https://doi.org/10.18502/jad.v20i3.23177

Original Article

Molecular Detection, Phylogenetic Analysis and Hematological Characterization of *Ehrlichia* spp. and *Anaplasma* spp. Infections in Clinically Suspected Dogs in Tehran, Iran, 2025

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(Received 31 May 2026; accepted 11 Aug 2026)

Abstract

Background: Canine tick-borne diseases from *Ehrlichia* and *Anaplasma* pose rising veterinary and zoonotic risks. In Iran, data on their prevalence in dogs and ticks are scarce. This cross-sectional study examined molecular rates, phylogenetic traits, and blood disorders in high-exposure dogs, aiming to clarify infection patterns and health consequences in this understudied region.

Methods: Blood samples and ticks were collected from 138 dogs in Tehran City, divided into 3 groups (tick-infested indoors, tick-infested outdoors and thrombocytopenics without ticks) and used for hematological/biochemical profiling, molecular detection and phylogenetic analysis targeting the 16S rRNA gene.

Results: The study found molecular prevalence of *Ehrlichia canis* and *Anaplasma platys* at 21.7% and 11.6%, with outdoor dogs highest; indoor dogs had no *A. platys*. Infection rates peaked in 1–3-year-old dogs. *Rhipicephalus sanguineus* was the sole tick collected. Thrombocytopenia signaled TBD exposure, prompting molecular screening. Phylogenetic 16S rRNA analysis confirmed genus identity and placed samples within global clusters, yet revealed no region-specific lineages due to gene conservation. Ehrlichiosis showed lower hematocrit, hemoglobin, neutrophils, and monocytes, with higher leukocytes and lymphocytes versus anaplasmosis; thrombocytopenia was prominent in both. Elevated Alanine transaminase, Aspartate Aminotransferase and globulin, and reduced albumin, were more prominent in Ehrlichiosis.

Conclusion: These findings confirm active circulation of *E. canis* and *A. platys* in Tehran's canine population, with outdoor exposure as a key risk factor, and demonstrate that *E. canis* induces more pronounced clinicopathological abnormalities than *A. platys*.

Keywords: Dog diseases; *Anaplasma platys*; *Ehrlichia canis*; Thrombocytopenia; *Rhipicephalus sanguineus*

Introduction

Canine vector-borne diseases (CVBDs), particularly tick-borne diseases (TBDs), represent a group of globally distributed illnesses that pose significant veterinary and zoonotic concern. Dogs act as hosts and reservoirs for TBDs and hosts/carriers of ticks (1, 2). Pathogen spread is facilitated by human and dog mobility and climate change, aligning CVBD control with One Health (1, 3, 4). Two important TBDs of dogs, *Anaplasma phagocytophilum* and *Ehrlichia canis*, are members of the family Anaplasmataceae. Canine monocytic ehrlichiosis (CME), caused by *E. canis*, was first identified in Algeria in 1935 (5) and gained atten-

http://jad.tums.ac.ir
Published Online: September 30, 2026

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https://doi.org/10.18502/jad.v20i3.23177

tion in the 1970s (6). *Ehrlichia canis* is a Gram-negative, pleomorphic, obligate intracellular rickettsia targeting platelets, monocytes and granulocytes, with dogs serving as reservoirs and the *Rhipicephalus sanguineus* tick as the main vector (1, 7, 8). Canine monocytic ehrlichiosis exhibits three stages: acute, subclinical and chronic. The acute phase starts with a high fever, lethargy, anorexia, lymphadenomegaly, splenomegaly, hemorrhagic diathesis and ophthalmological and neurological signs. The subclinical phase has no overt clinical symptoms; dogs unable to eliminate the infection become asymptomatic carriers. The chronic phase presents with recurrent but more severe symptoms, pale mucous membranes, weakness, bleeding, weight loss and death (7, 9). Moderate to severe thrombocytopenia is prominent in acute CME, while the subclinical phase shows mild thrombocytopenia without symptoms. *Ehrlichia canis* may hide in the spleen in this phase (4). The chronic stage presents severe thrombocytopenia, anemia, leukopenia and pancytopenia due to bone marrow hypoplasia. Hypoalbuminemia, hypergammaglobulinemia and mild increases in Alanine transaminase and Alkaline phosphatase are frequent in acute CME (7).

Anaplasma phagocytophilum is an obligate intracellular gram-negative granulocytotropic rickettsia from the same family and order as *E. canis*, transmitted by *Ixodes* spp. ticks, targeting neutrophils/platelets, causing canine granulocytic anaplasmosis (CGA) with fever, lethargy, inappetence, weight loss and musculoskeletal pain. Hematological/biochemical changes include thrombocytopenia, anemia, neutrophil inclusions and increased liver enzymes (1, 10, 11). *Anaplasma platys* multiplies inside platelets, causing infectious canine cyclic thrombocytopenia (ICCT), transmitted by *Rh. sanguineus* ticks. Clinical signs are mild, but fever, lethargy, pale mucous membranes, petechial haemorrhages, epistaxis and lymphadenopathy may be observed. Thrombocytopenia cycles every 1–2 weeks (3, 12). Immunofluorescence antibody test (IFAT) and ELISA are used for diagnosis, with the benefit of determining disease stage and intensity. PCR is invaluable in detecting small amounts of DNA and diagnosing active infection early when serology gives false negatives (8).

There have been multiple studies that evaluated the prevalence of ticks and *Anaplasma* spp. and *Ehrlichia* spp. in various regions of Iran. *Hyalomma* spp. ticks were the most common, with *Anaplasma* spp. infections being the most prevalent among the studied ticks (13–15). However, these studies mostly targeted livestock populations. Several studies have documented the prevalence of *Ehrlichia* spp. and *Anaplasma* spp. in dogs in Iran, but data on paired molecular testing of dogs and attached ticks alongside phylogenetic analysis are limited (16). The present study aims to: 1) determine molecular prevalence of *Ehrlichia* spp. and *Anaplasma* spp. in domestic dogs in Tehran with suspected high-exposure (tick infestation or thrombocytopenia) and their ticks, 2) determine associations between signalment/exposure with TBDs, 3) identify and confirm genus/species via phylogenetic analysis, and 4) characterize hematological/biochemical alterations and their correlation with demographic/risk factors.

