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Research article

Epidemiological characteristics of the ectoparasite infestation in domestic dogs in the Mekong Delta of Vietnam

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Abstract

Ectoparasites not only cause pruritus and dermatitis but also play a crucial role in vector-borne disease in domestic dogs. This study aims to investigate the prevalence of ectoparasite infestation and the molecular characteristics of ticks and fleas. A cross-sectional study was conducted on 720 dogs in two provinces and one city in the Mekong Delta of Vietnam: An Giang, Can Tho, and Kien Giang. The identification of ectoparasites was performed based on parasitological methods, conventional PCR, and sequencing. The findings revealed that domestic dogs were infected ectoparasites with a high infection rate of 57.92% (417/720). Age, lifestyle of dogs, and veterinary management practices of dog owners had a significant influence on the ectoparasite infestation in the surveyed dogs. Six different species of ectoparasites were identified by morphological characteristics, including ticks (*Rhipicephalus sanguineus*), fleas (*Ctenocephalides canis*, *Ctenocephalides felis felis*), demodectic mange (*Demodex canis*), louse (*Trichodectes canis*), and mites (*Sarcoptes canis*). In this study, ticks (*R. sanguineus*) were the most common ectoparasites in infected dogs (59.95%), followed by fleas (*Ctenocephalides* spp.) at 34.77%, *Trichodectes canis* at 6.71%, *Demodex canis* at 2.63%, and *Sarcoptes canis* 1.92%. Analyzing the 16S rDNA sequence of *R. sanguineus* in domestic dogs in the Mekong Delta showed high homologs to the *R. sanguineus* strains from Brazil and India. Furthermore, the *ITS-1* sequence of *Ctenocephalides* revealed a close relationship to the *C. canis* isolates in Spain.

Keywords: Domestic dogs, ectoparasites, Mekong Delta, molecular characteristics.

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INTRODUCTION

Ectoparasites, including ticks, fleas, mites, and louse, not only cause irritation, aesthetic problems, pruritus, and dermatitis in dogs but also play a vital role in vector-borne diseases to transmit zoonotic diseases and threats to animal health (Childs and Paddock, 2003; Eisen et al., 2017). Fleas and louse (*Ctenocephalides* spp., *Pulex irritans*, *Trichodectes canis*, and *Felicola subrostratus*) occupy an important position in the intermediate host stage of *Dipyllium caninum* life cycle (Low et al., 2017; Hu et al., 2021), which is a common parasite in domestic dogs and posing a threat to human health. Moreover, ticks such as *Ixodes* spp., *Rhipicephalus* spp., and *Dermacentor* are recognized as crucial vectors of Babesiosis (Gray et al., 2010), Rickettsia (Parola et al., 2013) or Rocky Mountain spotted fever to humans (McCollough, 2018).

Dogs have become an important part of human life in increasing the quality of mental life, especially for the emotional development of children (Gee et al., 2021). However, this close companionship with dogs might become a potential risk unless people ignore the presence of ectoparasites that can transmit hazardous pathogens to humans (Klimpel et al., 2010).

Moreover, ectoparasitic disease is one of the ubiquitous diseases in domestic dogs. Although there were many studies of ectoparasites in Vietnam (Nguyen and Nguyen, 2014; Doan and Vu, 2021), the molecular identification and genetic analysis of ectoparasites are still limited. Hence, this study aimed to investigate the epidemiology of ectoparasite infestation and analyze the genetic characteristics of common ectoparasitic species in domestic dogs in the Mekong Delta of Vietnam.

MATERIALS AND METHODS

Samples collection and morphological identifications

Ectoparasites were collected from 417 domestic dogs at 9 veterinary clinics in An Giang province (Long Xuyen, Cho Moi, and Thoai Son districts), Kien Giang province (Rach Gia, Giong Rieng, and Tan Hiep districts), and Can Tho city (Ninh Kieu, O Mon, and Thot Not districts) from March to October 2022.

To gather detailed information about dogs, such as sex, age, lifestyle, and veterinary practices, questionnaires were administered to the dog owners. In addition, the age of dogs was determined by dental formulary analysis. The domestic dogs were classified into 3 age groups, namely dogs under than 1-year-old (juvenile dogs), those between 1-3 years old (young dogs), and those older than 3 years old (adult dogs). Ectoparasites, such as ticks and fleas, were removed by using specialized forceps and grooming methods. Samples were then collected and preserved in 70% ethanol. Finally, the samples were examined under a stereomicroscope to identify the ectoparasites.

