Research Article

High Prevalence and Risk Factors for *Ehrlichia canis*, *Babesia* spp., and *Anaplasma* spp. Infections in Dogs Exhibiting Clinical Signs from Nghe An, Vietnam

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Abstract | This study was conducted to determine the prevalence of blood-borne parasitic infections and associated risk factors in symptomatic dogs in Nghe An Province, Vietnam. A total of 134 blood samples were collected from dogs exhibiting clinical signs and examined using rapid diagnostic tests (RDTs) for screening and polymerase chain reaction (PCR) assays for molecular confirmation. The results showed that the overall seroprevalence (RDT) for *E. canis*, *Anaplasma* spp., and *Babesia* spp. was 73.13%, 41.04%, and 26.12%, respectively, while the molecular prevalence (PCR) was 51.49%, 25.37%, and 0.00%. Multivariate logistic regression identified husbandry system and breed as independent risk factors for *E. canis* infection. Free-roaming dogs had a significantly higher risk (adjusted odds ratio [aOR] = 2.89, 95% CI: 1.38–6.05) compared with confined dogs for *E. canis*. Exotic breeds exhibited a higher infection rate than local breeds (aOR = 2.24, 95% CI: 1.05–4.78). No statistically significant differences were found regarding age or sex (P > 0.05). The most common clinical manifestations included lethargy and inappetence (86.57%), pale mucous membranes (75.37%), and high fever (67.91%). Characteristic hematological abnormalities were thrombocytopenia (85.40%), decreased hemoglobin (50.40%), and anemia (47.20%). These findings provide critical data for the clinical diagnosis and prevention of canine tick-borne diseases in hospital-based settings in Central Vietnam.

Keywords | *Anaplasma* spp., *Babesia* spp., *Ehrlichia canis,* Nghe An, Symptomatic dogs, Tick-borne pathogens

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INTRODUCTION

Canine vector-borne diseases (CVBDs) represent an important group of infectious diseases that significantly affect animal health and impose a substantial economic burden on dog owners and communities worldwide. Their impact is particularly pronounced in tropical and subtropical regions, where climatic conditions favor the survival and proliferation of ticks and the pathogens they transmit. Among CVBDs, the key pathogens belong to the genera *Ehrlichia* (notably *Ehrlichia canis*), *Anaplasma* spp., and *Babesia* spp. *Ehrlichia canis* is the etiological agent of canine ehrlichiosis, a major tick-borne disease characterized by systemic disorders and coagulation abnormalities. *Anaplasma* spp. (especially *Anaplasma platys*) and *Babesia* spp. (with *Babesia vogeli* being the most prevalent species in tropical regions) are also commonly detected in dogs and can cause clinical manifestations ranging from mild disease to severe health deterioration.

The prevalence of CVBD pathogens has been reported to vary considerably across geographic regions. Global studies indicate that the overall prevalence of *Babesia* spp. infection is approximately 12.07%, with marked differences among continents (Abdoli *et al*., 2024). In certain canine populations, the prevalence of *Ehrlichia* spp. infection can exceed 50%, whereas *Anaplasma* spp. generally have lower prevalence but remain a significant potential threat. For example, *Babesia canis* exhibits a relatively high prevalence in Europe, closely associated with the density of the tick vector *Dermacentor reticulatus*, while *B. vogeli* is widely distributed due to the broad geographic range of its vector and is typically associated with milder clinical disease (Zygner *et al*., 2023). Accurate estimation of prevalence and genetic diversity requires the application of molecular epidemiological approaches, such as polymerase chain reaction (PCR) and gene sequencing, which enable precise species identification and sensitive detection of co-infections.

In Vietnam, the tropical monsoon climate provides favorable conditions for the widespread distribution of ticks and CVBD pathogens. Several studies have reported the occurrence of canine blood-borne parasitic infections and associated factors such as age, breed, and husbandry practices (Bich *et al*., 2020; Tri *et al*., 2024; Dao *et al*., 2024). However, most studies to date have been conducted in major urban areas or have relied primarily on conventional diagnostic methods. Notably, Nghe An Province, the largest province in Vietnam, possesses a complex topography ranging from dense mountainous areas to coastal plains. This environmental diversity supports a wide range of tick habitats and diverse husbandry practices, including free-roaming dogs in rural areas and exotic breeds in urban centers. Furthermore, local veterinary practitioners in Nghe An have recently reported an increasing frequency of dogs presenting with severe anemia, persistent fever, and treatment failures, suggesting a high burden of tick-borne pathogens. Despite these concerns, there is a lack of comprehensive, up-to-date molecular data to guide clinical diagnosis and control strategies in this specific region.

