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Integrated Clinical, Hematobiochemical and Molecular Diagnosis of *Ehrlichia canis* in Dogs, Ho Chi Minh City, Vietnam

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Abstract | *Ehrlichia canis* (*E. canis*), the causative agent of canine monocytic ehrlichiosis (CME), poses a significant diagnostic challenge in endemic regions due to the nonspecific nature of its clinical signs and the limitations of conventional diagnostic methods. This study aimed to characterize the clinical features, hematobiochemical alterations, and evaluate the diagnostic efficiency of nested PCR in detecting *E. canis* infection in dogs in Ho Chi Minh City, Vietnam. Between December 2022 and April 2023, a total of 112 domestic dogs presenting with clinical signs suggestive of ehrlichiosis, including subcutaneous hemorrhages, mucosal bleeding, pale oral mucosa, epistaxis, pruritus, and dermatitis, were examined. Blood samples were collected aseptically for cytological evaluation (Diff-Quick-stained blood smears), hematology, biochemistry, and nested PCR targeting the 16S rRNA gene of *E. canis*. Cytological examination revealed intracytoplasmic morulae in 31.25% (35/112) of cases, while nested PCR confirmed active *E. canis* infection in 34.82% (39/112). Hematological analysis demonstrated normocytic-normochromic anemia, leukocytosis, and marked thrombocytopenia in PCR-positive dogs. Biochemical profiles revealed elevated levels of ALT, AST, BUN and creatinine, indicating hepatic and renal involvement. The combination of clinical signs, particularly epistaxis, subcutaneous hemorrhages, and pale mucous membranes, with hematological and biochemical abnormalities provides high diagnostic value for CME. However, nested PCR remains essential for definitive diagnosis, enabling detection of active infections that may be missed by cytology or serology alone. These findings underscore the need for integrated diagnostic approaches in the surveillance and management of canine ehrlichiosis in endemic regions.

Keywords | *Ehrlichia canis*, Canine monocytic ehrlichiosis, Nested PCR, Hematological, Biochemical, Ho chi minh city

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INTRODUCTION

Ehrlichia canis (*E. canis*), the causative agent of canine monocytic ehrlichiosis (CME), has become increasingly problematic in Ho Chi Minh City, Vietnam. The warm, humid climate of the region supports high tick activity year-round, particularly of *Rhipicephalus sanguineus*, the primary vector for *E. canis* (Kottadamane *et al.*, 2017).

Combined with rising pet populations and limited tick control practices, these factors contribute to a growing burden of tick-borne diseases in the area. Despite limited regional data, clinical observations suggest higher vector density and possible resistance to common acaricides, prompting a focused investigation in this geographic context.

E. canis is an obligate intracellular Gram-negative bacterium that infects monocytes and macrophages, forming morulae in the cytoplasm (Neer *et al*., 2002). The infection disrupts hematological and biochemical homeostasis, often causing thrombocytopenia, anemia, and elevated liver enzymes (Harrus and Waner, 2011; Parashar *et al*., 2016). Clinical manifestations vary with disease stage and host response, ranging from mild fever and lethargy to severe immune dysregulation in chronic cases (Alleman *et al*., 2001; Harrus and Waner, 2011).

Accurate diagnosis in the early stages is critical to prevent progression and complications. While serological tests such as ELISA and IFA are widely used, they detect antibodies that may persist post-infection and fail to differentiate active from past exposure. In contrast, PCR directly detects *E. canis* DNA in peripheral blood, offering greater specificity for current infection, particularly in acute cases. For this reason, PCR was selected as the primary diagnostic method in this study.

Given the ecological conditions in Ho Chi Minh City and the clinical importance of early detection, this study aims to assess the correlation between physiological and biochemical markers and PCR-confirmed *E. canis* infection in dogs. The goal is to improve early diagnostic accuracy and inform more targeted treatment strategies in an urban, tick-endemic setting.