Deep scraping had been performed in dogs with dermatologic lesions to look for the presence of mites (*Sarcoptes* or *Demodex*) by veterinarians. The skin scrapings were later treated with KOH 10% and heated for 30 minutes, followed by centrifuge and collecting the sediment. Then, the sediment from each sample was examined under a microscope. The identification is based on morphological characteristics of ectoparasites, as described by Wall and Shearer (2001), as well as Mathison and Pritt (2014).

DNA extraction and PCR performing for ticks and fleas

Ticks and fleas were further identified by using molecular techniques. To extract DNA from ticks and fleas, the DNeasy Blood and Tissue Kit (Qiagen) was utilized. The PCR amplifications were conducted by using Benchmark TC 9639. The primer sets consisted of primer set for *Ixodidae* 16S-F (5′-CTGCTCAATGATTTTTAAATTGCTGTGG-3′) and 16S-R (5′-CCGGTCTGAACTCAGATCAAGT-3′) with the predict PCR product around 460 bp (Black and Piesman, 1994). Another primer set for the detection of *Ctenocephalides* spp. included NC5 (5′-GTAGGTGAACCTGCGGAAGGATCATT-3′) and *ITS-1* R (5′-GCTGCGTTCTTCATCGACCC-3′) with the amplification products around 700 bp (Marrugal et al., 2013).

Each PCR reaction consisted of 0.5 µl of each primer (25 µM), 12.5 µl Master Mix 2X (Meridian Bioscience®), DNA template (2 µl), and DNase/RNase-free distilled water to get the total volume up to 25 µl. The cycling conditions involved an initial denaturation at 94°C for 5 minutes (1 cycle), followed by 35 cycles of 94°C denaturation for 30 seconds, 58°C annealing for 30 seconds, 72°C extensions for 40 seconds, and a final extension at 72°C for 7 minutes. After amplification, PCR products were loaded on 1.5% (w/v) agarose gel in 1X TAE 100V for 35 min with a DNA ladder of 100 bp (Invitrogen). The gel was visualized under UV light (UVP Multidoc-It) after being stained with ethidium bromide (0.5 µg/ml). Purified PCR products were delivered to Phu Sa Company for sequencing.

Phylogenetic analysis

The nucleotide sequences of ticks and fleas were analyzed by Bioedit software and BLAST in NCBI. Then, *ITS-1* and 16S rRNA sequences of *R. sanguineus* and *Ctenocephalus* spp. were aligned with other reference sequences by using ClustalW. The phylogenetic tree was constructed by using MEGA X, and the Maximum Likelihood method and Tamura-Nei model have been used to analyze the phylogenetic tree. The gene sequences of *R. sanguineus*, *C. canis*, and *C. felis* in this study were registered in GenBank under the accession numbers OQ626573, OQ626218, and OQ626238, respectively. Additionally, the *ITS-1* and 16S rRNA gene sequences of *Rhipicephalus* spp. and *Ctenocephalus* spp. used in the phylogenetic analysis were obtained through GenBank. Isolates including the corresponding accession number were described in more detail in the Supplementary table.

Statistical analysis

The prevalence of ectoparasite infestation among various locations, odd ratios, Chi-Square (χ^2) test, and 95% confidence interval (CI) were calculated by using GraphPad Prism 8. The obtained results indicate statistical significance at a *P*-value<0.05.

RESULTS

The prevalence of ectoparasite infestation in domestic dogs in the Mekong Delta

The study revealed that the general infestation rate of ectoparasites in domestic dogs in the Mekong Delta was 57.92% (Table 1). Dogs in Kien Giang province had the highest infestation rate of ectoparasites at 61.93%, compared to dogs in Can Tho (59.15%) and An Giang (53.56%). However, there were no significant differences in ectoparasite infestation rate in surveyed areas (P>0.05).

Table 1 The prevalence of domestic dogs infested by different ectoparasites based on each studied area in the Mekong Delta, Vietnam

Location	No. Surveyed dogs	No. Of infestation dogs	% Infestation dogs	*P*-value
An Giang	235	139	59.15	>0.05
Kien Giang	218	135	61.93	
Can Tho	267	143	53.56	
Total	720	417	57.92	

Risk factors of ectoparasite infestation in domestic dogs

Regarding potential risk factors of ectoparasite infestation in dogs, five factors were taken into consideration, including location, sex, age, lifestyle, and veterinary practices (Table 2). The results found that neither the geographic areas nor the sex of dogs influenced the ectoparasite infestation (P>0.05); however, the age of dogs, lifestyle, and veterinary practices had a substantial association with the ectoparasite infestation. The factor of veterinary practices containing acaricide treatment and the frequency of bathing had a significant influence on the prevalence of ectoparasite. The group of dogs without applying acaricide treatment and irregular bathing (<4 times/ month) had 11-fold higher risks (OR=11.34; 95% CI: 8.13 to 15.56) of ectoparasite infestation than those with acaricide treatment and regular bathing. Besides, juvenile dogs (<1 year) showed four times higher risk (OR=4.44; 95% CI: 2.91 to 6.66) of ectoparasite infestation than adult dogs. Moreover, the outdoor dogs were exposed to higher risk than indoor dogs (OR=1.42, 95% CI: 1.05 to 1.91). Therefore, the lifestyle of dogs had a slight impact on the dog infection of ectoparasites.