Therefore, this study was conducted to provide updated data on the molecular prevalence and seroprevalence of major canine blood-borne parasitic pathogens in symptomatic dogs in Nghe An Province. The aims of this study were to (1) determine the overall prevalence of *Ehrlichia canis*, *Babesia* spp., and *Anaplasma* spp. and (2) analyze potential risk factors associated with these infections. The findings are expected to support clinical diagnosis and contribute to the development of more effective prevention and control measures for canine tick-borne diseases in Nghe An Province and other regions of Vietnam.

MATERIALS AND METHODS

Study area and period

The study was conducted in Nghe An Province, Vietnam, from July 2024 to May 2025.

Animals and sample collection

A total of 134 dogs of various ages and breeds were selected using a purposive, hospital-based sampling approach. All included animals were symptomatic dogs presenting with clinical signs suggestive of hemoparasite infection at local veterinary clinics in Nghe An. Blood samples (1–2 mL) were collected in EDTA tubes (Henso Medical, China) via cephalic or saphenous venipuncture for hematology, rapid diagnostic tests (RDTs), and PCR analysis. Epidemiological data, including breed, age, sex, husbandry system, and tick exposure, were obtained through owner interviews and clinical examinations. By focusing on this high-risk population, the study aimed to evaluate the prevalence of these pathogens in a clinical context rather than in the general dog population.

Diagnostic procedures

Clinical examinations were performed by well-trained veterinarians at the participating to record key clinical signs, including fever, anorexia, dyspnea, and hemorrhage.

Hematological analysis was performed to evaluate white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), and platelet count (PLT) using a Mindray BC-30 Hematology Analyzer (Mindray, China). The procedure is summarized as follows: the analyzer was prepared according to the manufacturer's instructions; blood samples collected in EDTA tubes were gently inverted and then placed into the analyzer's sampling port. Results were recorded and stored immediately after each analysis was completed.

Rapid Diagnostic Tests (RDTs) were used for the preliminary screening of antibodies against *E. canis*, *Anaplasma* spp., and *Babesia* spp. using the Ehrlichia/Babesia Gibsoni/Anaplasma Antibody Combo Test Cassette (WB/S/P) (Testsealabs, China). The test kits were allowed to bring to room temperature before use. EDTA-anticoagulated blood samples were centrifuged using an EBA 20 centrifuge (Hettich) to obtain plasma. Then, 20 µL of plasma was transferred into the kit's supplied buffer tube and mixed thoroughly. Three drops of the mixture were then added to each of the three sample wells (S) on the test cassette. The cassettes were kept on a flat surface for 8–10 minutes before the results were interpreted. A result was considered negative if only the control (C) line appeared, and positive if both the control (C) and test (T) lines were visible. The test was considered invalid if the

C line did not appear, regardless of the presence of the T line. In this study, RDT served as a primary screening tool; however, for *E. canis* and *Anaplasma* spp., only samples subsequently confirmed by PCR were recorded as positive cases in the final analysis. For *Babesia* spp., due to the absence of PCR-positive results, findings were reported strictly as seroprevalence based on RDT detection.

Molecular confirmation was performed using PCR targeting the *16S rDNA* gene for *E. canis*, 16S rDNA for *Anaplasma platys*, and 18S rRNA for *Babesia* spp. *(specifically B. canis and B. vogeli)*. The PCR procedure was performed according to the following protocol:

- DNA extraction: DNA was extracted from 200 µL of EDTA-anticoagulated whole blood using the MagMAX Core Nucleic Acid Purification Kit (A32702, Thermo Fisher Scientific, USA) on the KingFisher mL system (Thermo Fisher Scientific, USA), following the manufacturer's protocol. The extracted DNA (the purity (A260/280) range and specified that the template volume was optimized for high sensitivity) was stored at –20°C for further analysis.
- PCR amplification: The PCR reaction mixture (20 µL) consisted of 10 µL of 2X HRM Master Mix (Bioline), 0.4 µL of each of forward and reverse primer (10 µM each), 5 µL of template DNA, and nuclease-free water to a final volume of 20 µL. The primer pairs used in this study were based on Rucksaken *et al*. (2019) and are listed in Table 1. The thermal cycling conditions were: initial denaturation at 95°C for 3 minutes; followed by 40 cycles of denaturation at 95°C for 5 seconds, annealing at 56°C for 10 seconds, and a final extension at 72°C for 1 minute.
- Electrophoresis: PCR products were separated via gel electrophoresis on a 1.5% agarose gel at 70V for 45 minutes. Positive and negative controls included in each run to validate the results. Samples were considered positive for active infection only if they yielded the expected amplicon size on the gel.