MATERIALS AND METHODS

Sample Collection and Diagnostic Procedures

A total of 112 domestic dogs exhibiting clinical signs suggestive of ehrlichiosis, such as fever, lethargy, anorexia, pale mucous membranes, lymphadenopathy, and thrombocytopenia, were recruited from veterinary clinics in Ho Chi Minh City between December 2022 and April 2023. Inclusion criteria required dogs of any breed, sex, or age to present with at least two clinical signs commonly associated with CME, including fever, lethargy, anorexia, mucosal bleeding, lymphadenopathy, and thrombocytopenia. All clinical signs were considered equally during inclusion; no weighting or severity scoring was applied. Dogs were excluded from the study if they had received antimicrobial treatment within the previous 14 days, showed signs of non-infectious systemic illness (e.g., autoimmune or neoplastic diseases), or tested positive for co-infection with vector-borne pathogens such as *Babesia* spp., *Anaplasma* spp., and *Hepatozoon canis*. Co-infections were ruled out by microscopic examination of blood smears, identifying intraerythrocytic parasites for *Babesia*, platelet-associated inclusions for *Anaplasma*, and characteristic gamonts within neutrophils or monocytes for *Hepatozoon*, as described by Irwin (2010), Ferreira *et al*. (2007) and Alves *et al*. (2014).

Peripheral blood samples were collected from all included dogs using aseptic techniques and transferred to EDTA-coated tubes for further analysis. Thin blood smears were prepared, stained with the Diff-Quick method (Medion Diagnostics, Germany), and examined under oil immersion (1000× magnification) to detect intracytoplasmic morulae within monocytes.

Additionally, genomic DNA was extracted from all EDTA samples and subjected to nested PCR targeting the 16S rRNA gene of *E. canis* to confirm active infection. All diagnostic approaches, including hematological and biochemical analyses, cytological examination, and nested PCR, were uniformly applied across the study cohort to enable direct comparison of diagnostic accuracy and clinical relevance.

Blood sample collection: Whole blood was collected from each clinically suspected dog under aseptic conditions. Samples were drawn from the cephalic or saphenous veins using sterile 23G needles and transferred into EDTA-coated tubes (minimum 2 mL per dog). Tubes were gently inverted to prevent clot formation and labeled with the corresponding patient identification information. Samples were processed immediately or stored at 2–8°C and analyzed within 8 hours to preserve integrity. This collection protocol was designed to support multiple downstream assays, including cytological examination, hematology, biochemistry, and molecular diagnostics.

Hematological and biochemical analyses: Aliquots of EDTA-anticoagulated blood were analyzed using an automated hematology analyzer (Dymind DH 36, Jilin Sinoscience Technology Co., Ltd., China) to assess key parameters including Red Blood Cell Count (RBC), White Blood Cell Count (WBC), and Platelet Count (PLT). Additional blood samples were processed for biochemical evaluation of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Creatinine (CRE), and Blood Urea Nitrogen (BUN) using a semi-automated biochemistry analyzer (MNCHIP Technology Co., Ltd., Tianjin, China), following standard protocols described by Parashar *et al*. (2016). DNA was also extracted from blood samples for nested PCR analysis, enabling molecular confirmation of *E. canis* infection.

Cytological examination: Thin blood smears were prepared from EDTA blood, air-dried, and stained using

the Diff-Quick method (Medion Diagnostics, Germany). Slides were examined under oil immersion (1000×) for intracytoplasmic inclusion bodies (morulae) in monocyte, as described by Alves *et al.* (2014).

DNA extraction and molecular confirmation: DNA was extracted from all 112 samples using the TopPURE® RNA/DNA Viral Extraction Kit (ABT, Vietnam), following the manufacturer's protocol. The concentration and purity of DNA were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA), and samples were stored at –20°C until further analysis.