Materials and Methods

This cross-sectional study was conducted in Tehran, Iran, by collecting blood samples and/or ticks from dogs (presented to a private veterinary clinic, residing in shelters, or strays) between April 2025 and September 2025, as previous research suggests spring and summer have the highest tick prevalence in Tehran (17). Three groups of dogs with suspected high TBD exposure were included: 1) tick-infested indoor dogs, 2) tick-infested outdoor dogs, 3) dogs with marked thrombocytopenia. Sample size was determined based on a 95% confidence level, 80% test power and an estimated molecular prevalence of 10% (16), resulting

http://jad.tums.ac.ir
Published Online: September 30, 2026

https://doi.org/10.18502/jad.v20i3.23177

in the selection of 138 dogs between the ages of 7 months and 15 years. 2ml of blood was collected in Ethylenediaminetetraacetic acid (EDTA) tubes for complete blood count (CBC), 2ml in non-EDTA tubes for biochemistry, and 1ml stored at –27 °C for DNA extraction. Due to the fact that informed consent was acquired from owners and no additional procedures other than routine clinical diagnostics (blood samples and tick collection) were performed, ethical approval from the review board was not required.

CBC was performed using an automated hematology analyzer (Nihon Kohden Cell Tac Alfa). Serum was analyzed for Alanine transaminase, Aspartate Aminotransferase, Alkaline phosphatase, total protein, albumin and creatinine using the Mindray BS-200 automated chemistry Analyzer (Mindray Shenzhen, China) with standard commercial kits (Biorexfars, Iran). Engorged and semi-engorged adult ticks were collected from hosts (n=93), immersed in 70% ethanol and maintained at –27 °C, and identified based on identification keys (18), then homogenized and stored. DNA was extracted from ticks and whole blood using the SinaClon commercial tissue total DNA extraction kit and DNA Extraction kit (Blood) (SinaClon, Iran), respectively, per manufacturer's instructions. PCR assays were carried out using a BIO-RAD thermocycler model T100 (Bio-Rad, Germany) with 12.5 µL of 2× conventional PCR Master Mix (Ampliqon, Denmark), 0.5 µL (10 pmol/µL) of forward and backward primers (SinaClon, Iran), 3 µL of the DNA template (10ng/µL) and nuclease-free water, up to the final reaction volume of 25 µL. PCR settings were as follows: an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 30 seconds. A final extension phase was carried out at 72 °C for 5 min. Negative controls were utilized in all PCR runs to ensure accuracy and contained a PCR mixture without any DNA template. The efficiency of the PCR process and DNA extraction kits was considered 100%. Primer sequences (19, 20) are provided in Table 1. PCR products were analyzed on 1.5% agarose gel (21), as shown in Fig. 1.

Three positive PCR products for each gene were randomly selected and Sanger sequencing was performed using the same PCR primers and an ABI-PRISM 377 sequencer (ThermoFisher Scientific, USA). The obtained gene sequences were submitted to GenBank and analyzed using Geneious Prime 2025.0 and compared via BLAST. MEGA7 (Pennsylvania, USA) was used with 1000-replicate bootstrap. The maximum likelihood procedure was employed to infer phylogenetic relationships, and the Tamura-Nei model was automatically selected by MEGA7 because it had the lowest BIC value among all tested models, indicating the best fit for our dataset. *Wolbachia endosymbiont* of *Myzus persicae* (MG707967) and *Neorickettsia risticii* (NR029162) were used as outgroup taxa to root the trees, as they both were phylogenetically distinct enough from members of the genus in their respective trees, yet close enough to provide a proper outgroup (22–24).

Chi-square and Fisher's exact tests were used; $P \leq 0.05$ was considered significant. Hematological and biochemical parameters were compared between *Ehrlichia*-positive (n=30) and *Anaplasma*-positive (n=15) dogs using descriptive statistics (mean ± SD). Normality was evaluated using Shapiro-Wilk and Kolmogorov-Smirnov tests ($P>0.05$), and visual inspection of Q-Q plots (all variables were normally distributed) and parametric statistical tests (Welch's t-test, suitable for unequal sample sizes and unequal variances) were applied. Bonferroni correction was applied to account for multiple comparisons (Bonferroni-corrected alpha level: $P \leq 0.003$). GraphPad Prism version 10 for Windows (GraphPad Software, Boston, Massachusetts, USA, www.graphpad.com) was used for analysis. In addition, severe thrombocytopenia was defined as $<50 \times 10^3/\mu L$ and the reference range for Total protein was 5–7.2 g/dl.

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Published Online: September 30, 2026

https://doi.org/10.18502/jad.v20i3.23177

Results

138 dogs were included: 46 tick-infested outdoor dogs, 47 tick-infested indoor dogs, and 45 thrombocytopenic dogs. General characteristics of the population are provided in Table 2. All 93 ticks collected were identified as *Rhipicephalus* (55% male, 45% female, all adults).

Agarose gel electrophoresis of PCR products of 16S rRNA genes of both pathogens is shown in Fig. 1.

Molecular analysis revealed a prevalence of 21.7% (30/138) for *Ehrlichia* spp. and 11.6 % (16/138) for *Anaplasma* spp. in the studied dog population. Infection was significantly associated with group (p=0.018 for *Ehrlichia* and *p*=0.008 for *Anaplasma*). The "Outdoors" group had the highest prevalence of *Ehrlichia* infection (34.8%, 16/46), followed by "Thrombocytopenics" (20.0%, 9/45), then the "Indoors" group (10.6%, 5/47). For *Anaplasma*, only "Outdoors" (19.6%, 9/46) and "Thrombocytopenics" (15.6%, 7/45) were infected. Infections were significantly more prevalent in the 1–3 years age group (36.2%, 25/69 for *Ehrlichia*, p< 0.001 and 18.8%, 13/69 for *Anaplasma*, p= 0.024). Gender was not associated with infection (*Ehrlichia*: p= 0.518, *Anaplasma*: p= 0.683). Co-infection was observed in 6 dogs with no association between the two pathogens (p= 0.104).