Data analysis

Data were managed using Microsoft Excel and analyzed with SPSS version 26.0. The prevalence (%) of each pathogen was calculated as (number of positive samples / total samples examined) × 100. Associations between epidemiological variables (breed, husbandry system, age, and sex) and infection status were initially assessed using univariate analysis with chi-square tests. Risk levels were quantified using crude odds ratios (OR) with 95% confidence intervals (CI). Variables that showed significant associations ($P < 0.05$) in the univariate analysis were subsequently entered into a multivariate logistic regression model to identify independent risk factors and calculate adjusted odds ratios (aOR). For hematological parameters, data were presented as mean ± standard deviation (SD) for normally distributed variables or median (interquartile range, IQR) for non-normally distributed data. A P-value < 0.05 was considered statistically significant.

Table 1: Primer sequences used for PCR detection of pathogens.

Primer name	Sequence (5′ – 3′)	Expected amplicon size (bp)
16SF-E	CTGCTAGACTAGAGGTCGAA	181 (*Ehrlichia canis*)
16SR	CTCATCGTTTACAGCGTGGA	
16SF-A	GGGCATGTAGGCGGTTCG	250 (*Anaplasma platys*)
16SR	CTCATCGTTTACAGCGTGGA	
BH18SF	AATTGGAGGGCAAGTCTGGT	356 (*Babesia canis vogeli*). 357 (*Babesia canis canis*)
BH18SR	TGCTTTCGCAGTAGTTYGTC	

RESULTS AND DISCUSSION

Overall prevalence and diagnostic results

The diagnostic screening of 134 symptomatic dogs revealed a high burden of vector-borne pathogens, with RDT-based seroprevalence consistently exceeding PCR-based molecular prevalence for all investigated pathogens. *Ehrlichia canis* was the most predominant pathogen, exhibiting a seroprevalence of 73.13% (98/134) and a molecular prevalence of 51.49% (69/134). *Anaplasma* spp. followed with a seroprevalence of 41.04% (55/134) and a molecular prevalence of 25.37% (34/134). In contrast, *Babesia* spp. was detected only via RDT with a seroprevalence of 26.12% (35/134), while all samples tested negative for *Babesia* spp. DNA (0.00%). The molecular presence of *E. canis* and *Anaplasma* spp. was confirmed by the detection of specific amplicons via agarose gel electrophoresis (Figure 1).

A consistent discrepancy was observed between the RDT and PCR results across all three pathogens. This diagnostic gap, where seroprevalence significantly higher than molecular prevalence, is characteristic of the clinical stages of infection in endemic regions. While RDTs detect antibodies that persist during chronic phases or after the pathogen has been cleared, PCR identifies active parasitemia. The high seroprevalence compared to the lower molecular detection rates suggests that a substantial proportion of the symptomatic dogs were likely in a chronic stage of infection or had experienced prior exposure, resulting in parasitemia levels below the molecular detection threshold. This was most evident for *Babesia* spp., where no active infections were molecularly confirmed despite a high exposure rate.

