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Molecular Prevalence and *16s rRNA* Gene Analysis of *Ehrlichia canis* in Dogs in Ho Chi Minh City, Vietnam

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ABSTRACT

Ehrlichiosis has been recognized as one of the leading tickborne vector diseases affecting the canine population. The present study aimed to determine the infection rate of *Ehrlichia canis* (*E. canis*) in dogs in Ho Chi Minh City, Vietnam. From June 2024 to December 2024, a total of 3,752 dogs were brought for initial veterinary examination due to various health concerns at the K9 Veterinary Clinic System, which operates in Districts 1, 4, 5, 7, and 8 of Ho Chi Minh City, Vietnam. These dogs represented a wide range of breeds, including indigenous dogs, Poodles, Corgis, Chihuahuas, and French Bulldogs, among others. The age of dogs ranged from 3 months to 12 years, with a mean age of approximately 4 years. Body weights varied depending on breed and age. Dogs exhibiting clinical signs suggestive of *E. canis* infection were further evaluated using a combination of diagnostic methods, including a rapid antibody test, blood smear staining for morulae detection, and polymerase chain reaction (PCR). The results showed that 87 out of 3,752 dogs (2.32%) were infected with *E. canis* based on PCR. The most common clinical signs observed in *E. canis* infected dogs included lethargy and anorexia (49.42%), pale mucous membranes (28.73%), fever (24.14%), epistaxis (16.09%), icterus (14.94%), petechiae (13.79%), and hindlimb weakness (5.75%). Hematological analysis revealed that thrombocytopenia (90.80%) and anemia (54.02%) were the most common hematological abnormalities. The *16S rRNA* gene sequences from 15 *E. canis* isolates in this study exhibited a very high degree of similarity. Phylogenetic analysis showed considerable genetic diversity among these isolates. These findings provide valuable insights into the clinical, hematological, and molecular characteristics of *E. canis* infection in dogs in Vietnam and underscore the importance of molecular surveillance for improved diagnosis and disease management.

Keywords: Dog, *Ehrlichia canis*, Hemorrhage, Polymerase chain reaction, Phylogenetic analysis

INTRODUCTION

Ehrlichia canis has been recognized as one of the significant infectious diseases affecting dogs. The disease is primarily transmitted through the brown dog tick (*Rhipicephalus sanguineus*) and occurs concurrently in many regions with hot and humid climate conditions that are ideal for vector proliferation (Dantas-Torres et al., 2024). Canine Ehrlichiosis caused by *E. canis* presents with a wide range of clinical signs, including high fever, anemia, lethargy, lymphadenopathy, hemorrhages, and, in severe cases, may lead to death if not diagnosed and treated promptly (Kabir et al., 2024). In Asian countries, *E. canis* infection is considered an important veterinary concern due to favorable environmental conditions for vector transmission. According to global studies, the infection rate of *E. canis* in dogs has been reported to range from 10% to 60%, depending on geographic location, climate, and the implementation of parasite prevention strategies (Aziz et al., 2022). In Vietnam, Van Hai and Khuong (2021) reported a prevalence rate of 21.7% in clinically suspected canine cases based on blood smear staining for *E. canis* morulae. Although primarily a canine pathogen, *E. canis* has demonstrated zoonotic potential, with some human infections reported, particularly in immunocompromised individuals (Perez et al., 2006). This raises public health concerns, particularly in areas with high tick densities and close human-animal interactions. In addition, canine ehrlichiosis imposes a significant economic burden on pet owners due to the costs of diagnosis, long term treatment, and tick prevention measures (Beugnet and Marié, 2009). However, studies on the prevalence and phylogenetic analysis of *E. canis* remain limited in Vietnam. This lack of data has posed challenges in accurate diagnosis, effective treatment, and the development of appropriate prevention and control strategies. To the best of the authors' knowledge, this is the first study to determine the molecular prevalence and perform phylogenetic analysis of *E. canis* in dogs in Ho Chi Minh City, Vietnam.

MATERIALS AND METHODS

Ethical approval

This study was conducted in accordance with animal welfare guidelines and approved by the Council for Science and Education, College of Agriculture, Hutech University, Vietnam. All procedures involving animals were carried out

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under the supervision of licensed veterinarians, and informed consent was obtained from all dog owners prior to sample collection.