Nested PCR was performed to amplify a fragment of the 16S rRNA gene specific to *E. canis*. The first round of amplification used external primers ECC and ECB, followed by a second round using internal primers ECAN5 and HE3, as previously described by Murphy *et al.* (1998). Extracted DNA samples were subjected to nested PCR using GoTaq® DNA Polymerase (Promega, USA). The PCR reaction mixture (25 µL total volume) contained 5 µL of 5× BlasTaq Buffer, 0.5 µL of 10 mM dNTP, 1 µL each of forward and reverse primers (25 µM), 15.5 µL PCR-grade water, and 2 µL of DNA template.

The first-round PCR used ECC (AGAACGAACGCTGGCGGCAAGC) and ECB (CGTATTACCGCGGCTGCTGGCA) primers to amplify a 477 bp product. The second-round (nested) PCR used ECAN5 (CAATAATTTATAGCCTCTGGCTATAGGA) and HE3 (TATAGGTACCGTCATTATCTTCCCTAT) primers, generating a 389 bp fragment specific to *E. canis*. The thermal cycling conditions for both PCR rounds included an initial denaturation at 94°C for 3 minutes; 35 cycles of denaturation at 94°C for 15 seconds, annealing at 60°C for 15 seconds, and extension at 72°C for 45 seconds; followed by a final extension at 72°C for 5 minutes.

PCR products were separated by electrophoresis on a 1.5% agarose gel prepared with 0.5× TAE buffer. The gel was stained with 6× GelRed® DNA loading dye (ABT, Vietnam) at a final working concentration of 1X. A total of 10 µL per sample (8.5 µL PCR product + 1.5 µL loading dye) was loaded into each well. A 100 bp DNA ladder (Vivatis, Malaysia), along with positive and negative controls, was included for validation.

Electrophoresis was conducted at 100 V for 30 minutes. Gels were visualized under UV transillumination using a ViLber Bioprint system with BioVision software to confirm the presence of target amplicons.

Data Processing Methods

All data were initially organized and processed using Microsoft Excel 2016. Statistical analyses were performed using Minitab software version 21.0. The association between categorical variables was evaluated using the Chi-square (χ^2) test, while comparisons of means between groups were assessed using analysis of variance (ANOVA). Statistical significance was determined at a 95% confidence level ($p < 0.05$).

RESULTS AND DISCUSSION

Survey on the Infection Rate of *E. canis* in Dogs in Ho Chi Minh City

The findings of this study revealed that among 112 clinically suspected dogs, 39 cases (34.82%) were confirmed to have active *Ehrlichia canis* infection by nested PCR, while cytological examination detected intracytoplasmic morulae in 35 dogs (31.25%) (Table 1, Figures 1 and 2). Although the detection rates of cytology and PCR were relatively similar, this may reflect potential false positives in cytology due to staining artifacts or misinterpretation of platelet clumps as morulae. Despite the close numerical values, PCR remains more reliable due to its higher sensitivity, especially in chronic or low-bacteremia cases.

Table 1: Prevalence of *E. canis* infection among clinically suspected dogs in Ho Chi Minh City.

Category	Number of dogs (n)	Proportion (%)
Clinically suspected *Ehrlichia* cases	112	-
Diff-Quick stained blood smears	35	31.25
PCR-confirmed *E. canis* cases among seropositive dogs	39	34.82