Ticks' *Anaplasma* was significantly associated with corresponding dogs' *Anaplasma* (p< 0.001) or *Ehrlichia* (p= 0.043), while ticks' *Ehrlichia* showed a borderline non-significant association with dogs' *Ehrlichia* (p= 0.057) and no association with dogs' *Anaplasma* (p= 0.803). *Anaplasma* was more prevalent in ticks from outdoor versus indoor dogs (30.4% vs. 6.3%, p= 0.003), but *Ehrlichia* prevalence wasn't different (54.3% versus 42.5%, p= 0.255). *Ehrlichia* or *Anaplasma* infection was not significantly different in tick-infested dogs versus thrombocytopenics (p= 0.730, p= 0.312, respectively). Age and gender were not associated with tick presence (p= 0.336, p= 0.301, respectively).

Following hematological/ biochemical changes were observed: *Ehrlichia*-positive dogs (n= 30) showed thrombocytopenia (24/30, 80%), Anemia (20/30, 66.6%), leukopenia (3/30, 10 %), elevated Alanine transaminase (ALT) (11/ 30, 36%), elevated AST (5/30, 16.6%), low-normal (lower end of normal range) total protein range (9/30, 30%), decreased albumin (7/ 30, 23.3%) and elevated globulin (12/30, 40%). *Anaplasma* positive dogs (n=15) showed thrombocytopenia (9/15, 60.00%), leukocytosis/ neutrophilia (8/15, 53.3%), mild-moderate nonregenerative anemia (4/15, 26.6%), severe thrombocytopenia (<50× $10^{\Lambda}3$/µL, 2/15, 13%), mild ALT elevation (5/15, 33.33%), mild AST/ ALP elevation (15/15, 100%), Hypoalbuminemia (4/15, 26.66%) and mild hyperglobulinemia (3/15, 20.00%). Co-infection sample size (n= 6) was too small for reliable comparison. Comparison of hematological/biochemical alterations revealed *Ehrlichia* was associated with lower hematocrit (Mean Difference=3.617, 95 % Confidence Interval=2.153 to 5.082), hemoglobin (MD=6.479, 95% CI=5.912 to 7.046), neutrophil (MD=13.45, 95% CI=12.99 to 13.91) and monocyte (MD=4.748, 95% CI=4.330 to 5.166) values and higher leukocyte (MD=–0.8007, 95% CI= –0.9579 to –0.6435) and lymphocyte (MD= –15.15, 95% CI= –16.32 to –13.98) counts than *Anaplasma* (p< 0.0001). Platelet counts (p= 0.4675, MD=3.390, 95% CI= –6.149 to 12.93) and total protein (p= 0.01, MD= 0.3920, 95% CI= 0.09970 to 0.6843) levels were not significantly different between the two parasites. Higher ALT (MD= –18.50, 95% CI= –26.09 to –10.91), AST (MD= –19.02, 95% CI= –25.57 to –12.47), globulin (MD= –0.9047, 95% CI= –1.180 to –0.6297), creatinine (MD= –0.5980, 95% CI= –0.8210 to –0.3750), and lower albumin (MD= 0.9747, 95% CI= 0.7092 to 1.240) (p< 0.0001) levels were also observed in Ehrlichiosis (Figs. 2–3).

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Published Online: September 30, 2026

https://doi.org/10.18502/jad.v20i3.23177

GenBank submissions were made under the following accession numbers: Ehr1 (PX022686), Ehr2 (PX022789) and Ehr3 (PX022799) for *Ehrlichia* spp. and Ana1 (PX022804), Ana2 (PX022800), Ana3 (PX022802) for *Anaplasma*.16S rRNA sequences of *Ehrlichia*- and *Anaplasma*-positive dog blood samples were 389bp and 429bp in length, respectively. Phylogenetic analysis identified and placed the isolates within the *Anaplasma platys* and *Ehrlichia canis* clades, with other *Anaplasma* and *Ehrlichia* species forming separate clades. *E. canis* isolates FA2024E, FA2024E2, FA2024E3 clustered with others from Iran, India, Brazil, China, USA, Turkey, Greece, Japan and Spain (bootstrap value: 100%), while *A. platys* isolates FA2024A, FA2024A2, FA2024A3 grouped with global strains from Italy, Japan, China, Iraq, Taiwan, Colombia, Germany, Turkey, Spain, Cuba, India, South Africa, Portugal and Croatia (bootstrap value: 94%). *Neorickettsia risticii* and *Wolbachia* spp. were used as outgroups and clearly separated from the main clusters in respective trees (Figs. 4–5).

Table 1. Primer sequences targeting 16s rRNA as previously described by Seong et al. (2015) for *Ehrlichia canis* and Park et al. (2018) for *Anaplasma platys*

Organism	Primer Type	Sequence (5′–3′)
Ehrlichia canis	Forward	CGGAATTCCTAGTGTAGAGG
	Reverse	AGGAGGGATACGACCTTCAT
Anaplasma platys	Forward	TACCTCTGTGTTGTAGCTAACGC
	Reverse	CTTGCGACATTGCAACCTATTGT

Table 2. General characteristics of the studied population (n=138), tested for *Anaplasma* spp. and *Ehrlichia* spp. along with ticks found on their body, analyzed by group, sex, age range and tick pathogen load

Variables	Outdoors with ticks†	Indoors with ticks	Thrombocytopenic	Total
Sex				
Male	24/46 (52.2%)	21/47 (44.7%)	26/45 (57.8%)	71/138 (51.5%)
Female	22/46 (47.8%)	26/47 (55.3%)	19/45 (42.2%)	67/138 (48.5%)
Age				
<1 y	15/46 (32.6%)	15/47 (31.9%)	12/45 (26.7%)	42/138 (30.4%)
1–3 y*	24/46 (52.2%)	24/47 (51.1%)	21/45 (46.7%)	69/138 (50.0%)
>3 y	7/46 (15.2%)	8/47 (17.0%)	12/45 (26.7%)	27/138 (19.6%)
Infection (dogs)				
***Anaplasma* positive**	9/46 (19.6%)	0/47 (0.0%)	7/45 (15.6%)	16/138 (11.6%)
***Ehrlichia* positive**	16/46 (34.8%)	5/47(10.6%)	9/45 (20.0%)	30/138 (21.7%)
Co-infection	3/46 (6.5%)	0/47 (0.0%)	3/45 (6.6%)	6/138 (4.3%)
Tick pathogen load				
Tick's *Anaplasma*	14/46 (30.4%)	3/47 (6.3%)	-	17/93 (18.3%)
Tick's *Ehrlichia*	25/46 (54.3%)	20/47 (42.5%)	-	45/93 (48.3%)
Co-infection	6/46 (13.0%)	1/47 (2.1%)	-	7/93 (7.52%)

*Infections were significantly more prevalent in the 1–3 years age group ($p< 0.001$ for *Ehrlichia*, and $p= 0.024$ for *Anaplasma*).

