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Evaluation of the *3-minute search and collect* Protocol for Dog Ectoparasite Surveys in the Domestic-Wildlife Interface Area

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Abstract

Background and Aim: Dog ectoparasites are a major concern regarding the emergence of several vector-borne zoonotic diseases associated with domestic dogs. Information on the quantified assessment of ectoparasite collection methods from dogs remains limited. Therefore, this study aimed to evaluate the effectiveness of the ”3-minute method” for collecting dog ectoparasites in the human and wildlife border interface in Northern Thailand.

Materials and Methods: The ”3-minute method” (TMM) was compared with the bathing-combing method (BCM) in 31 domestic dogs in the domestic-wildlife interface area, comprising 4 villages in Nan province, Thailand, from July 2022 to July 2023. All ectoparasites were collected, and morphological identification was confirmed. The percentage of agreement between TMM and BCM was calculated using Kappa. A seasonal comparison of ectoparasite infestation was conducted using the TMM method.

Results: Comparatively, the diversity of ectoparasites collected by TMM was revealed to be similar to the BCM method: ticks (*Rhipicephalus sanguineus* (Ixodida: Ixodidae), *Haemaphysalis* spp. (Ixodida: Ixodidae), *Dermacentor* spp. (Ixodida: Ixodidae)), fleas (*Ctenocephalides felis orientis* (Siphonaptera: Pulicidae), *Ctenocephalides felis* (Siphonaptera: Pulicidae)), and lice (*Heterodoxus* spp. (Phthiraptera: Boopiidae)). More ectoparasites were collected by the BCM than by the TMM method. The average efficiency percentages of TMM and BCM were 12.8% and 87.2%, respectively. The observed percentage agreement between BCM and TMM was very good (K = 0.9) for ticks, good (K = 0.7) for fleas, and moderate (K = 0.5) for lice. The diversity of ectoparasites in dogs living in the domestic dogs-wildlife interface area showed that there were 4 species of ectoparasites collected in the dry season compared to 6 species in the rainy season.

Conclusion: The fast-body search for 3 minutes is a fast, inexpensive, and effective method for the identification and study of the diversity and abundance of ectoparasites from owned dogs when compared to bathing and combing methods with Amitraz. This method can be used as a non-invasive technique to collect ectoparasites from domesticated dogs for further study. Sharing ectoparasites from wildlife to domestic dogs in the domestic-wildlife interface area has reported.

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INTRODUCTION

Ectoparasite infestations in dogs cause important vector-borne diseases (VBDs) in the hosts [1]. Studies in ectoparasite collection have been conducted for several purposes, such as (1) to identify ectoparasites [1–12], and (2) to study the diversity [12], seasonality [4], and prevalence of ectoparasites [3–11]. Several ectoparasite collection methods used as diagnostic approaches for locating ectoparasites have been documented, such as skin scraping, acetate tape impression, coat brushing and floatation, trichoscopy, and ear swabs. Convenient methods for the collection of ectoparasites from dogs have also been used [2,3]. Previous studies in dogs focused on the investigation of ectoparasite infestation [4,5], the measurement of ectoparasite prevalence [3, 6,7,8, 9,10,11], and the study of ectoparasite diversity [12] as well as the investigation of vector-borne parasites [13,14,15,16]. The duration of time for the collection of ectoparasites usually varied from 5 min [16], 10 min [15], and 20 min, though some did not indicate the time for collection [6], previously [5,10,11,15]. The World Association for the Advancement of Veterinary Parasitology (WAAVP) guideline for evaluating the efficacy of drugs on ectoparasites is also available [17]. However, information concerning the time to assess the ectoparasite collection methods from dogs remains limited.