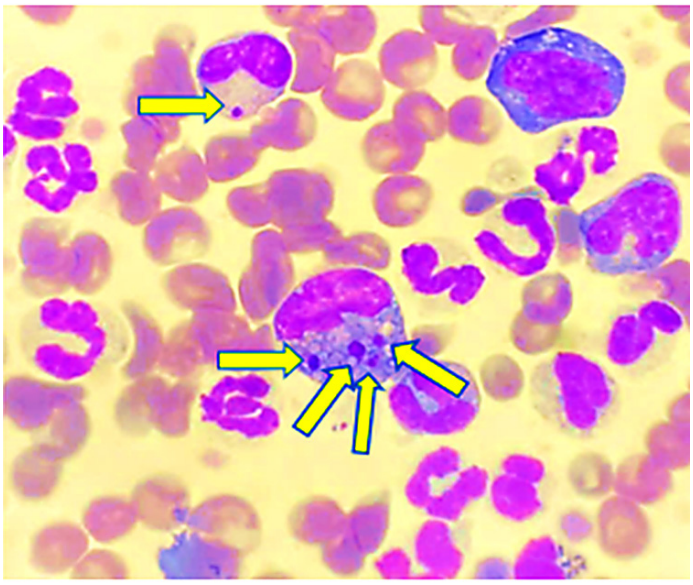

Figure 1: Photomicrograph of *Ehrlichia canis* morulae (arrows) in a monocyte from a naturally infected dog in Ho Chi Minh City, Vietnam. Blood smear stained with Diff-Quick (1000× magnification).

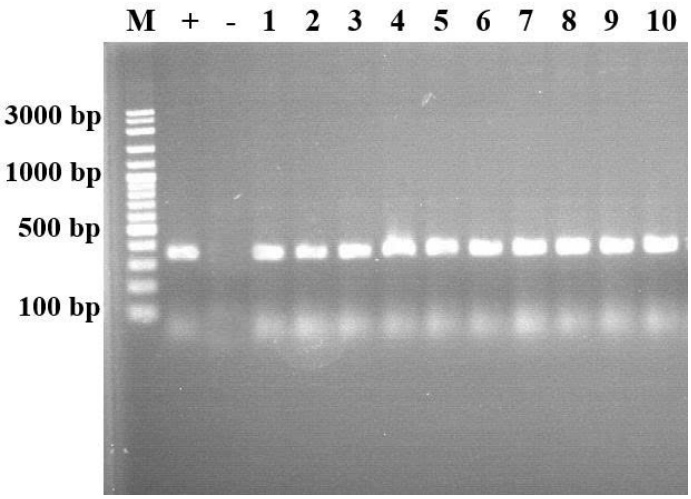

Figure 2: Agarose gel (1.5%) showing nested PCR amplification of the *Ehrlichia canis* 16S rRNA gene (389 bp). Lanes: M = 100 bp ladder (Vivatis, Malaysia), + = positive control, – = negative control, 1–10 = clinical samples.

The comparative application of multiple diagnostic techniques allowed for a robust evaluation of cytology versus molecular testing. The 31.25% cytological positivity rate, while useful for rapid, point-of-care screening, highlights the limitations of microscopy, including its dependence on observer expertise, quality of blood smears, and the transient presence of morulae. Staining artifacts and subjective interpretation also increase the likelihood of false positives.

Nested PCR, in contrast, directly detects pathogen DNA and yields a comparable but slightly higher detection rate (34.82%). This result aligns with prior research; for example, Wongtawan *et al.* (2024) reported a PCR detection rate of 21.07% in Thailand. Additionally, the current study outperforms cytology-based studies such as those by Nakaghi *et al.* (2008) in Brazil (3.3%) and Kottadamane *et al.* (2017) in India (14.28%), reinforcing the superior sensitivity of PCR in endemic settings. The prevalence reported in this study may be overestimated, as case selection was limited to dogs exhibiting clinical signs suggestive of ehrlichiosis, rather than random or broad-spectrum sampling.

Notably, the discrepancy between cytological and PCR-based results reflects the diagnostic limitations of blood smear examination, including inter-observer variability and the transient presence of morulae. These observations are consistent with findings by Alves *et al.* (2014) and Wongtawan *et al.* (2024), who documented that cytology may fail to detect up to 50% of PCR-positive cases.

The molecular confirmation of *E. canis* DNA in over one-third of suspected cases provides strong evidence of active pathogen circulation within the canine population in Ho Chi Minh City. This finding corresponds with the high local prevalence of tick vectors and ecological conditions conducive to disease transmission. Consequently, the results advocate for the incorporation of nested PCR into routine diagnostic protocols, especially in regions with endemic ehrlichiosis.