Blood collection and subsequent laboratory analyses

The study was conducted through a continuous survey of 3,752 dogs presented by their owners for initial clinical examination or routine health checkups at the K9 Veterinary Clinic system located in Districts 1, 4, 5, 7, and 8 of Ho Chi Minh City, Vietnam, from June to December 2024. These dogs represented various breeds, including indigenous dogs, Poodles, Corgis, Chihuahuas, and French Bulldogs, among others. The age of dogs ranged from 3 months to 12 years, with a mean age of approximately 4 years. Body weights varied depending on breed and age. A total of 1.5 mL of blood was aseptically collected from the cephalic vein of each of the 185 dogs exhibiting clinical signs suggestive of *E. canis* infection, including fever, anorexia, jaundice, pale mucous membranes, epistaxis, subcutaneous hemorrhages, hind limb weakness, and transferred into EDTA tubes for further analysis. The following diagnostic procedures were subsequently performed: complete blood count analysis using an automated hematology analyzer (BC-5000Vet, Mindray, China); detection of anti *E. canis* antibodies using a commercial rapid test kit (IDEXX Laboratories, USA); and microscopic examination of Giemsa-stained blood smears prepared from the buffy coat layer (Merck, Germany) to detect *E. canis* morulae.

Polymerase chain reaction (PCR)

Based on the aforementioned diagnostic tests, a total of 107 blood samples that tested positive by rapid antibody test and/or Giemsa-stained blood smear for morulae detection were subsequently subjected to PCR analysis at Can Tho University, Vietnam. DNA from each blood sample was amplified by PCR targeting a specific fragment of the *16S rRNA* gene, yielding a product of 396 base pairs. The primer sequences were adopted from Murphy et al. (1998) and have been applied in subsequent studies on *E. canis* by Lakshmanan et al. (2007) and Pinheiro et al. (2013).

Ehr-16S-HE3-F: 5'-TATAGGTACCGTCATTATCTTCCCTAT-3'

Ehr-16S-ECAN5-R: 5'-CAATTATTTATAGCCTCTGGCTATAGGA-3'

The PCR thermal profile included a preliminary denaturation step at 95°C for 3 minutes, then 40 amplification cycles comprising denaturation at 95°C for 30 seconds, annealing at 54°C for 30 seconds, and extension at 72°C for 30 seconds, concluding with a final elongation phase at 72°C for 7 minutes.

Sequencing

The PCR products were purified using the FastGene® Gel/PCR Extraction Kit (NIPPON Genetics EUROPE, Düren, Germany) according to the manufacturer's instructions. Purified PCR products were then submitted to DNA SEQUENCING Company (Vietnam) for Sanger sequencing using the same primer pair employed in the PCR reaction. The *16S rRNA* gene nucleotide sequences of *E. canis* obtained from this study were manually reviewed, primer sequences were removed, and the resulting sequences were compared to publicly available sequences in the GenBank database to assess nucleotide similarity. Sequence similarity searches were performed using the Basic Local Alignment Search Tool (BLAST). Pairwise sequence similarity values between the studied strains and reference strains were calculated using the SIAS tool (http://imed.med.ucm.es/Tools/sias.html). A phylogenetic tree was constructed using the Neighbor Joining method with 1,000 bootstrap replicates, implemented in MEGA software (Tamura et al., 2013).

Statistical analysis

Data were collected and analyzed using Microsoft Excel 2016 and Minitab version 17. Quantitative variables were expressed as mean ± standard deviation ($\overline{X} \pm SD$).

RESULTS AND DISCUSSION

Prevalence of *Ehrlichia canis* infection in dogs in Ho Chi Minh City, Vietnam, based on molecular diagnosis

A total of 3,752 clinically sick dogs were presented for initial consultation and treatment at the K9 Veterinary Clinic system in Ho Chi Minh City between June 2024 and December 2024. Based on clinical history, physical examination, hematological analysis, rapid antibody testing, and Giemsa-stained blood smear evaluation, a preliminary diagnosis was identified in 107 cases suspected of *E. canis* infection, corresponding to a proportion of 2.85%. Subsequent confirmation by PCR revealed 87 positive cases, yielding an overall prevalence of 2.32%. Representative PCR positive samples are presented in Figure 1.

The prevalence of *E. canis* infection observed in this study was lower compared to recent studies conducted in other geographic regions. For example, in Colombia, Forero-Becerra et al. (2021) reported that among 114 cases, 66% of dogs tested positive for *E. canis* using Enzyme linked Immunosorbent assay (ELISA), while 41% were confirmed by PCR. In

Bangladesh, Kabir et al. (2024) recorded a prevalence rate of 6.9% among 246 dogs using PCR. In India, Chakraborty et al. (2024) conducted a study in the state of Odisha and found that 31.46% of 178 dogs tested positive for *E. canis* through blood examination and PCR analysis. These findings highlight the considerable variability in *E. canis* prevalence across different geographical regions, which may be attributed to factors such as diagnostic methodology, environmental conditions, the presence of *R. sanguineus* ticks, and the implementation of ectoparasite control measures in canine populations (Dantas-Torres et al., 2012).