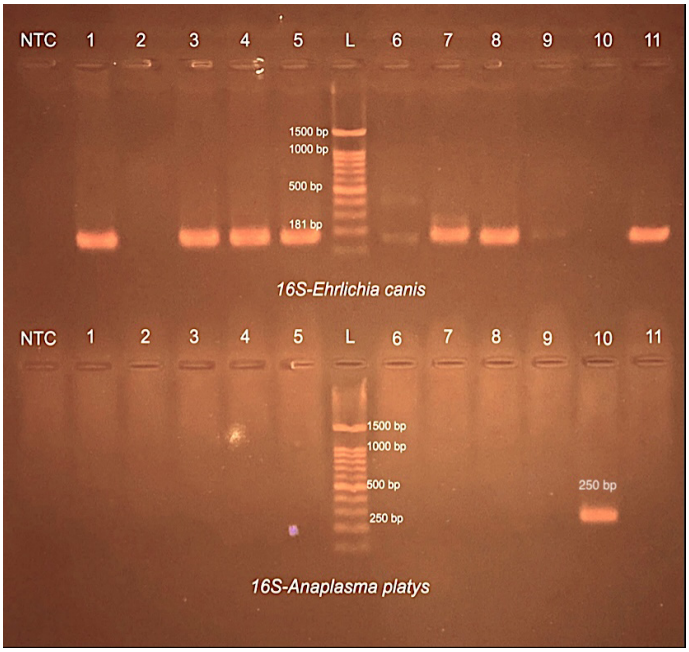

Figure 1: Agarose gel electrophoresis (1.5%) for the molecular detection of *Ehrlichia canis* and *Anaplasma platys*. (Lane L: 1500 bp DNA ladder; Lanes 1–11: Clinical samples tested for pathogen DNA; Lane NTC: Negative control (nuclease-free water). Positive samples for *E. canis* are identified by a specific DNA band at approximately 181 bp, while positive samples for *A. platys* exhibit a band at approximately 250 bp. The absence of bands in the NTC lane confirms the lack of DNA contamination during the PCR process.)

Consequently, these findings underscore the importance of a combined diagnostic approach. While RDT provides a rapid assessment of exposure history, PCR remains the gold standard for identifying active, acute infections. To ensure clinical and epidemiological precision, our subsequent analysis of risk factors was based exclusively on PCR-confirmed cases, as this identifies the factors specifically associated with active parasitemia and clinical disease state in the study population.

Ehrlichia canis infection and associated risk factors

Molecular analysis using PCR confirmed that the prevalence of active *E. canis* infection among the symptomatic dog population was 51.49% (69/134). The detailed associations between epidemiological variables and the molecular infection status are summarized in Table 2. Based on the univariate analysis, the husbandry system and breed were identified as significant risk factors ($P < 0.05$), whereas age and sex showed no statistically significant correlation with *E. canis* infection ($P > 0.05$). To account for potential confounding effects, a multivariable logistic regression model was employed, which further confirmed that both breed and husbandry system serve as independent risk factors for the molecular presence of the pathogen.

Regarding husbandry practices, free-roaming dogs had a significantly higher infection rate (66.15%) than confined dogs (37.68%), with an crude odds ratio (OR) of 3.23 (95% CI: 1.59–6.56, P = 0.001). After adjusting for breed, the multivariate model confirmed husbandry as an independent risk factor with an adjusted odds ratio (aOR) of 2.89 (95% CI: 1.38–6.05, P= 0.005). This aligns with Mitpasa *et al.* (2022) and reflect increased tick exposure in uncontrolled environments. Interestingly, the relatively high prevalence observed even among confined dogs (37.68%) suggests that *Rhipicephalus sanguineus* can adapt and reproduce in domestic environments, maintaining a persistent infection risk if hygiene and tick control measures are neglected.

Table 2: Molecular prevalence and risk factors associated with *Ehrlichia canis* infection.

Investigated factors	Total samples (n)	Positive samples (n)	Prevalence (PCR +) (%)	Adjusted odds ratio (aOR) (95% CI)	P value
Breed					0.037*
Local	44	16	36.36	1.00 (Ref)	
Exotic	90	53	58.89	2.24 (1.05 – 4.78)	
Husbandry system					0.005*
Confined	69	26	37.68	1.00 (Ref)	
Free-roaming	65	43	66.15	2.89 (1.38–6.05)	
Age (years)					0.170
< 1	22	9	40.91	NS	
1 – 3	75	44	58.67	NS	
3 – 5	37	16	43.24	NS	
Sex					0.415
Female	79	43	54.43	NS	
Male	55	26	47.27	NS	
Total	134	69	51.49		

Note: CI: Confidence Interval; Ref: Reference group; NS: Not significant

In terms of breed, exotic dogs had a higher prevalence (58.89%) and a 2.52-fold increased risk of infection (95% CI: 1.19–5.28, P= 0.014) compared with local breeds (36.36%). In the multivariable analysis, breed remained a significant independent predictor of infection (aOR= 2.24, 95% CI: 1.05–4.78, P= 0.034). This disparity suggests that exotic breeds may exhibit higher susceptibility to clinical infections in the local environment. While these findings might reflect differences in innate biological susceptibility or long-term environmental adaptation, they could also be influenced by detection bias. Exotic breeds often receive more intensive veterinary monitoring, potentially leading to more frequent clinical presentations and higher detection rates when symptoms occur. However, the fact that breed remained an independent risk factor even after adjusting for husbandry practices suggests that breed-specific factors are significant drivers of infection status in this clinical population.