Furthermore, variations in detection between cytology and PCR may be influenced by disease stage. Dogs in the acute phase often present with detectable morulae due to active monocyte infection, whereas those in subacute or chronic stages may harbor the pathogen at levels below the microscopic detection threshold but still within the detection capacity of PCR. This finding also suggests that some PCR-positive but cytology-negative dogs may have been in subclinical or chronic phases of infection, with low parasitemia not evident on microscopy.

Table 2: Frequency of clinical manifestations in *Ehrlichia canis*-infected dogs (n = 39).

Clinical sign	Number of positive dogs (n)	Frequency (%)
Tick exposure	34	87.18
Fever, lethargy, anorexia	30	76.92
Vomiting	9	23.08
Pale mucous membranes	25	64.10
Subcutaneous hemorrhages	15	38.46
Epistaxis (nosebleeds)	22	56.41
Ocular hemorrhage/orbital cellulitis	5	12.82
Gingival bleeding	7	17.95
Erythematous dermatitis	22	56.41

Clinical Manifestations Observed in *Ehrlichia canis*-Infected Dogs

Among the 39 dogs molecularly confirmed to be infected with *E. canis*, a variety of clinical signs were recorded (Table 2 and Figure 3). Ticks were detected on 87.18% of infected dogs. The ticks were morphologically identified as *Rhipicephalus sanguineus*, predominantly in the adult stage. Common systemic signs included fever, lethargy, and anorexia (76.92%). Pale mucous membranes were observed in 64.10% of infected dogs. Hemorrhagic manifestations included subcutaneous petechiae or ecchymoses (38.46%), epistaxis (56.41%), and gingival bleeding (17.95%). Less frequent signs included ocular hemorrhage or orbital cellulitis (12.82%) and uterine inflammation (10.26%). Ocular findings were confirmed through basic ophthalmoscopic examination during clinical evaluation.

The high rate of tick exposure (87.18%) among *E. canis*-infected dogs strongly supports the established role of tick vectors, particularly *R. sanguineus*, in the transmission of canine monocytic ehrlichiosis, consistent with previous studies (Harrus and Waner, 2011; Bich *et al.*, 2020). Tick

infestation remains a major epidemiological risk factor for *E. canis* infection in endemic areas. The emergence of acaricide resistance in *R. sanguineus*, as reported in recent studies, may contribute to persistent infestations despite preventive efforts, thereby sustaining the risk of E. canis transmission in endemic areas (Siriporn *et al*., 2023). Systemic clinical signs, particularly fever, lethargy, and anorexia, were present in approximately 77% of infected dogs, aligning with findings from Sosa-Gutierrez *et al*. (2014) and Igarashi *et al*. (2024), who described these nonspecific symptoms as hallmark presentations during the acute phase of CME. The high frequency of pale mucous membranes (64.10%) corroborates the hematological findings of anemia commonly associated with *E. canis* infection, likely resulting from bone marrow suppression and immune-mediated hemolysis (Saito and Walker, 2016; Navarrete *et al*., 2022).

Figure 3: Representative clinical signs in dogs naturally infected with *Ehrlichia canis*. Clinical signs in *E. canis*-infected dogs: **A:** Bilateral epistaxis; **B:** Gingival petechiae; **C:** Ventral abdominal dermatitis; **D:** Rhipicephalus sanguineus infestation on the pinna.

The high proportion of dogs exhibiting erythematous dermatitis (56.41%) highlights the importance of cutaneous manifestations in the clinical diagnosis of *E. canis* infection. In this study, dermatitis included petechiae, ecchymosis, alopecia, and localized erythema. While *E. canis* is primarily known for hematological and systemic effects, skin lesions have been increasingly recognized in association with subacute or chronic ehrlichiosis. These dermatological signs are believed to result from immune-mediated vasculitis, platelet dysfunction, or endothelial damage, all of which are characteristic pathophysiological consequences of *E. canis* infection. This finding is consistent with previous studies, such as Mylonakis *et al*. (2004), which also reported skin lesions in naturally infected dogs.