† Infection was significantly associated with group ($p= 0.018$ for *Ehrlichia* and $p= 0.008$ for *Anaplasma*), with "Outdoors with ticks" showing the highest infection rate for both pathogens

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https://doi.org/10.18502/jad.v20i3.23177

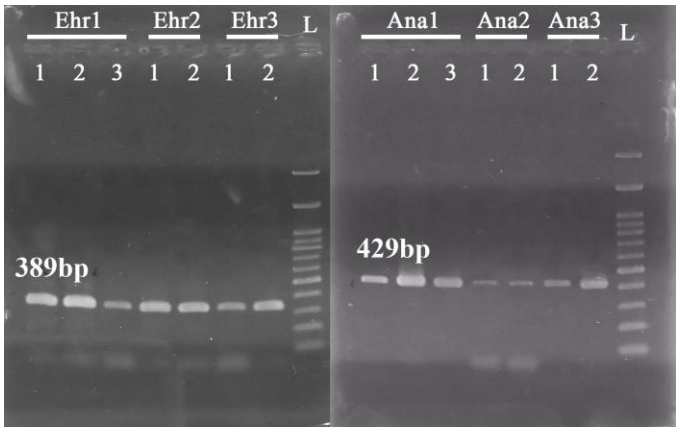


Fig. 1. Agarose gel electrophoresis of PCR products of 16S rRNA genes of *Ehrlichia canis* (left, 389bp) and *Anaplasma platys* (right, 429 bp). Ehr1, Ehr2 and Ehr3 for *Ehrlichia* and Ana1, Ana2 and Ana3 for *Anaplasma* are the sequences selected at random from pathogen-positive dog blood samples used for PCR. Each sample in the gel was used in either 2 or 3 technical replicates, shown here by the number under each well. The accession numbers are provided in the text. L is ladder

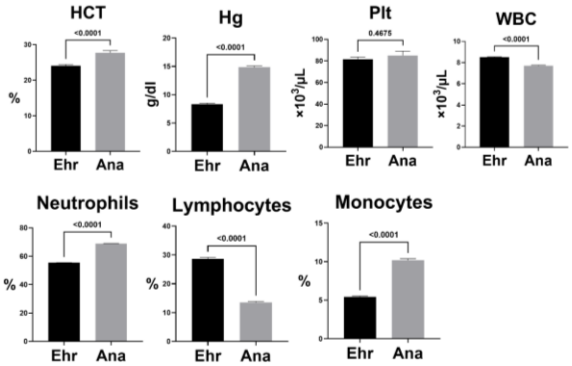


Fig. 2. Comparison of hematological factors between Ehrlichiosis (n=30) and Anaplasmosis (n=15). Bars represent mean ± SD. HCT: hematocrit; Hg: hemoglobin; Plt*: platelets; WBC: white blood cells; Ehr: *Ehrlichia*; Ana: *Anaplasma*
*Platelet counts were not significantly different between groups (Mean±SD: 81.61±10.69 for *Ehrlichia* and 85.00±16.04 for *Anaplasma*)

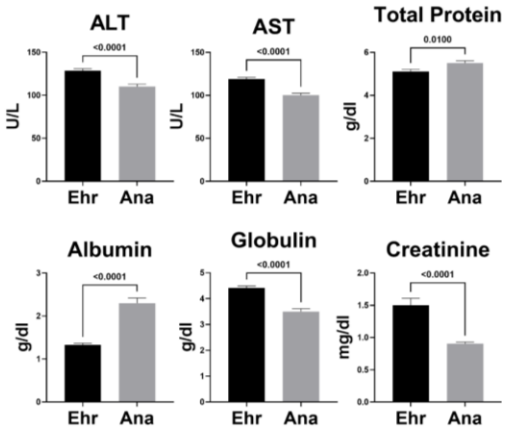


Fig. 3. Comparison of biochemical factors between Ehrlichiosis (n=30) and Anaplasmosis (n=15). Bars represent mean ± SD. ALT: Alanine transaminase; AST: Aspartate aminotransferase; Ehr: *Ehrlichia*; Ana: *Anaplasma*

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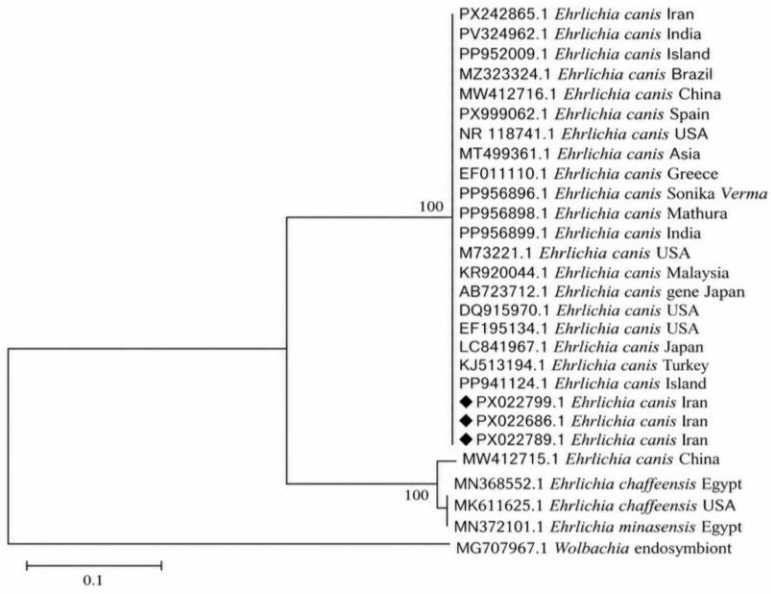