A dataset from 2000 to 2019 indicated a positive correlation between the increasing number of livestock and the number of outbreaks of human diseases. It also showed a link between infectious diseases, biodiversity, and livestock expansion, which is important for public health and conservation [18]. A study in Madagascar showed that a large portion of parasites was host-specific. However, some ectoparasite species were shared either by several endemics or by several introduced species [19]. The link surveying ectoparasites in domestic animals is one of the useful tools for studying changing habitats and moving patterns of wildlife species near rural barrier areas where there are links between domestic animals, wildlife, and humans living in zones with potential emerging diseases. An example of the sharing of ectoparasites between wildlife and domestic dogs can be seen in a study that showed ticks found in red foxes (*Vulpes vulpes*) were also found in domestic dogs [20]. However, that study was conducted with ectoparasite samples from wildlife carcasses [20]. Therefore, a fast, convenient, and effective method for surveying ectoparasites in domestic animals living in this unique area is important. A time-consuming ectoparasite collection method can be performed with domestic dogs in the city and urban areas, but a fast and effective collection method is required in rural areas where the owner may be busy with agricultural work and have no time to wait for the collection of ectoparasite samples from their dogs. The sharing of ectoparasites between domestic dogs and wildlife animals has been reported in the domestic and wildlife border interface area and known spillover. The spillover of ectoparasites between domestic and wild animals might introduce pathogens through insect vectors as well as the restoration of pathogens in wild animals. Domestic animals and pet dogs interact closely with humans and may act as reservoir hosts that spread parasites to wildlife [21]. Global warming has been influenced by changes in temperature, and the increase of agriculture and livestock farming is required to produce food, requiring the invasion of land use for livestock and agriculture. It has also increased the chance of ectoparasite sharing between domestic dogs and wildlife. Therefore, surveying ectoparasites from these dog populations in many medical and veterinary aspects is important.

This study aims to measure the effectiveness of the 3-minute search and collect method (TMM) compared with the bathing-combing method (BCM) for ectoparasite collection, and to study the diversity of ectoparasites in the domestic dogs-wildlife interface area. The importance and benefits of this study are to help the researchers choose a timely and effective method for ectoparasite collection in dogs.

MATERIALS AND METHODS

Ethics and Form of Consent

This study involved animal restraint for sample collection, so ethical approval was provided by the Kasetsart University Institutional Animal Care and Use Committee (approval number ACKU-VTN-001). The owners of all dogs in this study were informed about the procedures and associated risks before the study was conducted. Dogs were restrained under the supervision of experienced certified veterinary staff of Faculty of Veterinary Medicine, Kasetsart University.

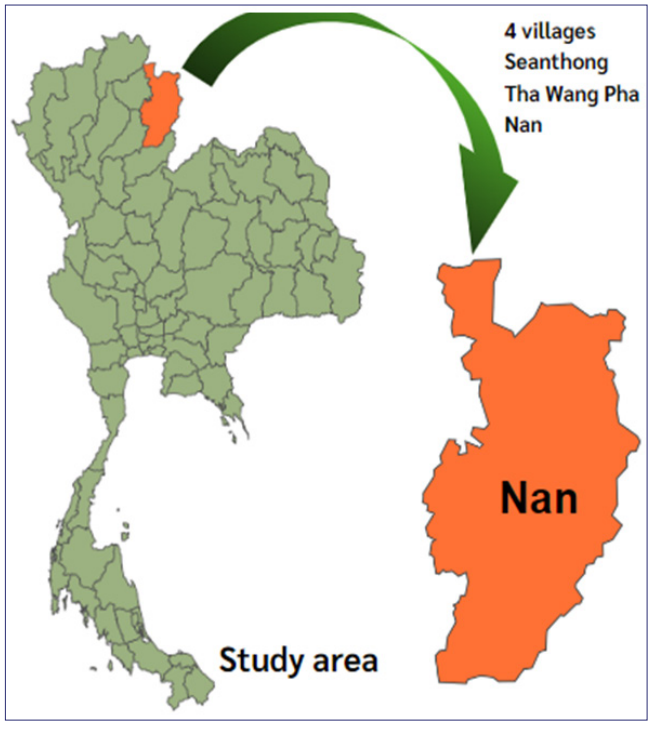

Figure 1. The study area in Tha Wang Pha District, Nan Province, Thailand, including 4 villages

Study Areas, Animals, and Time of Collection

This study was performed in Saengthong sub-district, Tha Wang Pha district, Nan Province, Thailand (Figure 1). Thirty-one dogs from four villages in Saengthong sub-district participated and were selected for the SEA DOG SEA project included in this study [22]. The comparison of the "3 minutes method" (TMM) with the BCM was conducted in July 2022. The comparison of TMM in different seasons was conducted in January 2023 (dry season) and July 2023 (rainy season).