Analysis of other factors revealed that infection was independent of age (P= 0.170) and sex (P= 0.415). Although the highest prevalence was in dogs aged 1–3 years (58.67%) with an OR of 2.05, the association was not statistically significant. The wide 95% CI (0.79–5.28) for this age group, which crosses the null value of 1.0, indicates a degree of uncertainty likely stemming from the relatively small sample size of the reference group (dogs < 1 year, n=22). Nevertheless, our results suggest that age is not a primary determinant of infection risk in this population, consistent with Chuan *et al.* (2021). Similarly, the lack of a sex-based predisposition (54.43% in females vs 47.27% in males) further aligns with Chuan *et al.* (2021), indicating that the influence of sex may be mitigated by standardized local management practices in the study area.

The overall prevalence of *E. canis* (51.49%) is markedly higher than the prevalence found in Ho Chi Minh City by Hai and Khuong (2021) (21.7%) and Loan *et al.* (2025) (2.32%), but remains within the global range of 10–60% reported by Aziz *et al.* (2022). This higher prevalence compared to previous studies in Vietnam may be attributed to several factors. First, the sampling frame focused exclusively on symptomatic dogs in a clinical setting, whereas other studies often included healthy or random populations. Second, the use of both RDT and PCR in this study allowed for the detection of both active infections and previous exposures, providing a more comprehensive assessment of the disease burden in Nghe An.

Beyond methodological differences, local management practices in North-Central Vietnam likely contribute to this disparity. Unlike the highly urbanized environment of Ho Chi Minh City, dog husbandry in Nghe An frequently involves free-roaming practices in semi-rural or garden-based settings, which increases the likelihood of contact with environmental tick reservoirs. Furthermore, access to routine veterinary preventive care, such as consistent long-term tick prophylaxis (e.g., isoxazolines or spot-on treatments), is generally less systemic in this region compared to major southern metropolitan hubs. These socio-economic and behavioral factors, combined with the favorable humid subtropical climate for *Rhipicephalus sanguineus*, create a high-pressure environment for the transmission of tick-borne pathogens.

Anaplasma platys infection and associated risk factors

Molecular screening identified an active *Anaplasma platys* infection prevalence of 25.37% (34/134) within the symptomatic dog population. The relationships between epidemiological factors and the molecular infection status are summarized in Table 3. Unlike the findings for *E. canis*, univariate analysis revealed that the prevalence of *A. platys* was independent of breed, husbandry system, age, and sex (P > 0.05). Consequently, no variables met the criteria for inclusion in a multivariable logistic regression model for this pathogen.

Table 3: Molecular prevalence of *Anaplasma platys* infection and comparison of epidemiological factors.

Investigated factors	Total samples (n)	Positive samples (n)	Prevalence (PCR +) (%)	P value
Breed				0.355
Local	44	9	20.45	
Exotic	90	25	27.78	
Husbandry system				0.546
Confined	69	16	23.19	
Free-roaming	65	18	27.69	
Age (years)				0.054
< 1	22	5	22.73	
1 – 3	75	25	33.33	
3 – 5	37	4	10.81	
Sex				0.222
Male	55	17	30.91	
Female	79	17	21.52	
Total	134	34	25.37	

Regarding age groups, the highest prevalence was in dogs aged 1–3 years (33.33%), followed by the < 1 year (22.73%), and the lowest rate in the 3–5-year group (10.81%). Although this variation approached statistical significance (P = 0.054), it did not meet the statistical threshold. This trend differs from the findings in Can Tho by Tien *et al.* (2021), where puppies younger than 6 months had the highest prevalence (70.00%), suggesting that local pathogen pressure and the timing of exposure may vary geographically.

Regarding husbandry system, the infection rate in free-roaming dogs (27.69%) was only slightly higher than in confined dogs (23.19%, P= 0.546). The relatively high prevalence among confined dogs suggests that *Rhipicephalus sanguineus* can maintain transmission within domestic environments in Nghe An Province if tick control is neglected. Similarly, no significant sex-based predisposition was found (30.91% in males vs 21.52% in females, P= 0.222).

The prevalence of 25.37% is higher than previous reports in Can Tho by Do *et al.* (2024) (11.42%) and Tien *et al.* (2021). This discrepancy likely arises from differences in the study populations; while previous surveys in Can Tho often included a broader or random sample of dogs, our data specifically represents a hospital-based population of symptomatic animals. This focused sampling frame, combined with the use of dual diagnostic methods (RDT and PCR), provides a more targeted assessment of the pathogens actively contributing to clinical cases in Nghe An. These results reinforce the widespread distribution of *Anaplasma* spp. in the region and emphasize its status of this pathogen as a significant veterinary concern in central Vietnam.