Fig. 4. Phylogenetic analysis of *Ehrlichia canis* strains isolated in this study (marked with ♦) based on 16S rRNA sequences (389bp), compared to globally reported strains, using the Maximum Likelihood (ML) method with 1000 bootstrap replicates (Bootstrap support values are shown at the nodes) with the Tamura-Nei model. *Wolbachia* endosymbiont was used as an outgroup. GenBank accession numbers are shown. The scale bar represents 0.1 nucleotide substitutions per site

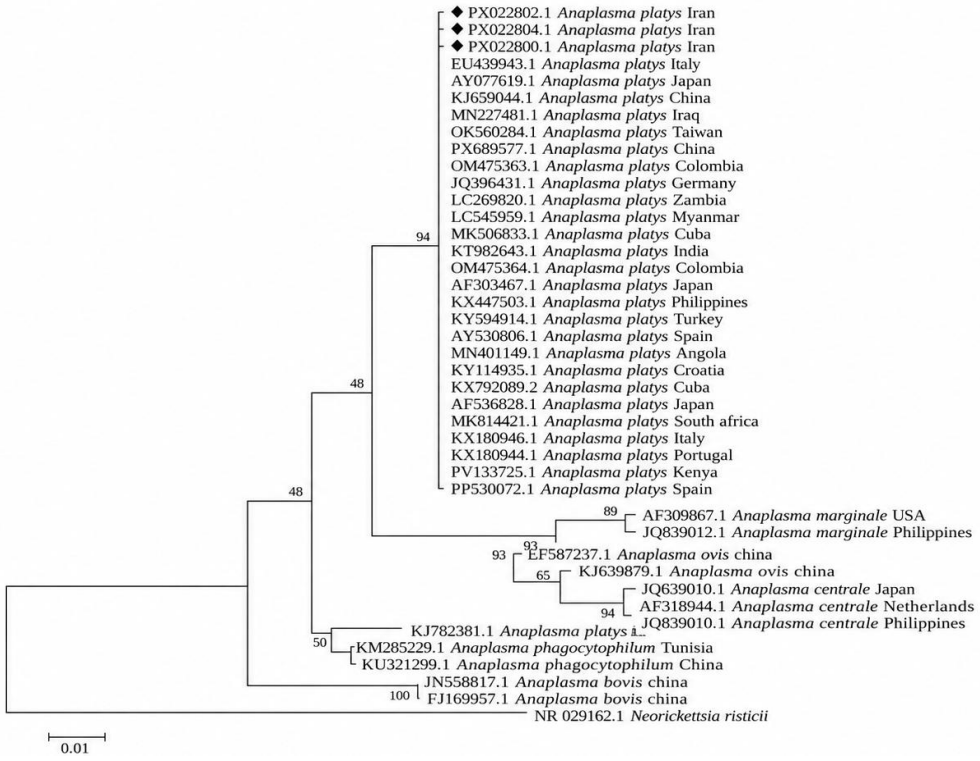


Fig. 5. Phylogenetic analysis of *Anaplasma platys* strains isolated in this study (marked with ♦) based on 16S rRNA sequences (429bp), compared to globally reported strains, using the Maximum Likelihood (ML) method with 1000 bootstrap replicates (Bootstrap support values are shown at the nodes) with the Tamura-Nei model. *Neorickettsia risticii* was used as the outgroup. GenBank accession numbers are shown. The scale bar represents 0.01 nucleotide substitutions per site

https://doi.org/10.18502/jad.v20i3.23177

Discussion

This study investigated prevalence, hematological/biochemical alterations, and molecular detection of *Ehrlichia* spp. and *Anaplasma* spp. in dogs with suspected high TBDs exposure and attached ticks from Tehran, Iran. Overall prevalence of *Ehrlichia* (21.7%) was higher than *Anaplasma* (11.6%), contrary to the general Iranian trend (20.1% for *Anaplasma*, 10% for *Ehrlichia*) (16), likely due to differences in diagnostic methodology, sampling strategy, definition of the studied population, regional geographic conditions and publication bias. All identified ticks were *Rh. sanguineus*, the most prevalent tick species in Tehran (17) and the 2nd most prevalent in Iran (25), and none were *Ixodes* spp., which explains why *Anaplasma phagocytophilum* wasn't detected, as it is primarily transmitted by *Ixodes* spp. ticks. The heterogeneous prevalence of canine ehrlichiosis and anaplasmosis observed across Iran may be due to regional differences in tick fauna. *Rh. Sanguineus* is the primary vector transmitting *Ehrlichia canis* in dog populations. In contrast, *Anaplasma* spp. can be transmitted by various vectors; the ones affecting dogs are most likely also transmitted by *Rh. Sanguineus*, as the most prevalent dog tick in Iran and Tehran; of course, this needs further evaluation, as most literature and meta-analyses are focused on livestock ticks as well (17, 25).

Infection was significantly associated with living environment: the "Outdoors" group had the highest infection rate (34.8% for *Ehrlichia* and 19.6% for *Anaplasma*), and "Indoors" the lowest (10.6% for *Ehrlichia* and 0% for *Anaplasma*), with "Thrombocytopenics" showing intermediate infection rates. Higher infection rates in outdoor dogs can be due to exposure to vectors during roaming behavior (8, 26). Contact with outdoor dogs is also a risk factor (11), though since no *Anaplasma* infections were found in indoor dogs in this study, this may not be a major transmission route in their population. Indoor dogs benefit from owner vigilance (shorter tick attachment duration), routine antiparasitic treatment and decreased tick survival indoors. Interestingly, "Indoors" dogs showed no *Anaplasma* infections, possibly due to lower prevalence, detection limits, different transmission mechanics, limited sample size, or temporal dynamics (indoor dogs clearing the infection before being sampled).

Dogs aged 1–3 years exhibited significantly higher pathogenic prevalence, possibly due to limited environmental access (puppies), or sampling heterogeneity. Some studies report increased infection rate with age (27), but certain Iranian studies (28, 29) found similar results as ours, while others claim no connection (11, 16). Gender of dogs was not a risk factor, but some report higher prevalence in males (28, 29), while others (16) suggest exposure is more influenced by environmental/behavioral factors than demographics.