Comparison of Two Ectoparasite Collection Methods

TMM

According to the SEA DOG SEA protocol [22], dogs were restrained by their owners. They were visually checked, and the operators removed as many ectoparasites as they could see within 3 minutes. Ectoparasites were removed by using fingers and forceps (Figure 2). Collected ectoparasites were placed in collection bottles (3 mL) containing 95%

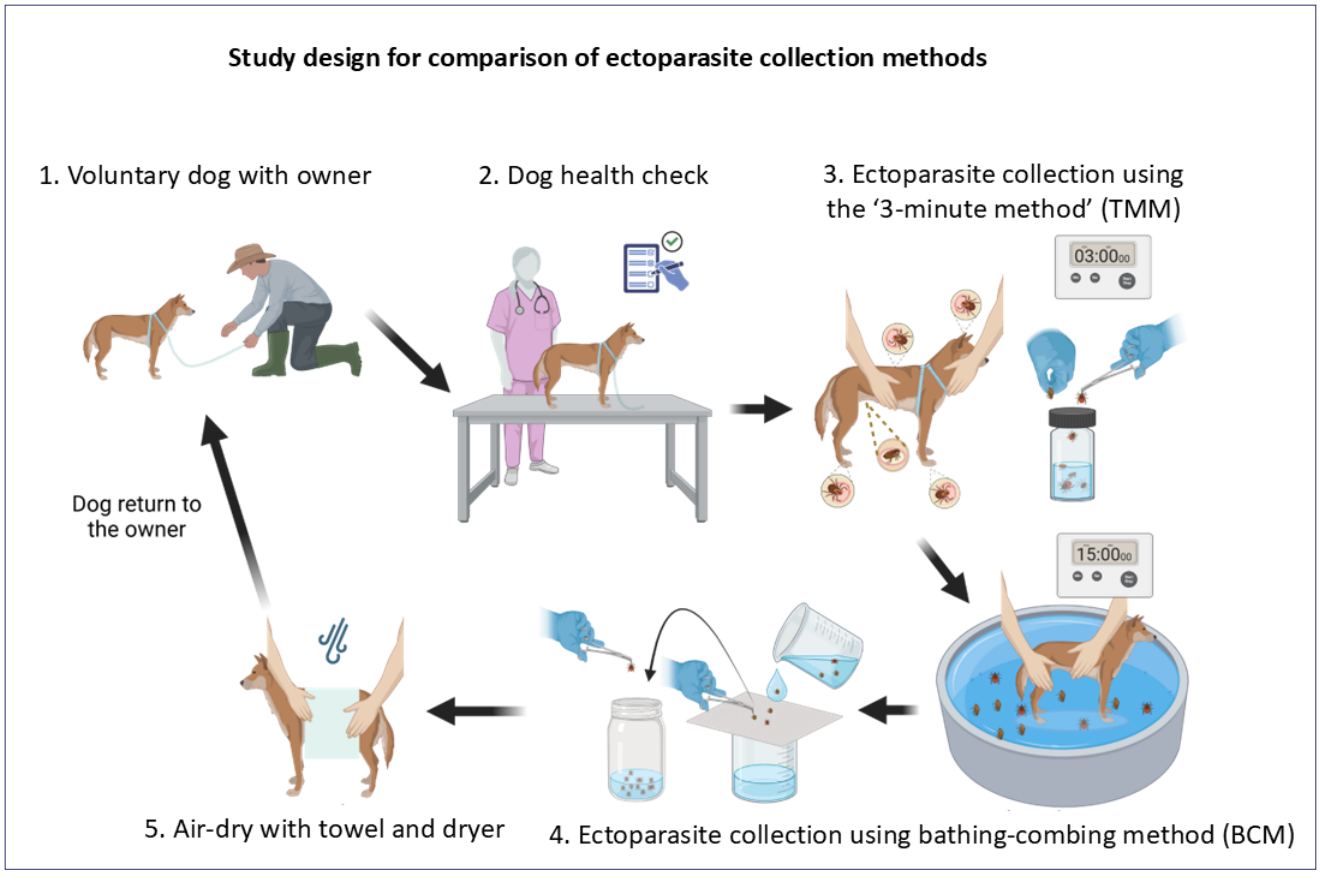

Figure 2. Study design and procedure of ectoparasites collection from each voluntary dogs using TMM and BCM methods

ethanol for preservation before morphological identification and ectoparasite counting.

BCM

The total ectoparasite burden on each dog was collected using the BCM method. Briefly, each of 31 dogs were placed in a plastic tub, rinsed with water, and washed with 250 ppm Amitraz-containing shampoo while combing and washing with water to remove all the ectoparasites from the body. Dogs were air-dried with towels and a hair dryer. The water residue remaining in the bathtub was filtered using nylon filters to collect ectoparasites in the water residue. The nylon filters that may contain small ectoparasites were immersed into a bottle with 80% alcohol and used for ectoparasite counting and morphological identification (Figure 2).

Morphological Identification of Ectoparasite

Ectoparasites collected by the TMM and BCM methods were sorted, counted, and identified under a stereomicroscope (Olympus, Japan) using the morphological key for ticks, fleas, and lice [23].

Statistical Analysis

Ectoparasite types and numbers collected by two methods were compared for each dog. The percentage of agreement between TMM and BCM was calculated using the kappa statistic (*K*), as described previously [24]. The correlation of TMM and BCM on ectoparasite diversity (no. of species) was calculated using RStudio software. Student's *t*-test was used, and results $P < 0.05$ were considered significant.

RESULTS

Ectoparasites Collected from Two Methods

Number of Ectoparasites Collected