Table 2 Potential risk factor of ectoparasite infestation in domestic dogs in the Mekong Delta of Vietnam

Factor	No. Examine	No. positive	Prevalence (%)	OR (95% CI)	*P*-value
Location					
An Giang	235	139	59.15	1.26 (0.88 to 1.78)	0.21
Kien Giang	218	135	61.93	0.71 (0.49 to 1.02)	0.06
Can Tho*	267	143	53.56		
Sex					
Male	381	223	58.50	1.13 (0.84 to 1.52)	0.43
Female*	339	194	57.20		
Age					
< 1 year	319	217	68.03	3.66 (2.42-5.40)	<0.01
1-3 year	246	143	58.13	2.56 (1.70- 3.83)	<0.01
> 3 year*	155	57	36.77		
Lifestyle					
Outdoor	326	204	62.58	1.42 (1.05 to 1.91)	0.02
Indoor*	394	213			
Veterinary practices					
No Acaricides treatment Bathing <4 times/ month	379	301	79.40	11.34 (8.13 to 15.56)	<0.01
Acaricides treatment Bathing at least 4 times/ month*	341	116	34.00		

**reference criteria, OR: Odds ratio, CI: confidence interval*

Morphological ectoparasite identification

Six different species have been identified such as *R. sanguineus*, *Ctenocephalides* spp. (*C. canis* and *C. felis*), *D. canis*, *S. canis*, and *T. canis* (Table 3). The current study revealed that *R. sanguineus* was the most common ectoparasite in infestation dogs in surveyed areas in the Mekong Delta, occupying 59.95%. *Ctenocephalides* spp. were the second most common ectoparasite, accounting for 34.77%, followed by *T. canis* at 6.71%, *D. canis* at 2.63%, and the lowest ones belonged to *S. canis* at 1.92%.

Table 3 The prevalence of ectoparasite species in domestic dogs in the Mekong Delta of Vietnam based on morphological identification

Species	No. of infected dogs	% infection dogs	*P*-value
Ticks			
R. sanguineus	250	59.95	<0.05
Fleas			
Ctenocephalides spp.	145	34.77	
Demodex			
D. canis	11	2.63	
Mites			
S. canis	8	1.92	
Louse			
T. canis	28	6.71	

Morphological ectoparasite identification

In this study, ticks and fleas were the two most common species, which were further performed PCR to validate the results of morphological identification and gain a deeper understanding of their genetic characteristics. The PCR and sequencing results proved that the collected ticks were *R. sanguineus* and two different types of fleas, namely *C. canis* and *C. felis*.

Phylogenetic tree analysis of ticks and fleas in domestic dogs

A profound analysis of the phylogenetic tree of *R. sanguineus* species, based on the sequences of target gene 16S rRNA revealed that *R. sanguineus* formed 2 distinct clades, which represent tropical lineages and temperate lineages (Figure 1). *R. sanguineus* from this study belonged to the tropical lineages, which shared a 99% genetic diversity with those *R. sanguineus* specimens found in Brazil (EU346682.1), India (ON428308.1, MH765331.1). Another lineage consists of temperate species including *R. sanguineus* from Germany, Spain, France, Argentina, and Chile.

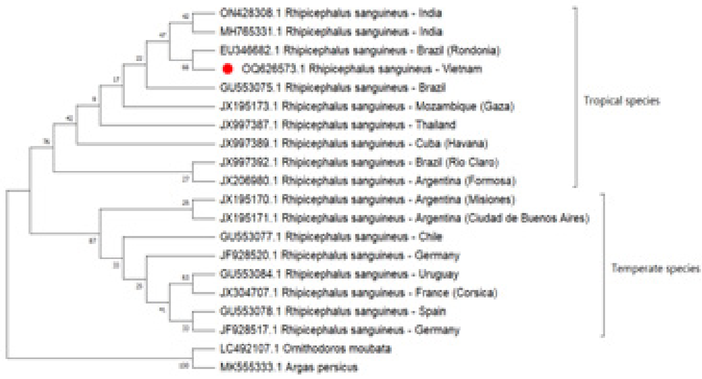

Figure 1 A phylogenetic tree was built using the 16S rDNA sequence of *Rhipicephalus* spp. by applying the Maximum Likelihood method with the Tamura-Nei model. Bootstrap analysis was performed using 1,000 replicates, and the numbers in the branches indicated the bootstrap values. *Rhipicephalus* spp. in this research is represented by a red dot