Parallel testing of dogs and ticks showed significant correlation between *Anaplasma*-positive ticks and dogs demonstrating active host-vector-pathogen circulation (though transmission mechanics cannot be interpreted from PCR alone) and putting *Rh. sanguineus* as the primary source of Anaplasmosis in the studied population (30). The marginally significant association between ticks' and dogs' *Ehrlichia* may suggest detection limits or alternative infection routes; temporal transmission dynamics may also have a role here, as ticks may attach, transmit the pathogen while feeding, then detach, creating a situation where the tick later collected and studied belongs to a different population than the one which originally caused the infection. Significant relationship between ticks' Anaplasmosis and dogs' Ehrlichiosis suggests mixed infections within ticks, variable pathogen load and detection sensitivity, or differences in vector competence.

Higher prevalence of *Anaplasma* spp. in "Outdoors" ticks reinforces the environment's role in the infection process. The non-signifi-

https://doi.org/10.18502/jad.v20i3.23177

cant relationship between environment and Ehrlichiosis may be due to broader circulation of *Ehrlichia* spp. in dogs. Co-infection was found in 6 dogs without any significant association. Higher co-infection rates are reported in certain geographical regions, which may be a factor herein (11). Diagnostic methodology (false positives due to previous exposure) may also influence the reported rate of co-infection (11). Prevalence rates were not significantly different between tick-infested and thrombocytopenic dogs, suggesting that infection is independent from current presence of ticks and that exposed dogs may present with hematological abnormalities long after tick detachment. Thus, thrombocytopenia is a strong indicator of exposure to TBDs and screening PCR panels should be considered in non-tick-infested thrombocytopenic dogs. Magnitude of thrombocytopenia has been suggested as a screening test for CME (7).

Phylogenetic analysis placed all isolates within the same clade as other global strains in their respective trees, confirming genetic consistency. The high bootstrap value (100% for *E. canis* and 94% for *A. platys*) confirms the reliability of the groupings (Figs. 4–5). However, 16s rRNA sequencing revealed no region-specific lineages, which is to be expected due to: 1) homogeneity in strains discovered in different geographical regions (31, 32), 2) the 16S rRNA gene is highly conserved and lacks enough variation for strain separation (12, 33), 3) the sample size (n= 3) was too small for characterization of Iranian strains. While regional lineage identification was not a primary goal in this study, higher-resolution markers such as groEL, gltA and TRP36 (12, 24) are recommended in future studies to resolve fine-scale genetic relationships.

Marked disturbances in hematological/ biochemical parameters demonstrated in Figures 2 and 3, are consistent with the known tropism of these pathogens for hematopoietic/ immune cells. Anemia and leukocyte abnormalities are common features of ehrlichiosis and anaplasmosis (34–36). Variable leukocyte responses (leukopenia/leukocytosis), depending on pathogen, infection stage and host response, have been reported (37, 38). Comparison between *E. canis* and *A. platys* infections revealed lower hematocrit, hemoglobin, neutrophil and monocyte values and higher leukocyte/ lymphocyte counts in Ehrlichiosis, consistent with previous studies (39). *Anaplasma platys* has distinct tropism towards platelets and doesn't produce severe anemia, leukopenia, or systemic inflammatory response (11, 39). Thrombocytopenia due to immune-mediated platelet destruction, hemorrhage-induced consumption, and decreased production is a hallmark of TBDs (11), which explains why platelet counts did not differ significantly between groups.

Hepatic involvement (elevated ALT/ AST), attributed to systemic inflammation, hypoxia secondary to anemia, or immune-mediated hepatic injury, is frequently reported in canine Ehrlichiosis and Anaplasmosis (35, 40, 41). Hyperglobulinemia accompanied by hypoalbuminemia is associated with TBDs, reflecting sustained antigenic stimulation and disturbed protein metabolism (34, 40, 42). Higher ALT/ AST, globulin, creatinine and lower albumin/ total protein in Ehrlichiosis support the concept that *E. canis* is associated with more severe hepatic injury compared to *A. platys* (39).

Conclusion

This study provides a comprehensive assessment of *E. canis* and *A. platys* in dogs with suspected high TBD exposure from Tehran, Iran, integrating phylogenetic characterization with hematological/biochemical profiling. *Rhipicephalus sanguineus* was the dominant tick species in the investigated study population in Tehran, Iran and the primary vector for Ehrlichiosis and Anaplasmosis. *Anaplasma platys* was the most prevalent *Anaplasma* species, but contrary to observed trends in Iran, *E. canis* was comparatively more prevalent.

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Published Online: September 30, 2026

https://doi.org/10.18502/jad.v20i3.23177

Ehrlichiosis produced more severe alterations in hematological/biochemical factors compared to Anaplasmosis.

Limitations of this study include: 1) Small sample size of co-infected animals (n=6) prevented accurate analysis of co-infection mechanics and hematological/biochemical parameters, 2) Study was geographically limited to Tehran which limits generalizability to the whole country; in addition, Tehran's specific climate, urban density and dog management practices (including the affordability of and access to high-quality veterinary care) are factors that could influence the prevalence and distribution of TBDs, 3) Phylogenetic analysis based on 16s rRNA sequencing lacked the resolution for strain-level discrimination, 4) The cross-sectional design limits the ability to establish causal relationships between infection and clinical abnormalities, 5) The study did not include serological testing (for example, IFAT or ELISA), which could have provided additional information on past exposure and disease stage, 6) The study did not assess other co-infections (for example, *Babesia* spp., *Leishmania* spp.), which could confound the hematological and biochemical findings. It's recommended that higher-resolution genetic markers be used in a geographically diverse multicenter and multi-pathogen study with greater sample size and varying diagnostic methods, providing an accurate picture of TBDs epidemiology in Iran.

Acknowledgements

The authors are grateful to Dr Ghazale Faridfar for her help with the Phylogenetic analyses of the study.

Ethical considerations

Ethical approval was not sought for this study. Informed consent was obtained from all dog owners, and all biological samples (blood and ticks) were collected exclusively as part of routine clinical diagnostic procedures. The blood used for parasitic disease detection was residual material from samples drawn for standard health evaluations; no additional blood was collected for research purposes. Therefore, this study qualified for an exemption from institutional ethical review.