A total of 8,600 parasites were collected from 31 dogs, of which 290 (3.37%) were collected using the TMM method (Table 1).

Diversity of Ectoparasites

There were a total of 8,600 ectoparasites collected using both methods, which included 7,303 (84.9%) lice, 429 (5.00%) fleas, and 868 (10.1%) ticks. *Heterodoxus spiniger* (Phthiraptera:Boopiidae) (Figure 3A, 3B) was the only lice species collected in this study.

Table 1. Diversity of ectoparasites collected from dogs by TMM and BCM methods in Saengthong village, Tha Wang Pha District, Nan province, Thailand.

Ectoparasite species	TMM	BCM	Total number (TMM+BCM)
Lice	40	7263	7303
Heterodoxus spiniger	*40*	7263	7303
Fleas	46	374	429
Ctenocephalides felis orientis	45	363	418
Ctenocephalides felis felis	1	10	11
Ticks	204	664	868
Rhipicephalus sanguineus	204	661	865
Haemaphysalis spp.	0	3	3
Total	290	7210	8600

There were 40 (0.55%) lice, collected by the TMM method. For fleas, there were a total of 429 (5.00%) collected, 46 (10.7%) of which were collected by the TMM method. Among 429 fleas, morphological identification revealed that 418 (97.4%) fleas were *Ctenocephalides felis orientis* (Siphonaptera:Pulicidae) (Figure 3C), and 11 (2.6%) fleas were *Ctenocephalides felis felis* (Siphonaptera:Pulicidae) (Figure 3D). The TMM method was used to collect 45 (97.8%) *C. felis orientis* and 1 (2.2%) *C. felis felis*. Only adult fleas were collected by both the TMM and BCM methods according to fleas' life cycle on dogs.

For ticks, there were 868 (10.1%) ticks collected in total. Among those ticks, there were 204 (23.5%) ticks collected by TMM, and 664 (76.49%) ticks collected by BCM for both nymphal and adult stages, respectively. Morphological identification of ticks revealed that there were 865 (99.7%) *Rhipicephalus sanguineus* (Ixodida: Ixodidae) (Figure 3E, 3F) and 3 (0.3%) *Haemaphysalis* spp. (Ixodida: Ixodidae) (Figure 3G) collected from the dogs in this study. There were 204 (23.5%) *R. sanguineus* and no *Haemaphysalis* spp. ticks collected by the TMM method (Table 1). Only the BCM method collected 3 *Haemaphysalis* spp. ticks in the nymphal stage from 1 dog (Table 1).

