi.org/10.1007/BF00145082>
- Salinas-Estrella, E., Ueti, M.W., Lobanov, V.A., Castillo-Payró, E., Lizcano-Mata, A., Badilla, C., Martínez-Ibáñez, F. & Mosqueda, J., 2022. *Serological and molecular detection of Babesia caballi and Theileria equi in Mexico: a prospective study*. *PLoS ONE*, 17(3), e0264998. <https://doi.org/10.1371/journal.pone.0264998>
- Sasser, D., Beninati, T., Bandi, C., Bouman, E.A.P., Sacchi, L., Fabbri, M. et al., 2006. Candidatus *Midichloria mitochondrii*, an endosymbiont of the tick *Ixodes ricinus* with a unique intramitochondrial lifestyle. *International Journal of Systematic and Evolutionary Microbiology* 56, 2535–2540. <https://doi.org/10.1099/ijs.0.64386-0>
- Tirosh-Levy, S., Gottlieb, Y., Fry, L.M., Knowles, D.P., Steinman, A. 2020. Twenty years of equine piroplasmosis research: global distribution, molecular diagnosis, and phylogeny. *Pathogens* 9(11), 926. <https://doi.org/10.3390/pathogens9110926>
- Tsachev, I., Baymakova, M., Pantchev, N. 2019. *Seroprevalence of Anaplasma phagocytophilum, Ehrlichia spp. and Borrelia*

burgdorferi infections in horses: first report from Northern Bulgaria. *Acta Veterinaria Hungarica* 67(2), 197-203. <https://pubmed.ncbi.nlm.nih.gov/31518572/>

WOAH (formerly OIE) Terrestrial Manual: CHAPTER 3.6.8. 2021 <https://doi.org/10.1556/004.2019.021>

Tyrrell, J.D., Quorollo, B.A., Tornquist, S.J., Schlaich, K.G., Kelsey, J., Chandrashekar, R. & Breitschwerdt, E.B., 2019. Molecular identification of vector-borne organisms in Ehrlichia seropositive Nicaraguan horses and first report of *Rickettsia felis* infection in the horse. *Acta Tropica*, 200, p.105170. Available at: <https://doi.org/10.1016/j.vetpar.2012.10.019>

Equine Piroplasmosis. [https://www.woah.org/fileadmin/](https://www.woah.org/fileadmin/Home/fr/Health_standards/tahm/3.06.08_EQUINE_PIROPLASMOSIS.pdf)

Vougiouka, M., Papadopoulos, E., Koutinas, A.F., Tzora, A., Psaroulaki, A. 2013. Seroprevalence of *Theileria equi* and *Babesia caballi* in Greek horses. *Veterinary Parasitology* 191, 293–297. <https://doi.org/10.1016/j.vetpar.2012.10.019>