Through analyzing the *ITS-1* sequences of fleas (*Ctenocephalides* spp.), the results indicated that *Ctenocephalides* spp. was divided into two primary clades: *C. canis* and C. *felis*. *C. canis* isolated from the Mekong Delta belongs to *C. canis* species obtained from Iran, Spain, and China (Figure 2). It showed a close relationship to *C. canis* isolate (LS992469.1) from Spain. On the other hand, *C. felis* originated in the Mekong Delta and belonged to *C. felis* species in Spain, India, and China. *C. canis* and *C. felis* demonstrated a high homologous to each other.

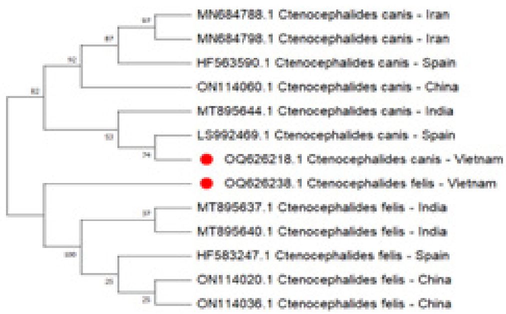

Figure 2 A phylogenetic tree was built using the *IST-1* sequence of *Ctenocephalides* spp. by applying the Maximum Likelihood method with the Tamura-Nei model. Bootstrap analysis was performed using 1,000 replicates, and the numbers in the branches indicated the bootstrap values. *Ctenocephalides* spp. in this research is represented by the red dots.

DISCUSSION

Ectoparasites are the main cause of irritation, dermatological responses, and hypersensitivity disorders (Ettinger and Feldman, 1995) in domestic dogs. Besides the direct effects on hosts, ticks can induce anemia and paralysis through blood feeding and excreting toxins. Mites, especially *Demodex*, can trigger allergic reactions. Consequently, it is necessary to investigate the prevalence and risk factors of ectoparasitic infestation. The overall infestation of ectoparasite in domestic dogs in the Mekong Delta was rather high at 57.92%. This result was considerably higher than in other parts of Eastern and Southern Asia, as shown by investigation from 2017-2018 with 42.4% (Colella et al., 2020), in Thai Nguyen province (North of Vietnam) with 33.28% (Doan and Vu, 2021). There was a difference in tick infestation in Thai Nguyen and surveyed areas (South Vietnam). One possible explanation could be attributed to the difference in seasons of North and South Vietnam. While the North of Vietnam experiences four distinct seasons, the South of Vietnam has only rainy and sunny seasons. As a result, the consistent hot climate and high humidity throughout the year might create favorable conditions for tick development.
Conversely, the dog population in the Philippines had 67% infected with tick infestation (Colella et al., 2020), and the high prevalence of tick infestation in Nigeria was documented, ranging from 71.2% to 96.0%. The divergences among studies might be attributed to the disparate ecological climate and geographical conditions.

Morphological identification of ectoparasites revealed that the dog population in the Mekong Delta infected by *R. sanguineus*, *C. canis*, *C. felis*, *D. canis*, *S. canis*, and *T. canis*. *R. sanguineus* was found to be the predominant tick in dogs, which was consistent with previous studies in Vietnam (Nguyen and Nguyen, 2014) and worldwide (Nijhof et al., 2007; Abdullah et al., 2016; Maurelli et al., 2018).

Regarding associate risk factors of ectoparasites, the study revealed that age, lifestyle, and veterinary practices had a significant influence on the infestation of ectoparasites. In our study, locations and the sex of dogs did not contribute to the ectoparasite infestation. Despite some differences in the altitude of An Giang, Kien Giang, and Can Tho, those locations share similarities in eco-climate and average temperature; therefore, this factor did not affect the ectoparasite infestation in dogs. Regarding sex and ectoparasite, namely tick infestation, there has been a controversial issue. Some studies found that female dogs had a higher infestation of ticks than male counterparts (James-Rugu and Jidayi, 2004; Zeb et al., 2023) because of the changes in hormones and immune system during gestation or lactation period of female dogs (James-Rugu and Jidayi, 2004). In contrast, our results were in line with other studies (Adetayo et al., 2021), which proved that there was no difference between males and females in tick infestation.