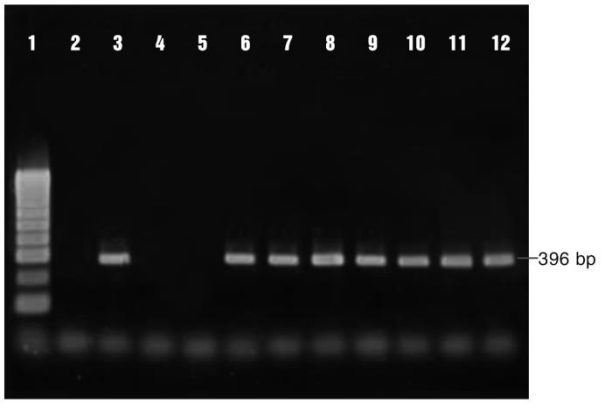

Figure 1. Presence of *E. canis* in certain dogs' blood samples collected from clinical cases in Ho Chi Minh City, Vietnam, from June to December 2024. Lane 1: DNA ladder; Lane 2: negative control; Lane 3: positive control; Lanes 4 - 5: Negative samples; Lanes 6-12: positive samples.

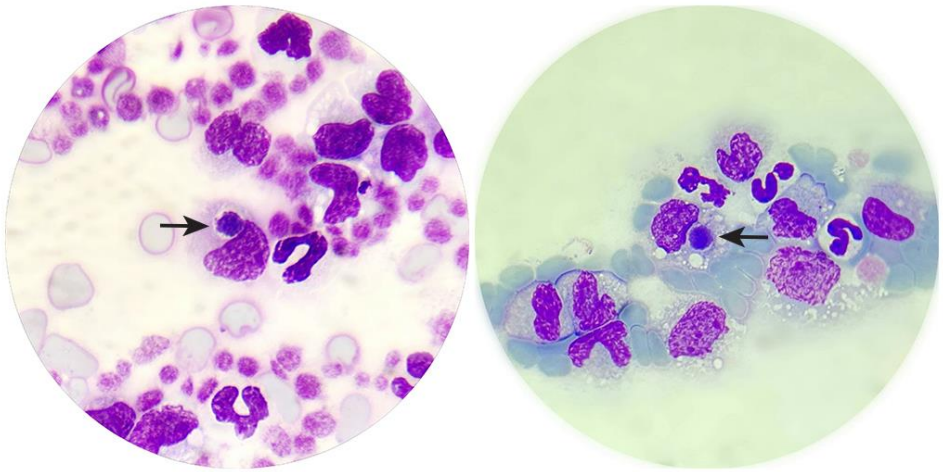

Figure 2. Morulae of *E. canis* within the cytoplasm of a monocyte in certain dog's blood samples collected from clinically suspected dogs in Ho Chi Minh City, Vietnam, from June to December 2024. Giemsa stain, 100x magnification (Black arrow).

Table 1. Distribution of *E. canis*-positive cases by diagnostic method in dogs aged 3 months to 12 years, examined in Ho Chi Minh City, Vietnam, from June to December 2024

Diagnostic method	Number of positive cases
Rapid antibody test	75
Giemsa-stained blood smear	68
Polymerase chain reaction	87

Table 1 illustrates the variation in sensitivity among the three diagnostic methods used to detect *E. canis* infection in dogs, including rapid antibody tests, morula detection via Giemsa-stained blood smear, and PCR. The results indicated that PCR detected the highest number of positive cases (87), followed by the rapid antibody test (75 cases), and the Giemsa–stained smear (68 cases). These findings reflect the differing accuracy levels of each method in diagnosing the disease. While the rapid antibody test is widely used due to its ease of application, quick turnaround time, and affordability, it merely detects antibodies against *E. canis* and cannot distinguish between current infection and prior exposure (Dantas-Torres et al., 2012). As noted by Harrus and Waner (2011), the sensitivity of the rapid antibody test