Effectiveness Percentages of the TMM and BCM Methods

The collection of ectoparasites from a total of 32 dogs in July 2022 showed that the effective percentages of the TMM and BCM methods were 12.80% (0.0%-68.8%) and 87.2% (31.3%-100%), respectively. There were 4, 9, 6, and 10 dogs from villages

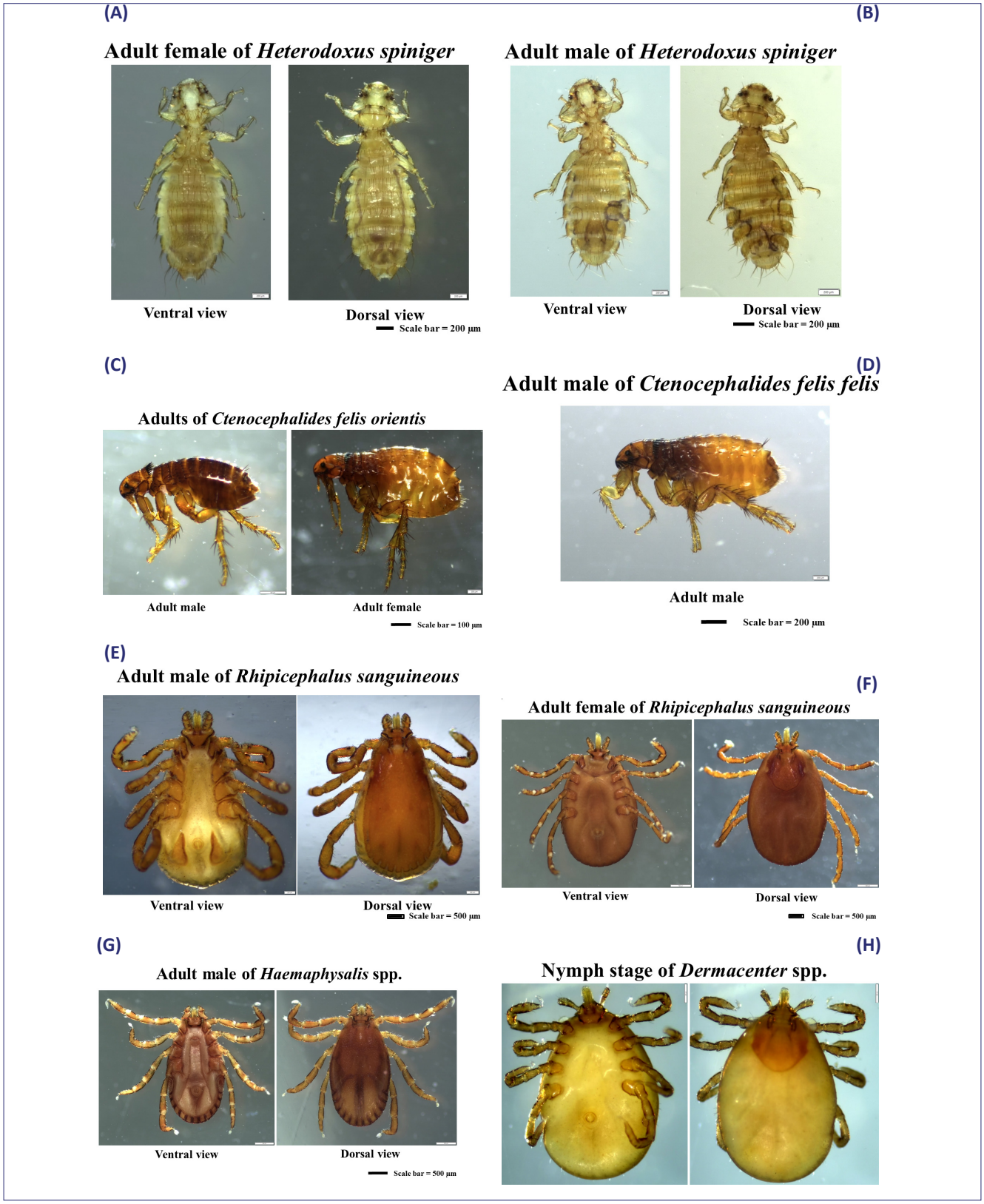

Figure 3. Ectoparasites found in dogs in Saengthong sub-district, Tha Wang Pha district, Nan province, Thailand collected by the "3-minute method" (TMM). (A) Adult female of *Heterodoxus spiniger*, (B) Adult male of *Heterodoxus spiniger*, (C) Adults of *Ctenocephalide sfelis orientis*, (D) Adult male of *Ctenocephalides felis felis*, (E) Adult male of *Rhipicephalus sanguineous*, (F) Adult female of *Rhipicephalus sanguineous*, (G) Adult male of *Haemaphysalis* spp., and (H) Nymph stage of *Dermacentor* spp.

Table 2. The effectiveness percentage of the TMM and BCM methods concerning the total number of ectoparasites.

Villages	Number of dogs	Effectiveness percentage of TMM (%)	(Range)	Effectiveness percentage of BCM (%)	(Range)
Village 4	7	11.60%	(0.2% - 37.9 %)	88.40%	(62.1% - 99.8%)
Village 5	9	25.70%	(5.6% - 68.8%)	74.30%	(31.3% - 94.4%)
Village 6	6	5.00%	(0.0% - 10.8%)	95.00%	(89.2% - 100.0%)
Village 7	10	9.00%	(0.0% - 27.0%)	91.00%	(73.0% - 100.0%)
Total	32	12.80%	(0.0% - 68.8%)	87.20%	(31.3% - 100.0%)

A to D, respectively. Ectoparasite infestation varied in each dog in this study. Due to the variation of ectoparasite infestation levels in each dog, TMM collected from 0 to 32 ectoparasites/dog, while BCM collected 3-1,891 ectoparasites/dog.

Percentage Agreement between the BCM and TMM Methods