Babesia spp. infection and associated risk factors

The seroprevalence of *Babesia* spp. in this study, determined exclusively via RDT, was 26.12% (35/134), whereas all samples tested negative by PCR analyses. Statistical analysis of epidemiological factors revealed no significant associations among any of the investigated factors (P > 0.05), indicating relatively uniform exposure pressure across the studied population. Detailed results are presented in Table 4.

Table 4: Seroprevalence of *Babesia* spp. infection and comparison of epidemiological factors.

Investigated factors	Total samples (n)	Positive samples (n)	Prevalence (RDT+) (%)	P value
Breed				0.835
Local	44	11	25.00	
Exotic	90	24	26.67	
Husbandry system				0.686
Confined	69	17	24.64	
Free-roaming	65	18	27.69	
Age				0.191
< 1	22	3	13.64	
1 – 3	75	20	26.67	
3 – 5	37	12	32.43	
Sex				0.799
Male	55	15	27.27	
Female	79	20	25.32	
Total	134	35	26.12	

Regarding age, although not statistically significant (P = 0.191), a cumulative trend was observed: the rate was lowest in dogs under 1 year (13.64%), increased in the 1–3 years group (26.67%), and peaked in the 3–5-year age group (32.43%). This trend supports the hypothesis of cumulative antibody acquisition over time, as noted by Long *et al.* (2023) and Obeta *et al.* (2020). Older dogs typically have longer periods of exposure to tick vectors, which may result in sustained antibody levels or a higher probability of reinfection compared with younger animals.

In terms of husbandry and breed, the seropositivity rate in free-roaming dogs (27.69%) was only marginally higher than in confined dogs (24.64%, P= 0.686), and no significant predisposition was found related to breed types (P= 0.835). The lack of significant differences suggests that the tick vector, *Rhipicephalus sanguineus*, is widely distributed in the local environment of Nghe An Province, so antigen exposure remains relatively constant across different management systems.

The seroprevalence of 26.12% is consistent with the results from Hanoi by Do *et al.* (2024), and higher than the 16.09% reported by Long *et al.* (2023) in Can Tho. It is crucial to emphasize that these values reflect seroprevalence, encompassing both active infections and previous exposures, due to the use of rapid diagnostic tests (RDTs). Notably, the absence of *Babesia* DNA in all samples via PCR, despite the 26.12% seropositivity, suggests that the sampled dogs might have been in a chronic stage of infection with low parasitemia levels below the PCR detection limit, or had previously cleared the parasite while maintaining detectable antibody levels. Furthermore, in this symptomatic population, the observed clinical signs were likely primarily driven by *E. canis* and *Anaplasma* spp., which showed high molecular prevalence, rather than an active *Babesia* infection.

Clinical presentation of the symptomatic study dog population

The clinical signs observed in the 134 dogs diagnosed with blood-borne parasites are summarized in Table 5 and illustrated in Figure 2. The most frequently clinical manifestations were lethargy, inappetence, and weakness (86.57%), pale mucous membranes (75.37%), and high fever (67.91%). Notably, an extremely high tick infestation rate of 92.53% was observed among the infected dogs. In contrast, epistaxis was recorded at a very low frequency, occurring in only 3.73% of the cases.

The high tick infestation rate (92.53%) found in this study is highly consistent with the epidemiological characteristics of Nghe An Province. The tropical hot and humid climate of this region provides an ideal environment for *Rhipicephalus sanguineus* to proliferate, the principal vector for

Table 5: Frequency of clinical signs observed in the cohort study (N=134).

Clinical signs	Examined (n)	Affected (n)	Frequency (%)
Tick infestation	134	124	92.53
Lethargy, inappetence, and weakness	134	116	86.57
Pale mucous membranes	134	101	75.37
High fever (> 39,5°C)	134	91	67.91
Subcutaneous hemorrhage	134	43	32.09
Joint swelling	134	28	20.89
Difficulty in ambulation	134	18	13.43
Epistaxis (nosebleed)	134	5	3.73

Figure 2: Representative clinical manifestations and diagnostic screening in infected dogs.
(A) Severe infestation of *Rhipicephalus sanguineus*, the primary vector for canine blood-borne pathogens in the study area; (B) Clinical presentation of epistaxis (nosebleed), a common symptom of canine monocytic ehrlichiosis; (C) A symptomatic dog during clinical examination with a corresponding positive Rapid Diagnostic Test (RDT) result, indicating serological exposure to blood parasites.