Hemorrhagic complications were prominent, with epistaxis (56.41%) being more frequent than subcutaneous hemorrhages (38.46%) and gingival bleeding (17.95%). These findings reflect the profound thrombocytopenia induced by *E. canis*, which impairs primary hemostasis (Ybañez *et al*., 2016). Notably, the rate of epistaxis observed here was higher than that reported in certain earlier studies (Sainz *et al*., 2015), suggesting potential differences in disease severity, vector burden, or host response in the surveyed population.

Although less common, ocular hemorrhages (12.82%) and uterine inflammation (10.26%) were clinically significant. Ocular involvement in CME, though rarely reported, is increasingly recognized as part of systemic vasculitis and coagulopathy induced by ehrlichial infection (Harrus and Waner, 2011). The occurrence of uterine inflammation may represent secondary bacterial infections or immune-mediated tissue damage, necessitating further investigation.

The overlapping clinical signs observed here underscore the diagnostic challenges posed by CME, as the manifestations are often nonspecific and may mimic other infectious or immune-mediated diseases. Therefore, early suspicion based on clinical presentation, combined with molecular diagnostic confirmation, remains essential for timely intervention.

Physiological Alterations Associated with *E. canis* in Dogs

The physiological changes in hematological parameters observed in *E. canis*-infected dogs are summarized in Table 3. Leukocyte abnormalities were frequent, with 38.46% of cases presenting leukocytosis (WBC>17×10^6/mm^3) and 10.26% showing leukopenia. The mean WBC count among leukocytotic dogs was markedly elevated (35.56±6.75×10^6/mm^3). Neutrophil alterations followed a similar trend, with 35.90% of dogs exhibiting neutrophilia (mean: 26.53 ± 5.30×10^6/mm^3), while 15.38% showed neutropenia (neutrophil count < 4×10^6/mm^3). Lymphocyte variations were also notable: 20.51% of cases demonstrated lymphocytosis, and 5.13% presented with lymphopenia. In addition, monocytosis was identified in 20.51% of dogs, with a high coefficient of variation (CV = 133.13%), suggesting a heterogeneous monocyte response possibly reflecting diverse stages of infection or individual immune variation.

Red blood cell parameters showed a substantial reduction in 43.59% of infected dogs, indicating anemia. Mean RBC values in anemic dogs dropped to 3.75±0.40×10^6/mm^3. Hemoglobin (HGB) concentration was decreased in 28.21% of cases, with a mean of 66.18±9.80 g/dL in the low group, while hematocrit (HCT) reduction was even more pronounced, affecting 48.72% of infected dogs. Platelet count (PLT) reductions were similarly observed in 92.31% of cases,

Table 3: Physiological alterations associated with *E. canis* infection in dogs.

Parameter	Reference Range*	Deviation	Range	Mean±SE	Cv(%)	No. of cases	Proportion (%)
White blood cell count (×10³/µL)	6-17	↓	2.59-5.64	4.04±0.77	38.03	4	10.26
		↑	18.29-105.10	35.56±6.75	73.54	15	38.46
Neutrophils (×10³/µL)	4-12.60	↓	0.95-3.90	2.61±0.42	39.68	6	15.38
		↑	13.66-80.09	26.53±5.30	74.68	14	35.90
Lymphocyte (×10³/µL)	0.80-5.10	↓	0.5-0.62	0.56±0.06	15.15	2	5.13
		↑	5.96-19.08	10.87±1.99	51.78	8	20.51
Monocyte (×10³/µL)	0-1.80	↑	1.89-30.20	7.29±3.43	133.13	8	20.51
Red blood cell count (×10⁶/µL)	5.50-8.50	↓	0.85-5.47	3.75±0.40	44.12	17	43.59
Hemoglobin (g/dL)	110-190	↓	23-109	66.18±9.80	49.13	11	28.21
Hematocrit (%)	39-56	↓	6.4-38.9	26.48±2.47	40.62	19	48.72
Platelet count (×10³/µL)	117-460	↓	2-112	66.21±9.40	61.88	36	92.31