ranges from 75% to 90%, implying that a proportion of infected dogs may remain undetected. Similarly, the detection of *E. canis* morulae in monocytes using Giemsa-stained blood smears enables direct visualization of the pathogen but is limited in diagnostic value. This method is most effective during the acute phase and requires a high level of technical expertise. In Figure 2, morulae of *E. canis* were detected in blood smears using the buffy coat method. Samples were collected from clinically suspected dogs presenting with acute manifestations such as epistaxis and subcutaneous hemorrhages. Reported sensitivities vary from 30% to 60%, with a significant risk of false-negative results, especially in cases with low bacteremia (Singh et al., 2021). In contrast, PCR offers a major advantage in terms of sensitivity and specificity. By directly amplifying specific DNA sequences of *E. canis*, PCR can detect the pathogen even at minimal concentrations in the bloodstream, including in chronic or subclinical infections. According to Chua et al. (2020), PCR achieves sensitivity rates exceeding 95%, surpassing both serological and cytological methods. The optimal performance of PCR in detecting *E. canis* was evident in this study, where it identified the greatest number of positive cases (87), as detailed in Table 1, reinforcing its essential role as the most reliable diagnostic tool among the methods employed. Given its high diagnostic performance, PCR should be considered the gold standard for confirming *E. canis* infection, especially in epidemiological studies or clinical cases requiring definitive diagnosis. While rapid antibody tests may serve as a convenient tool for preliminary screening, and Giemsa-stained smears may assist in diagnosis during the acute stage, both methods have inherent limitations. Optimal diagnostic outcomes are best achieved through the integration of PCR testing, where available, and careful selection of diagnostic tools based on laboratory capabilities and clinical objectives (Harrus and Waner, 2011).

Clinical signs

Ehrlichia canis infection causes a wide range of clinical manifestations that adversely affect the overall health status of affected animals, as presented in Table 2. Lethargy and anorexia were the most frequently observed clinical signs, attributed to the immunosuppressive effects of *E. canis*, which disrupt various physiological functions, leading to loss of appetite and rapid physical deterioration (Harrus and Waner, 2011). Pale mucous membranes, reported in 28.73% of infected dogs (Figure 3), are indicative of anemia, likely resulting from immune-mediated hemolysis, where the host's immune system targets and destroys red blood cells (Agnoli et al., 2024). Fever was recorded in 24.14% of cases and is associated with the host's inflammatory response to infection, often accompanied by fatigue and reduced immune function (Aguirre et al., 2004). More severe manifestations, such as epistaxis and subcutaneous hemorrhages, reflect the impact of *E. canis* on hematologic parameters, particularly thrombocytopenia. These signs are caused by a significant reduction in platelet count, impairing coagulation and leading to spontaneous bleeding. Icterus, observed in 14.94% of dogs, suggests hepatic dysfunction or excessive erythrocyte destruction, and is commonly seen in severe disease stages or cases of coinfection with Babesia. Additionally, hind limb weakness (5.75%) may result from neurologic involvement or joint inflammation caused by *E. canis*, leading to impaired mobility.

When compared with previous studies, these clinical findings are consistent with the commonly reported symptomatology of *E. canis* infection in dogs. According to Van Hai and Khuong (2021), the most frequent clinical signs included fever (33%), anorexia (28%), hemorrhage (27%), and pale mucous membranes (30%). Similarly, Espino-Solís et al. (2023) reported clinical manifestations such as anorexia, seizures, coughing, subcutaneous hemorrhages, epistaxis, and bloody diarrhea in dogs infected with *E. canis*. These findings underscore the broad clinical spectrum of the disease and its consistent presentation across diverse geographical regions (Aziz et al., 2022).

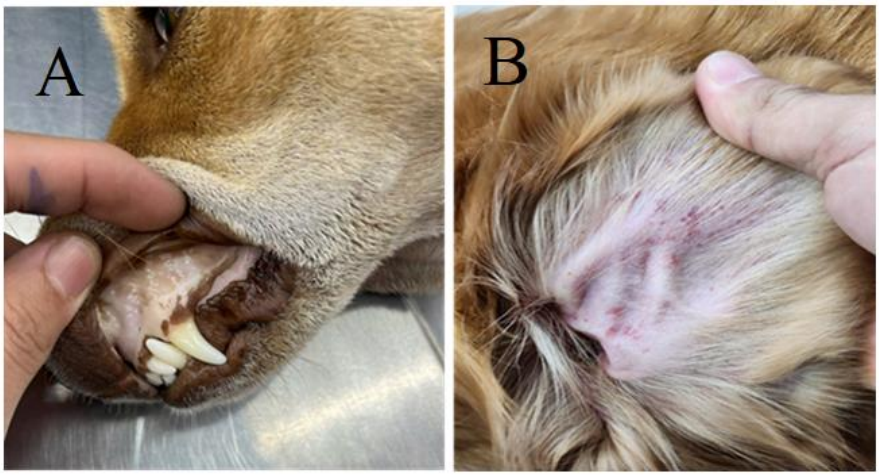

Figure 3. A 3-year-old male indigenous dog from Ho Chi Minh City, Vietnam, from June to December 2024, infected with *Ehrlichia canis*, showing pale mucous membranes (**A**) and subcutaneous hemorrhages (**B**).