E. canis, *B. vogeli*, and *A. platys*. This high vector density not only increases the risk of single pathogen exposure but also facilitates co-infections, underscoring the need for integrated vector control as a primary disease prevention strategy.

The predominance of lethargy, pale mucous membranes, and high fever as clinical signs aligns with previous reports by Anh *et al.* (2021) and Tri *et al.* (2024). These signs, particularly pale mucous membranes and hemorrhagic tendencies (observed in 32.09% of cases as subcutaneous hemorrhage), remain reliable clinical indicators for suspecting canine blood-borne parasitic infections in field conditions.

The low prevalence of epistaxis (3.73%) provides insight into the clinical stages of these infections. Since epistaxis is a hallmark of chronic ehrlichiosis often involving severe bone marrow suppression and coagulation disorders its rarity in our study suggests that the most diagnosed cases were likely in the acute or subacute stages. This finding highlights the critical importance of early diagnostic to identify infections before they progress to chronic, life-threatening forms.

Hematological profiles of the symptomatic dogs

Hematological analysis was performed on 123 infected dogs (after excluding 11 samples due to technical errors or duplicate records). Changes in hematopoietic cell lines are presented in Table 6, and the distribution patterns of these parameters are illustrated in Figure 3.

Table 6: Hematological parameters of the symptomatic dogs (N = 123).

Parameters (Unit)	Results	Reference range	Abnormal (%)
WBC (10^9/L)	9.66 (5.70-14.14)[a]	6.00-17.00	31.70
RBC (10^{12}/L)	5.20 ± 1.91[b]	5.10-8.50	47.20
HGB (g/L)	110.0 (77.5-142.5)[a]	110.0-190.0	50.40
HCT (%)	35.1 ± 14.0[b]	33.0-56.0	44.70
PLT (10^9/L)	67.0 (38.5 - 93.0)[a]	117.0-490.0	85.40

Notes: [a]Values are presented as median (interquartile range) due to non-normal distribution. [b]Values are presented as mean ± standard deviation due to normal distribution.

- Leukocyte lineage: Total leukocyte counts (WBC) showed substantial variation, with a median of 9.66 (IQR: 5.70–14.14) × 10^9/L. While the median remained within the reference range (6.00–17.00 × 10^9/L), 31.70% of the infected dogs exhibited abnormal WBC counts, indicating a heterogeneous immune response.
- Erythrocyte lineage: Anemia was a prominent clinical

feature. The mean red blood cell (RBC) count was 5.20 ± 1.91 × 10^{12}/L, with 47.20% of dogs falling below the physiological threshold. Similarly, abnormal levels were common for hemoglobin (50.40%) and hematocrit (44.70%), with a median HGB of 110.0 (77.5–142.5) g/L and a mean HCT of 35.1 ± 14.0%.

- Thrombocyte lineage: Thrombocytopenia was the most consistent and severe finding. The median platelet (PLT) count was only 67.0 (IQR: 38.5–93.0) × 10^9/L, which is markedly below the minimum reference threshold (117 × 10^9/L). Notably, 85.40% of the infected dogs had thrombocytopenia, and boxplot visualization (Figure 3) confirmed that the majority of these cases fell into the range of severe thrombocytopenia.

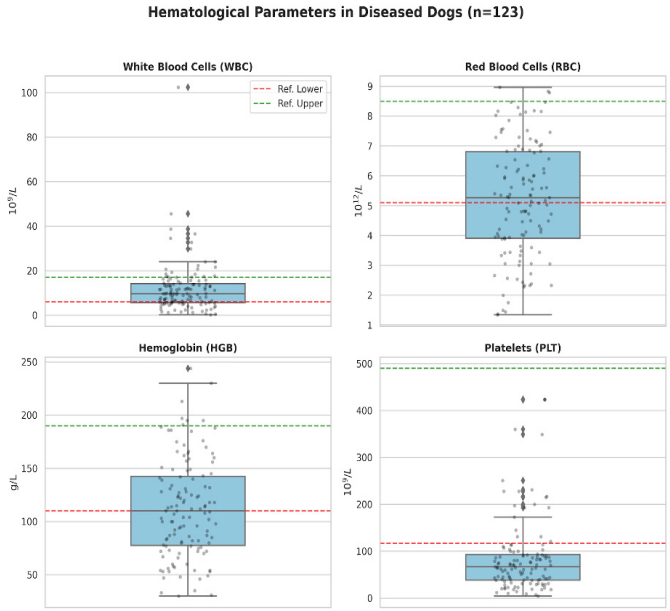

Figure 3: Hematological parameters of dogs infected with blood parasites.