The observed percentage agreement of BCM and TMM for collecting all ectoparasites shows the strength of agreement as very good (K = 0.9355) for collecting all ectoparasites, as well as good agreement for collecting ticks (K = 0.7419), and moderate agreement for collecting fleas and lice (K = 0.5) (Table 2). Lower agreement between TMM and BCM might involve the size and stage of ectoparasites, hair color, and thickness of the dog. However, further analysis and study should be conducted.

Correlation between TMM and BCM on Ectoparasite Diversity

The average ectoparasite diversity collected from TMM, BCM, and TMM+BCM methods was 1.484 (min = 0, max = 3), 2.774 (min = 1, max = 4), and 2.839 (min = 1, max = 4), respectively. Correlation between TMM and BCM was 0.3670 (p = 0.039, t = 2.16, df = 30), demonstrating a statistically significant moderate correlation between TMM and BCM (Figure 4).While the correlation between BCM and TMM + BCM methods was 0.9476 ($p < 0.05$, t = 15.988, df = 29). These results revealed that TMM could collect fewer ectoparasite species compared to BCM and BCM+TMM methods. Some ectoparasite species may be missed using the TMM collection method.

Seasonal Abundance of Ectoparasite in Dogs in Saengthong Sub-district

Applying TMM for ectoparasite collection from the same population of dogs in Saengthong sub-district during the dry and rainy seasons revealed the diversity of ectoparasite infestation in dogs. There were 4 and 6 species of ectoparasites in the dry and rainy seasons, respectively. In the dry season, four species of ectoparasites were collected from dogs, which included *R. sanguineus* tick, *Haemaphysalis* spp. tick, *Heterodoxus spiniger* lice, and *Ctenocephalides felis orientis*(Figure 3A, 3C, 3E, 3F, 3G). In the rainy season, apart from 4 species that were found in the dry season, two more species of ectoparasites, including *Dermacentor* spp. (Ixodida: Ixodidae) (Figure 3H) tick nymphal stage [14] and *C. felisfelis* (Figure 3D) fleas were also collected from the same dog population (Figure 3).