Table 2. Frequency of selected clinical signs observed in *Ehrlichia canis* infected dogs in Ho Chi minh City, Vietnam from June to December 2024

Clinical Symptom	Number of cases	Proportion (%)
Lethargy, anorexia	43	49.42
Pale mucous membranes	25	28.73
Fever	21	24.14
Nasal bleeding	14	16.09
Jaundice	13	14.94
Subcutaneous hemorrhage	12	13.79
Hindlimb weakness	5	5.75

Alterations in selected hematological parameters

The blood test results of 87 dog's positive for *E. canis*, presented in Table 3, revealed significant impacts on hematological indices, indicating notable alterations in white blood cell count, red blood cell count, hemoglobin concentration, hematocrit, and platelet count.

Changes in white blood cell counts reflect the host's immune response to infection. Bhadesiya and Raval (2015) reported a marked increase in neutrophils and a concurrent decrease in lymphocytes in dogs infected with *E. canis*. Leukopenia may occur during the chronic phase of ehrlichiosis as a result of bone marrow suppression and depletion of leukocyte reserves. Anemia is a common complication of *E. canis* infection, evidenced by reductions in red blood cell count (54.02%), hemoglobin concentration (36.78%), and hematocrit levels (44.83%). This condition is primarily attributed to blood loss associated with thrombocytopenic hemorrhage or immune-mediated hemolysis (Lachungpa et al., 2020). Thongsahuan et al. (2020) noted that anemia in *E. canis* infected dogs generally ranges from mild to moderate in severity, though in some cases it may progress to prolonged debilitation. Thrombocytopenia (90.80%) was the most prominent hematological abnormality associated with ehrlichiosis, and is commonly responsible for clinical signs such as epistaxis, subcutaneous hemorrhages, and mucosal bleeding. The underlying mechanisms include immune-mediated platelet destruction and impaired platelet production in the bone marrow (Harrus and Waner, 2011). Siriporn and Juasook (2022) also highlighted anemia and thrombocytopenia as the principal hematological features of all major tick-borne diseases in dogs.

These findings demonstrated that *E. canis* infection has a profound impact on the hematological system, particularly by inducing severe thrombocytopenia and persistent anemia. Such alterations not only compromise overall health status but also increase the risk of hemorrhage and immunosuppression, making affected dogs more susceptible to secondary infections (Wongtawan et al., 2024). Monitoring hematological parameters is therefore essential in the diagnosis of ehrlichiosis, as it aids in assessing disease progression, identifying potential complications, and formulating appropriate treatment plans. However, the extent of hematological alterations may vary depending on the disease stage, the individual immune response, and the animal's underlying health status. As such, hematological evaluation should be integrated with other diagnostic methods to ensure timely and accurate disease management (Haryanto and Tjahajati, 2020).

Table 3. Proportion of dogs aged 3 months to 12 years with alterations in selected hematological parameters associated with *Ehrlichia canis* infection in Ho Chi Minh City, Vietnam, from June to December 2024

Parameter	Unit	Reference range	Level	Number of dogs	Quantification (Mean ± SD, Range)	Proportion (%)
Leukocytes	10^9/L	6.0-17.00	Decreased	19	3.44 ± 1.98 (0.17-5.94)	21.84
			Increased	22	24.70 ± 8.51 (17.30-50.68)	25.29
Erythrocytes	10^{12}/L	5.10-8.50	Decreased	47	3.07 ± 0.95 (0.98-4.58)	54.02
Hemoglobin	g/L	110-190	Decreased	32	61.72 ± 15.52 (27.00-83.00)	36.78
Hematocrit	%	33.0-56.0	Decreased	39	18.37 ± 5.16 (7.60-25.70)	44.83
Platelets	10^9/L	117-490	Decreased	79	32.91 ± 22.86 (6.00-98.00)	90.80