The hematological changes observed in this study reflect the typical pathological progression of canine vector-borne diseases. The high variability in leukocyte counts likely related to the different disease stages; leukopenia typically results from bone marrow suppression in chronic stages, whereas leukocytosis stems from acute inflammation or secondary bacterial infections. Our findings align with Sandeep *et al.* (2023), highlight the complex immune responses typical of *E. canis* and *Anaplasma* spp. infections.

Regarding the erythrocyte lineage, the trend toward anemia is consistent with the known pathogenesis of these pathogens. *Babesia* spp. cause direct hemolysis of erythrocytes, while *Ehrlichia canis* induces anemia through bone marrow suppression or hemorrhagic loss. These results corroborate findings by Zygner *et al.* (2009) and Thang and Tram (2023), confirming that reductions in erythrocyte indices are characteristic of these infections.

Thrombocytopenia emerged as the principal biological hallmark in this study (85.40% prevalence), which is consistent with research by Salem and Farag (2014) and Angkanaporn *et al.* (2022). The marked heterogeneity in platelet counts, ranging from near-normal to severe depletion (< 40 × 10^9/L), likely reflects differences in pathogen virulence and the presence of co-infections. In cases involving *E. canis*, *Babesia* spp., and *Anaplasma* spp. platelet consumption and destruction of platelets are often exacerbated.

Overall, the combination of severe thrombocytopenia and a concurrent tendency toward anemia represents the most critical diagnostic finding in the Nghe An canine population. These findings underscore the essential role of complete blood count (CBC) analysis as a primary tool for early diagnosis and prognostic assessment of canine blood parasite infections.

Finally, this study has some limitations that should be acknowledged. The sample size of 134 dogs, while sufficient for an initial prevalence survey, resulted in relatively small subgroups when stratified by age and breed. This limited the statistical power to identify significant risk factors for *Anaplasma* spp. and *Babesia* spp., potentially leading to Type II errors. Furthermore, the reliance on a clinical (symptomatic) dogs may not fully reflect the epidemiological situation in the general dog population of Nghe An. Future large-scale studies with larger cohorts and random sampling are warranted to further clarify the epidemiological drivers of these tick-borne diseases.

CONCLUSION

This study highlights a high prevalence of canine vector-borne pathogens in Nghe An Province, North-Central Vietnam. Molecular and serological evidence identified *Ehrlichia canis* as the predominant pathogen (51.49%), followed by *Anaplasma* spp. (25.37%) and a notable seroprevalence of *Babesia* spp. (26.12%). Multivariable analysis confirmed that exotic breeds and free-roaming husbandry practices are primary independent risk factors specifically associated with *E. canis* infection. The clinical presentation of the symptomatic population was characterized by hallmark signs such as lethargy, pale mucous membranes, and high fever. These manifestations were strongly associated with significant hematological abnormalities, most notably severe thrombocytopenia (85.40%) and anemia. These findings underscore the critical need for integrated tick surveillance and enhanced vector control programs to mitigate the disease burden and improve canine health management in the region.

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NOVELTY STATEMENT

This study provides the first comprehensive molecular and serological assessment of the canine blood-parasite complex in Nghe An, a strategic livestock region in North-Central Vietnam. We report a critically high and diverse prevalence: Ehrlichia canis (51.49%), Anaplasma spp. (25.37%), and Babesia spp. (26.12%). Unlike previous studies, this research uniquely correlates these molecular findings with severe clinical hematological failures, specifically identifying free-roaming practices and exotic breeds as the primary drivers of this triple-pathogen burden. These results establish a new diagnostic benchmark for regional veterinary surveillance.

AUTHOR'S CONTRIBUTION

VTHL conceptualized and designed the study and performed data analysis. TTM conducted the molecular biological experiments and was responsible for manuscript preparation and finalization. NTL and TDT performed rapid diagnostic tests (RDTs) and collected clinical data. All authors read and approved the final manuscript.

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Generative AI and AI-assisted technology statement

During the preparation of this work, the authors used generative AI technologies for language editing, structural refinement, and proofreading to improve the clarity and flow of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication. No AI was used for data collection, analysis, or interpretation.

Conflict of interest

The authors have declared no conflict of interest.

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