Table note: Reference ranges for physiological values are based on **The Merck Veterinary Manual* (2016). (↓) Decreased compared to the reference range; (↑) Increased compared to the reference range; Mean±SE represents the mean value and its standard error; CV% denotes the coefficient of variation.

with a critically low mean of 66.21±9.4×10³/mm³ among thrombocytopenic dogs.

Hematological alterations in *E. canis*-infected dogs observed in this study are consistent with previous reports describing the pathophysiology of canine monocytic ehrlichiosis (Harrus and Waner, 2011; Tajima and Wada, 2013). Leukocytosis, found in 38.46% of cases, reflects a strong inflammatory response typical of the acute phase of ehrlichiosis, characterized by activation of granulopoiesis and monocytosis in response to *E. canis* proliferation in mononuclear cells (Tajima and Wada, 2013; Parashar *et al*., 2016). Neutrophilia in 35.90% of cases supports the hypothesis of secondary bacterial infections or reactive inflammatory processes.

Conversely, leukopenia and neutropenia, although less frequent, are significant markers of bone marrow suppression during the chronic phase of infection, as described by Waner *et al*. (2014). Lymphocytosis (20.51%) and monocytosis (20.51%) observed here are hallmark features of immune activation during subclinical or chronic infection stages, reflecting antigenic stimulation and monocytic hyperplasia (Sainz *et al*., 2015; Parashar *et al*., 2016). Anemia was a major finding, with 43.59% of dogs exhibiting decreased RBC counts, in line with the non-regenerative anemia associated with CME. Reduced HGB and HCT values further corroborate the hematological impact of chronic infection. The anemia likely results from multiple mechanisms, including immune-mediated destruction of erythroid precursors, bone marrow hypoplasia, and chronic inflammation-mediated iron sequestration (Saito and Walker, 2016). Thrombocytopenia (48.72%) was also a hallmark abnormality, contributing to the hemorrhagic manifestations observed clinically. Platelet destruction in CME is multifactorial, involving immune-mediated clearance, splenic sequestration, and marrow production deficits (Saito and Walker, 2016; Navarrete *et al*., 2022). The high coefficients of variation (Cv%) recorded, particularly for WBC, Mon, and PLT parameters, highlight the substantial heterogeneity in host hematological response to *E. canis* infection, depending on infection stage, immune competence, and co-infection status.

Biochemical Alterations Associated with *E. canis* in Dogs

The biochemical abnormalities observed in dogs confirmed with *E. canis* infection are summarized in Table 4. Elevated liver enzyme levels were common, with alanine aminotransferase (ALT) increased in 28.21% of cases (mean 370.09±119.01 U/L) and aspartate aminotransferase (AST) elevated in 87.18% of dogs (mean 65±10.4 U/L). Renal function parameters were also frequently altered. Blood urea nitrogen (BUN) levels were elevated in 28.21% of cases (mean 67.55±14.26 mmol/L), while creatinine (CREA) concentrations were elevated in 10.26% of infected dogs (mean 7.49±2.14 µmol/L). Conversely, CREA levels were below the normal reference range in 20.51% of cases, and mild reductions in BUN were observed in 5.13% of dogs.

The biochemical alterations observed in *E. canis*-infected dogs highlight significant involvement of both hepatic and renal systems during the course of canine monocytic ehrlichiosis. Elevation of liver enzymes, particularly AST (87.18% of cases) and to a lesser extent ALT (28.21%), suggests hepatocellular

Table 4: Biochemical alterations associated with *E. canis* infection in dogs confirmed.