SD: Standard deviation

Genetic analysis of *Ehrlichia canis*

Fifteen PCR-positive samples of *E. canis* were selected for sequencing. The *16S rRNA* gene region was analyzed to assess genetic similarity, and a phylogenetic tree was constructed using reference strains from various species, including *Neorickettsia helminthoeca*, *Anaplasma phagocytophilum* strain Webster, *Anaplasma platys*, *Ehrlichia* spp, *E. chaffeensis*, *E. ewingii* strain Stillwater, *E. ruminantium* strain Welgevonden, and *E. canis* strains isolated from different geographical regions worldwide. The *16S rRNA* gene sequence analysis of 15 *E. canis* isolates obtained in this study revealed a high level of genetic similarity, with sequence identity ranging from 99.33% to 100%. This finding reflects the highly conserved nature of the *16S rRNA* gene within *E. canis* and supports the inference that the isolates likely belong to the same phylogenetic clade. The high degree of homogeneity in the *16S rRNA* region is consistent with previous reports. According to Selim et al. (2020), the *16S rRNA* gene of *E. canis* isolates infecting dogs typically exhibits low genetic variability among different strains, particularly those originating from the same geographic region. Similarly, the study by Purisarn et al. (2022) in Thailand reported a high degree of genetic homogeneity among *E. canis* strains circulating in dogs within the region.

When compared with reference strains from various countries, including Malaysia (KR920044.1), Taiwan (GU810149.1), China (KJ659037.1), Turkey (KJ513197.1), Thailand (OP164610.1), Japan (LC841966.1), the Czech Republic (KC479023.1), Italy (EU439944.1), and Brazil (EF195135.1), the local isolates maintained a high degree of similarity, ranging from 98.67% to 100%. Some reference strains, such as *E. canis* Tokyo-1 (LC841966.1) and *E. canis* isolate K35 (OP164610.1), exhibited near–identical sequences with several local samples, suggesting the presence of a common genotype circulating in the Asia Pacific region (Pinyoowong et al., 2008). Notably, one isolate (strain E52) showed the lowest similarity (98.67%) with certain reference sequences, which may be attributed to localized genetic divergence or minor mutations within the analyzed gene fragment. However, this level of variation remains within the accepted threshold for classification within the same *E. canis* species. Even strains from geographically distant regions - such as *E. canis* strain Brazil-CO2 (EF195135.1) and *E. canis* strain 171 (KC479023.1, Czech Republic) exhibited over 98% similarity, suggesting the presence of a globally distributed clade with broad host adaptability and environmental tolerance (Kabir et al., 2024). The high degree of similarity between local isolates and reference strains from other countries underscores the potential for *E. canis* dissemination across diverse geographic regions, likely facilitated by the widespread distribution of the tick vector *Rhipicephalus sanguineus* in tropical and subtropical climates. Such evidence of cross-regional genetic homogeneity has significant implications for epidemiological research, particularly in the context of climate change and increased animal movement, both of which may contribute to the global spread of the pathogen. Furthermore, the detection of slight sequence variations among isolates highlights the need for further studies on intra-species genetic diversity, which may aid in identifying region specific circulating genotypes.

Phylogenetic analysis based on the *16S rRNA* gene sequences revealed notable genetic diversity among the *E. canis* isolates obtained in this study, which were grouped into three major clades. The isolates, designated from E13 to E56, highlighted with red dots in Figure 4, did not cluster into a single monophyletic group but were instead distributed across multiple branches of the phylogenetic tree. The distribution of isolates across distinct clades suggests the coexistence of multiple genetic lineages within the studied population.

The first clade included isolates E52, E13, E49, E19, and E55, which demonstrated close genetic relationships with *E. canis* Tokyo-1 (LC841966.1) and reference strains from Turkey (TrKysEcan1, KJ513194.1) and Venezuela (VHE, AF373612.1). This clade was supported by moderate bootstrap values ranging from 62 to 41, indicating a relatively stable evolutionary relationship within the group. The clustering of study isolates with strains from geographically distant regions suggests the possible existence of a widely distributed *E. canis* lineage, or it may reflect genetic recombination and diversification events occurring during international transmission. These findings further support previous studies, such as that of Unver et al. (2001), which proposed that *E. canis* strains exhibit a high degree of genetic homogeneity even across distant geographical regions, likely due to cross-border movement of infected companion animals and vectors through global transport and trade routes.