The current study revealed that juveniles and young dogs were more vulnerable to infestation of ectoparasites than adult dogs, which corresponds to previous studies (Abdulkareem et al., 2019; Zeb et al., 2023). Juvenile and young dogs have underdeveloped immune systems, whereas adults have greater exposure to tick infestation, so their immune systems are more effectively resistant to tick infestation in comparison to juvenile dogs (Sahu et al., 2013; Abdulkareem et al., 2019; Zeb et al., 2023).

Altogether, age, lifestyle, and veterinary practices had a significant influence on the ectoparasite infestation. Dogs that were primarily kept indoors exhibited lower ectoparasite infestation than their outdoor counterparts. Moreover, regular baths (at least 4 times/ month) with acaricidal application were observed to decrease the ectoparasite infestation dramatically. These findings were consistent with the study of Zeb et al. (2023). Thus, applying good hygiene and prophylaxis (acaricides) are recommended to dog owners to prevent ectoparasites in their dogs. In this way, dog owners can help mitigate the risks of vector-borne diseases.

Ticks and fleas are predominant species found in domestic dogs in the Mekong Delta of Vietnam. As a result, this study focused on conducting a molecular characterization study on ticks (*R. sanguineus*) and fleas (*Ctenocephalides* spp.). Many studies have investigated the genetic diversity of ticks by analyzing the target sequences of 12S RNA and 16S RNA (Burlini et al., 2010; Jones et al., 2017). In this study, the 16S RNA has been used to analyze the phylogenetic tree of *R. sanguineus*. The taxon *R. sanguineus* specimens in the Mekong Delta of Vietnam were closely related to *R. sanguineus* in tropical lineages, consistent with the study of Nguyen et al. (2019) confirming that *R. sanguineus* in Vietnam belonged to tropical lineages. In principle, the tropical lineages showed a close association with the climate and temperature of tropical areas such as Thailand, India, Brazil, Mexico, and some areas in Argentina. In contrast, the temperate lineages included *R. sanguineus*, which was distributed in cold areas such as Germany and France. The study of Troughton and Levin (2007) proved that the ambient humidity and photoperiod had impacts on the growth and development of ticks, which might cause the adaptation and diversity of genetic characteristics of ticks as well as the pathogenicity (Moraes-Filho et al., 2015).

Together with ticks, fleas have been known as another vector-borne disease that impacts veterinary medicine and human health. The identification of fleas has been performed based on morphological characteristics such as the shape of the front head (Linardi and Santos, 2012), the structure of genitalia, and the distribution of spines, ctenidia, and setae (Marrugal et al., 2013). Later, the target genes *ITS-1* and *ITS-2* of fleas were developed to differentiate the population among species (Vobis et al., 2004; Marrugal et al., 2013). In this study, the phylogenetic tree analysis of the *ITS-1* sequence showed the distinct clades between *C. felis* and *C. canis*. The results from molecular identification corresponded to morphological parameters of *Ctenocephalides* spp. In the previous study of Marrugal et al. (2013), although *C. felis* was collected from different geographical locations in Spain, Iran, and South Africa and possessed some morphological differences, the molecular data showed 100% identity. Therefore, the *ITS-1* target gene is an effective tool to differentiate *Ctenocephalides* spp.

CONCLUSIONS

To our knowledge, this is the first molecular biology-based investigation of the prevalence and risk factors of ectoparasites in the Mekong Delta of Vietnam. The ectoparasite infestation rate in dogs was high, with *R. sanguineus* infestation being the most predominant. Age, lifestyle of dogs, and applying veterinary practices were identified as the risk factors for ectoparasite infestation. Therefore, raising attention to veterinary care and management practices and lifestyle of dogs is a crucial key to protecting dogs from ectoparasite infestation.

AUTHOR CONTRIBUTIONS

This study was performed by all of the authors. **Tran Nguyen-Ho-Bao** and **Hung Nguyen Huu** were responsible for the conceptualization of the study, designing experiments, and revising the manuscript. **Minh Anh Thi La**, **Hanh Huu Thi Tran**, **Tran Nguyen-Ho-Bao**, **Tien Ai Lu**, and **Tri Minh Nguyen** performed experiments. **Tran Nguyen-Ho-Bao**, **Hung Nguyen Huu**, **Chuc Thi Nguyen**, and **Bang Phi Nguyen** interpreted data and prepared the manuscript. The final manuscript was read and approved by all authors.

CONFLICT OF INTEREST

The authors declare that they do not have any competing interests.

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