Conflict of interest statement

The authors declare that there is no conflict of interest.

References

1. Fritz CL (2009) Emerging tick-borne diseases. Vet Clin North Am Small Anim Pract. 39(2): 265–278.
2. Otranto D, Dantas-Torres F, Breitschwerdt EB (2009) Managing canine vector-borne diseases of zoonotic concern: part one. Trends Parasitol. 25(4): 157–163.
3. Chomel B (2011) Tick-borne infections in dogs-an emerging infectious threat. Vet Parasitol. 179(4): 294–301.
4. Ferrolho J, Antunes S, Vilhena H, Anastácio S, Ramalho de Sousa S, Frouco G, Ferreira B, Domingos A (2025) The complexities of canine monocytic ehrlichiosis: insights into *Ehrlichia canis* and its vector *Rhipicephalus sanguineus*. Microbiol Res. 16 (4): 85.
5. Donatien A, Lestoquard F (1937) State of the present knowledge concerning rickettsiosis of animals. Arch Inst Pasteur Alger. 15: 142–187.
6. Walker JS, Rundquist JD, Taylor R, Wilson BL, Andrews MR, Barck J, Hogge AL Jr, Huxsoll DL, Hildebrandt PK, Nims RM (1970) Clinical and clinicopathologic findings in tropical canine pancytopenia. J Am Vet Med Assoc. 157(1): 43–55.
7. Harrus S, Waner T (2011) Diagnosis of canine monocytotropic ehrlichiosis (*Ehrlichia canis*): an overview. Vet J. 187 (3): 292–296.

8. Aziz MU, Hussain S, Song B, Ghauri HN, Zeb J, Sparagano OA (2023) Ehrlichiosis in dogs: a comprehensive review about the pathogen and its vectors with emphasis on south and east Asian countries. Vet Sci. 10(1): 21.
9. Ramakant, Kumar R, Verma HC, Diwakar RP (2020) Canine ehrlichiosis: A review. J Entomol Zool Stud. 8(2): 1849–1852.
10. El Hamiani Khatat S, Daminet S, Duchateau L, Elhachimi L, Kachani M, Sahibi H (2021) Epidemiological and clinicopathological features of *Anaplasma phagocytophilum* infection in dogs: a systematic review. Front Vet Sci. 8:686644.
11. López-Valencia G, Meza-Silva KM, Haro-Álvarez AP, Trasviña-Muñoz E, Garcia-Reynoso IC, Herrera-Ramírez JC, Gómez-Gómez SD (2024) Prevalence, risk factors and hematologic changes in dogs from Baja California with presence of *Ehrlichia* spp., and coinfection with *Anaplasma* spp. Austral J Vet Sci. 56(2): 75–84.
12. Nkosi NF, Byaruhanga C, Arega SM, Conan A, Knobel DL, Oosthuizen MC, Quan M (2025) Development and validation of a real-time PCR assay, and phylogenetic analysis of *Anaplasma platys*. Vet Parasitol. 337: 110475.
13. Choubdar N, Karimian F, Koosha M, Nejati J, Oshaghi MA (2021) *Hyalomma* spp. ticks and associated *Anaplasma* spp. and *Ehrlichia* spp. on the Iran-Pakistan border. Parasit Vectors. 14(1): 469.
14. Dehnoaliyan F, Akbarabadi S, Mohseni P, Mohammadi E, Nourollahifard S, Pourfatahi M, Khalili A, Jajarmi M (2024) Snapshot study of the family Anaplasmataceae, *Anaplasma* spp. and *Ehrlichia* spp. prevalence in ticks of sheep and cattle in Jiroft City, Iran. J Arthropod Borne Dis. 18(3): 189–196.
15. Ranjbar R, Anjomruz M, Enayati AA, Khoobdel M, Rafinejad A, Rafinejad J (2020) *Anaplasma* infection in ticks in southeastern region of Iran. J Arthropod Borne Dis. 14(2): 126–133.
16. Abdous A, Rahnama M, Shams F, Jokar M, Rahmanian V, Farhoodi M, Abbassioun A, Kamjoo MS (2024) Prevalence of *Anaplasma*, *Ehrlichia* and *Rickettsia* infections in dogs in Iran: a meta-analysis study. Vet Med Sci. 10(2): e1381.
17. Beik-Mohammadi M, Tavassoli M, Telmadarraiy Z, Vatandoost H, Dabiri F (2025) Ticks (Argasidae and Ixodidae) in Tehran Province, central Iran: distribution and seasonal activity. Vet Med Int. 2025: 7844575.
18. Estrada-Peña A, Bouattour AJLC, Camicas JL, Walker AR (2004) Ticks of domestic animals in the Mediterranean region. University of Zaragoza, Spain.
19. Seong G, Han YJ, Oh SS, Chae JS, Yu DH, Park J, Park BK, Yoo JG, Choi KS (2015) Detection of tick-borne pathogens in the Korean water deer (*Hydropotes inermis argyropus*) from Jeonbuk Province, Korea. Korean J Parasitol. 53 (5): 653–659.
20. Park J, Han DG, Ryu JH, Chae JB, Chae JS, Yu DH, Park BK, Kim HC, Choi KS (2018) Molecular detection of *Anaplasma bovis* in Holstein cattle in the Republic of Korea. Acta Vet Scand. 60(1): 15.
21. Bezerra-Santos MA, Nguyen VL, Iatta R, Manoj RRS, Latrofa MS, Hodžić A, Dantas-Torres F, Mendoza-Roldan JA, Otranto D (2021) Genetic variability of *Ehrlichia canis* TRP36 in ticks, dogs, and red foxes from Eurasia. Vet Microbiol. 255: 109037.
22. Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 33(7): 1870–1874.
23. Tamai IA, Staji H (2025) Molecular identification and associated risk factors of *Hepatozoon canis* among dogs visiting veterinary clinics in Tehran, Iran. BMC Vet Res. 21(1): 660.
24. Tamai IA, Staji H, Pakbin B (2025) Genotyping of *Ehrlichia canis* TRP36 isolated from ticks and dogs in Iran. Vet Microbiol.