The second clade consisted of isolates E37, E45, E56, E38, and E14, which clustered closely with *E. canis* KR920044.1 (Malaysia) and *E. canis* Nero (EU439944.1, Italy). This branch was supported by a bootstrap value of 49, indicating a moderate level of genetic differentiation between this group and other reference strains. The grouping of these isolates with strains from Southeast Asia and Southern Europe suggests the possible existence of an *E. canis* lineage exhibiting intermediate genetic characteristics between distinct geographical regions, or alternatively, the result of local adaptation following introduction. This observation aligns with the epidemiological context in Southeast Asia, where *E. canis* is known to be widespread and potentially co-circulating with multiple genetic variants (Purisarn et al., 2022). Additionally, the genetic proximity between Asian and European strains in this clade may reflect transregional genetic exchange facilitated by the movement of infected dogs or tick vectors through international transportation and trade routes (Siarkou et al., 2007).

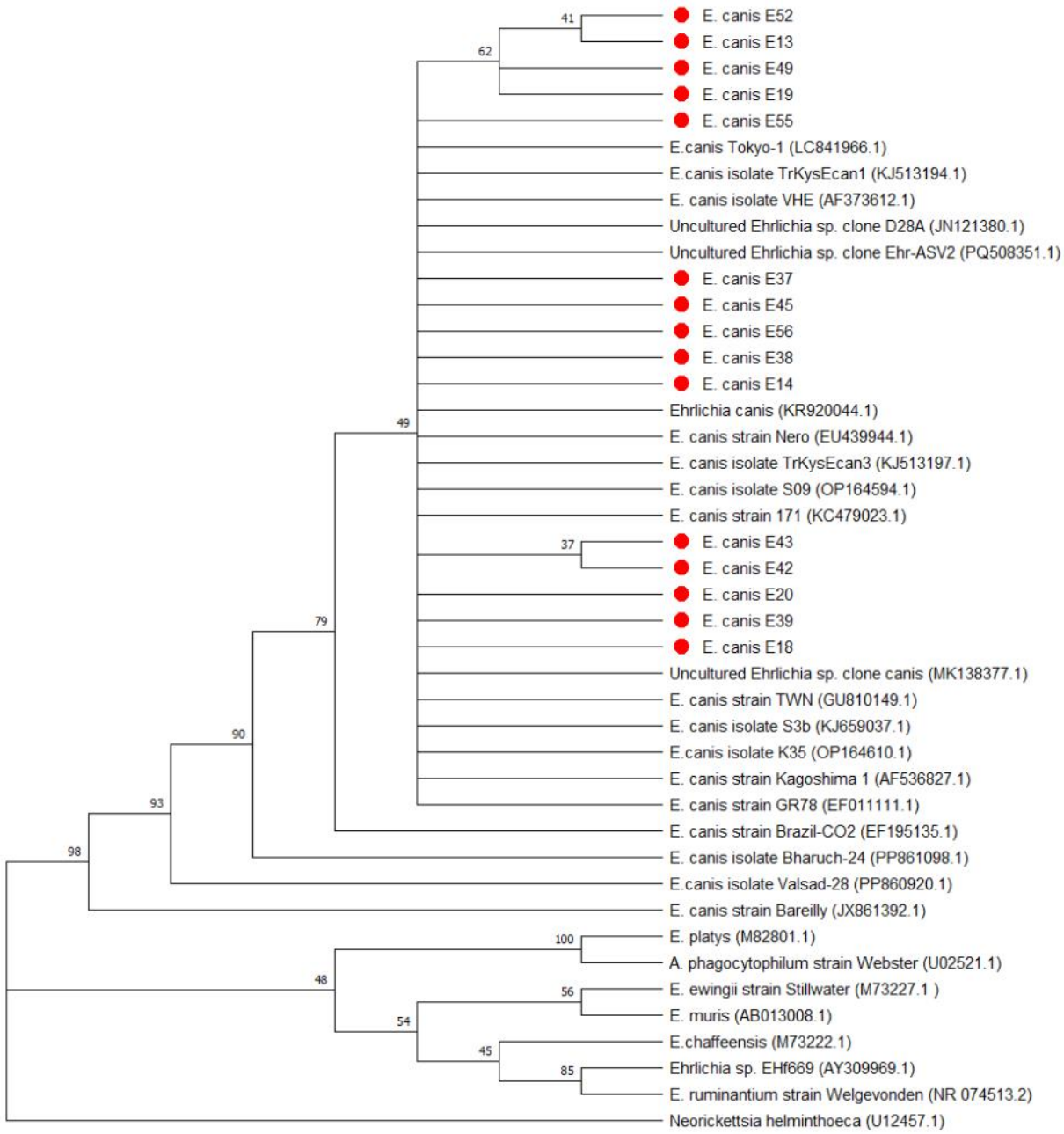

Figure 4. Phylogenetic tree of *Ehrlichia canis* isolates infecting dogs in Ho Chi Minh City, Vietnam, along with reference strains retrieved from GenBank. The 15 isolates obtained in this study are indicated by red dots.