Biochemical Parameter	Reference Range	Deviation	Range	Mean±SE	Cv(%)	No. of cases	Proportion (%)
ALT (U/L)	10-109	↑	143-1500	370.09±119.01	106.65	11	28.21
AST (U/L)	13-15	↑	16-303	65±10.4	93.31	34	87.18
BUN (mmol/L)	8-28	↑	28.2-140.1	67.55±14.26	70.01	11	28.21
Creatinine (CREA, µmol/L)	0.5-1.7	↑	2.3-11.01	7.49±2.14	57.24	4	10.26

Table note: ALT-Alanine aminotransferase; AST-Aspartate aminotransferase; BUN-Blood Urea nitrogen; Reference ranges for physiological values are based on **The Merck Veterinary Manual* (2016); (↓) Decreased compared to the reference range; (↑) Increased compared to the reference range; Mean±SE represents the mean value and its standard error; Cv% denotes the coefficient of variation.

damage or reactive hepatopathy secondary to systemic inflammation. This pattern aligns with previous reports by Villaescusa *et al*. (2012) and Navarrete *et al*. (2022), which identified hepatic injury as a frequent complication in CME due to either direct infection of hepatic macrophages (Kupffer cells) or immune-mediated vasculitis. The markedly high coefficient of variation (CV>90% for AST and ALT) indicates substantial inter-individual variability in the degree of hepatic involvement, likely reflecting differences in infection stage, immune response, or concurrent secondary infections.

Renal impairment was also evident. Elevated BUN (28.21%) and CREA (10.26%) levels indicate decreased glomerular filtration rate and potential progression to prerenal or renal azotemia. Renal dysfunction may be attributed to immune complex deposition in the glomeruli, as commonly reported in chronic ehrlichiosis (Sainz *et al*., 2015; Parashar *et al*., 2016). Interestingly, a notable proportion of dogs (20.51%) exhibited decreased creatinine concentrations, possibly attributable to muscle mass loss associated with chronic disease progression and cachexia (Villaescusa *et al*., 2012). Comparative studies in other endemic regions (Kottadamane *et al*., 2017 in India; Wongtawan *et al*., 2024 in Thailand) have similarly reported elevations in BUN and liver enzymes in CME, supporting the multi-organ involvement theory of *E. canis* pathogenesis.

Overall, these findings emphasize that *E. canis* infection is a systemic disease, affecting not only the hematopoietic system but also causing significant hepatic and renal dysfunction. Early recognition of biochemical alterations is crucial for staging disease severity, monitoring therapeutic response, and improving prognosis in affected dogs.

CONCLUSIONS AND RECOMMENDATIONS

This study confirms that *Ehrlichia canis* infection in dogs in Ho Chi Minh City is closely associated with high rates of tick exposure and a broad range of clinical signs, particularly anemia, thrombocytopenia, and hemorrhagic manifestations. Hematological and biochemical abnormalities reflect systemic involvement, notably of the hepatic and renal systems. Although serological testing was not performed in this study, the relatively low PCR confirmation rate (34.82%) among clinically suspected cases reinforces the need for molecular testing to improve diagnostic accuracy. These findings underscore the importance of combining clinical evaluation, hematobiochemical profiling, and PCR-based detection to enhance diagnostic reliability and support appropriate case management in endemic settings.

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AUTHORS' CONTRIBUTIONS

All authors contributed to the conduct of this research. The authors engaged in study design, conducted result analysis, interpreted findings, and prepared the manuscript. All authors reviewed and endorsed the final manuscript.

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Ethics Statement

This research was conducted on animals with natural infections. This study utilized diagnostic samples; no experimental procedures were conducted on animals. Informed consent was obtained from the owners for their animals' participation in this study.

Competing Interest

The authors declare no competing interests.

Conflict of Interest

We certify that there is no conflict of interest.

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