https://doi.org/10.18502/jad.v20i3.23177

307: 110592.
25. Khoobdel M, Jafari AS, Telmadarraiy Z, Sedaghat MM, Bakhshi H (2021) Tick-borne pathogens in Iran. Asian Pac J Trop Med. 14(11): 486–504.
26. Angelou A, Gelasakis AI, Verde N, Pantchev N, Schaper R, Chandrashekar R, Papadopoulos E (2019) Prevalence and risk factors for selected canine vector-borne diseases in Greece. Parasit Vectors. 12(1): 283.
27. Schäfer I, Kohn B, Silaghi C, Fischer S, Marsboom C, Hendrickx G, Müller E (2023) Molecular and serological detection of *Anaplasma phagocytophilum* in dogs from Germany (2008–2020). Animals (Basel). 13(4): 720.
28. Maazi N, Malmasi A, Shayan P, Nassiri SM, Salehi TZ, Fard MS (2014) Molecular and serological detection of *Ehrlichia canis* in naturally exposed dogs in Iran: an analysis on associated risk factors. Rev Bras Parasitol Vet. 23(1): 16–22.
29. Razi Jalali MH, Mosallanejad B, Avizeh R, Alborzi AR (2010) Seroprevalence of *Ehrlichia canis* in dogs referred to veterinary hospital of Shahid Chamran University of Ahvaz, Iran. Archives of Razi Institute. 65(1): 21–26.
30. Jafari A, Asadolahizoj S, Rasekh M, Saadati D, Faghihi F, FazlaliPour M, Lotfi A, Beik Mohammadi M, Hosseini-Chegeni A (2021) Hard ticks circulate Anaplasma spp. in South-Khorasan Province, Iran. Iranian J Vet Sci Technol. 13(1): 98–102.
31. Alhassan A, Hove P, Sharma B, Matthew-Belmar V, Karasek I, Lanza-Perea M, Werners AH, Wilkerson MJ, Ganta RR (2021) Molecular detection and characterization of *Anaplasma platys* and *Ehrlichia canis* in dogs from the Caribbean. Ticks Tick Borne Dis. 12(4): 101727.
32. Sipin Q, Mustaffa-Kamal F, Watanabe M, Abdul Rani PAM, Aziz NAA (2023) Low genetic diversity of vector-borne haemoparasites in dogs and their ticks revealed local and long-range transmission in Peninsular Malaysia. Pertanika J Trop Agric Sci. 46(2): 401–423.
33. Unver A, Rikihisa Y, Kawahara M, Yamamoto S (2003) Analysis of 16S rRNA gene sequences of *Ehrlichia canis, Anaplasma platys* and *Wolbachia* species from canine blood in Japan. Ann N Y Acad Sci. 990(1): 692–698.
34. Gaunt S, Beall M, Stillman B, Lorentzen L, Diniz P, Chandrashekar R, Breitschwerdt E (2010) Experimental infection and co-infection of dogs with *Anaplasma platys* and *Ehrlichia canis*: hematologic, serologic and molecular findings. Parasit Vectors. 3(1): 33.
35. Bonilla-Aldana DK, Gutiérrez-Grajales EJ, Osorio-Navia D, Chacón-Peña M, Trejos-Mendoza AE, Pérez-Vargas S, Valencia-Mejía L, Marín-Arboleda LF, Martínez-Hidalgo JP, Reina-Mora MA, González-Colonia LV, Cardona-Ospina JA, Jiménez-Posada EV, Diaz-Guio DA, Salazar JC, Sierra M, Muñoz-Lara F, Zambrano LI, Ramírez-Vallejo E, Álvarez JC, Jaramillo-Delgado IL, Pecho-Silva S, Paniz-Mondolfi A, Faccini-Martínez ÁA, Rodríguez-Morales AJ (2022) Haematological alterations associated with selected vector-borne infections and exposure in dogs from Pereira, Risaralda, Colombia. Animals (Basel). 12(24): 3460.
36. Boonhoh W, Sontigun N, Fungwithaya P, Wongtawan T (2023) Hematological analysis of naturally infecting blood parasites in dogs. Vet World. 16(4): 681–686.
37. Aguiar DM, Rodrigues FP, Ribeiro MG, Dos Santos B, Muraro LS, Taques IIGG, Campos ANS, Dutra V, Nakazato L, da Costa Vieira RF, Takahira RK (2020) Uncommon *Ehrlichia canis* infection associated with morulae in neutrophils from naturally infected dogs in Brazil. Transbound Emerg Dis. 67 Suppl 2: 135–141.
38. Özata F, Ural K (2014) Thrombocyte indices in dogs infected with *Ehrlichia canis*

http://jad.tums.ac.ir
Published Online: September 30, 2026

https://doi.org/10.18502/jad.v20i3.23177

and *Anaplasma phagocytophilum*. Revista MVZ Córdoba. 19(3): 4277–4288.
39. Nair AD, Cheng C, Ganta CK, Sanderson MW, Alleman AR, Munderloh UG, Ganta RR (2016) Comparative experimental infection study in dogs with *Ehrlichia canis*, *E. chaffeensis*, *Anaplasma platys* and *A. phagocytophilum*. PLoS One. 11(2): e0148239.
40. Costa MDP, Horta RDS, Coura FM, Mol JDS, Valente PCLG, Paes PDO (2015) Serum biochemistry in dogs infected with *Ehrlichia canis*, *Anaplasma platys* and *Leishmania* sp. Acta Sci Vet. 43: 1261.
41. Al Izzi S, Martin DS, Chan RY, Leutenegger CM (2013) *Babesia canis vogeli*, *Ehrlichia canis* and *Anaplasma platys* infection in a dog. Vet Clin Pathol. 42 (4): 471–475.
42. Wongtawan T, Sontigun N, Boonchuay K, Chiawwit P, Wongtawan O, Hayakijkosol O, Boonhoh W (2024) Improvements in blood profiles of canines naturally infected with triple blood pathogens (*Babesia vogeli*, *Ehrlichia canis* and *Anaplasma platys*) subsequent to Doxycycline monotherapy. Animals (Basel). 14(24): 3714.