The third clade comprised isolates E43, E42, E20, E39, and E18, which showed close genetic relationships with *E. canis* strains from Taiwan (TWN, GU810149.1), Japan (Kagoshima1, AF536882.1), India (Valsad-28, PP860920.1; Bharuch-24, PP861098.1), and Brazil (Brazil-CO2, EF195135.1). This clade was supported by the lowest bootstrap value (37), suggesting a relatively high degree of genetic similarity among the strains within the group, but an unclear or weakly supported evolutionary relationship between them. Nevertheless, the clustering of strains from both Asian and South American regions within the same branch reflects the high genetic polymorphism of *E. canis* and raises the possibility of genetic exchange among geographically dispersed strains, or the existence of a broadly evolved lineage capable of adapting to diverse ecological conditions. This distribution pattern is consistent with the findings of Aguiar et al. (2007), who reported significant genetic differences between *E. canis* strains from Brazil and those from Asia, suggesting independent evolutionary trajectories in different geographic regions.

All isolates analyzed in this study clustered within a major clade supported by a high bootstrap value (98), confirming that they all belong to the *E. canis* species, with no evidence of co-infection by other *Ehrlichia* species. However, the presence of several branches with relatively low bootstrap values highlights a limitation of relying solely on the *16S rRNA* gene for phylogenetic analysis. Previous studies by Gaunt et al. (2010) have emphasized that, to

improve phylogenetic resolution and reliability, additional genetic markers such as *groEL* (*chaperonin* gene), *gltA* (*citrate synthase*), *dsb* (*disulfide bond formation protein*), and *omp* (*outer membrane protein*) should be incorporated. These genes typically exhibit greater sequence diversity due to their functional roles and selective pressures, particularly *omp* and *dsb*, which are involved in host-pathogen interactions. Genetic markers *groEL* and *gltA* evolve faster than *16S rRNA* and provide more informative polymorphisms, making them suitable for resolving closely related strains and inferring more robust evolutionary relationships. Multi-locus sequence analysis (MLSA) using these markers has been shown to enhance phylogenetic discrimination and better reflect epidemiological and geographical variation among *E. canis* strains. Moreover, the application of more advanced analytical methods, such as Bayesian inference or Maximum Likelihood, is recommended to enhance the robustness of evolutionary relationship assessments.

CONCLUSION

This study represents the first molecular investigation of *E. canis* infection in dogs in Ho Chi Minh City, Vietnam, integrating clinical observation, hematological evaluation, and phylogenetic analysis based on the *16S rRNA* gene. The overall PCR-confirmed prevalence of 2.32% highlights the presence of *E. canis* infection in the local canine population, albeit at a lower rate compared to some other regions in Asia and South America. Clinically, infected dogs exhibited a wide spectrum of signs, with lethargy, anorexia, and pale mucous membranes being the most prevalent. Hematological findings were dominated by thrombocytopenia and anemia, underscoring the diagnostic value of blood profile monitoring in suspected cases. Phylogenetic analysis based on the *16S rRNA* gene revealed high genetic similarity with reference strains from other regions, suggesting the presence of a widely distributed *E. canis* lineage in the Asia-Pacific. The observed genetic clustering into three clades indicates multiple lineages circulating locally. While *16S rRNA* analysis was informative, additional genetic markers are recommended for improved phylogenetic resolution.

DECLARATION

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Author's contributions

Loan Vu Thuy Hong Nguyen and Vu Thanh Du conceptualized the study and proposed the diagnostic procedures. Dung Van Nguyen and Vu Thanh Du collected samples, performed laboratory tests, and supervised the research process. Loan Vu Thuy Hong Nguyen, Dung Van Nguyen, and Vu Thanh Du analyzed and interpreted the obtained data. All authors critically reviewed the entire study. All authors revised and approved the submitted manuscript.

Conflict of interests

The authors declare no conflict of interest.

Availability of data and materials

All data supporting the findings of this research are available upon reasonable request from the corresponding author.

Ethical considerations

This article was written originally without any copy from data from published articles and books.

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