Advantages and Disadvantages between TMM and BCM Methods

The advantages of TMM and BCM methods are based on the effectiveness percentages and time consumption. The diversity of ectoparasites collected can be explained as shown in Table 3.

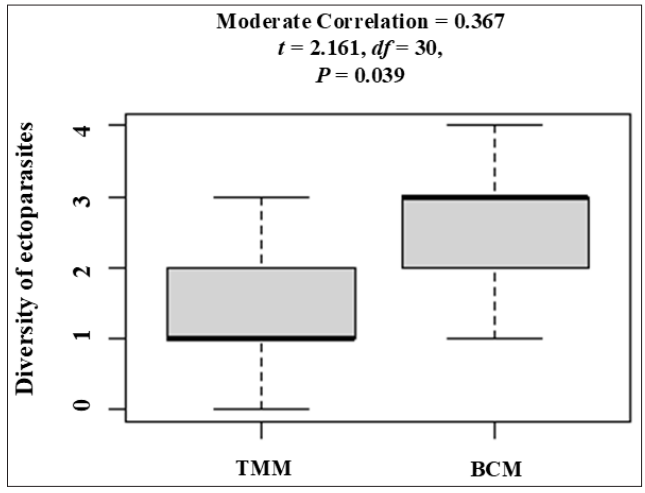

Figure 4. Boxplot revealing ectoparasite diversity collected from the TMM and BCM methods generated by RStudio software

Table 3. The observed percentage agreement of BCM and TMM for collecting of ectoparasites in dogs.

Type of ectoparasite collected	Kappa	Streng of agreement
All ectoparasites	0.9355	Very good
Ticks	0.7419	Good
Fleas	0.5161	Moderate
Lice	0.5484	Moderate

Table 4. Advantages and disadvantages between TMM and BCM methods.

Methods	Advantages	Disadvantages
TMM	Fast Easy	not suitable for aggressive dogs some species of ectoparasite may be missed
BCM	large number of ectoparasite were collected	time consuming several steps to be proceed use of acaricide may not suitable for environment

DISCUSSION

Ectoparasites such as ticks, fleas, lice, and mites are commonly found in both domestic and wild dogs. Some ectoparasites are host-specific but some are metropolitan species that can be found in several hosts. For example, cat fleas are incredibly host-generalist, likely exhibiting a host range that is among the broadest of all ectoparasites [25]. The sharing of ectoparasites between hosts such as domestic dogs and wildlife animals has also been reported in domestic and wildlife interface areas [26]. The spillover of parasites at the domestic animal-wildlife interface is a pervasive threat to animal health [25,27]. The sharing of emerging pathogens through insect vectors as well as the restoration of emerging pathogens in wild animals is still questionable. Domestic animals, and pet dogs, are closely associated with humans and may act as reservoir hosts that spread parasites to wildlife [21]. Global warming has been influenced by changing temperatures, increasing agriculture and livestock farming is required to produce food, and the invasion of land use for livestock and agriculture has increased [28]. For those reasons, the sharing of land occurs between wildlife habitats with domestic animals and human activity. It has also increased the chance of ectoparasite sharing between domestic dogs and wildlife [28].

Pet dogs are human-friendly friends, which might also carry zoonotic pathogens to their human owners. Ectoparasite sharing between domestic dogs, wildlife, and human owners also becomes an interesting issue in the human and wildlife border interface area to study the possibility of dogs carrying wildlife ectoparasites and transmitting emerging zoonotic pathogens [25]. Therefore, surveying ectoparasites from these dog populations in various medical and veterinary aspects is important.

The ectoparasite collection method for the evaluation of ectoparasite diversity, prevalence, and prognosis of virus, bacterial, and protozoan diseases in dogs can vary depending on the purpose of the study. Some studies have mentioned how to and how long to collect ectoparasites from animals. However, there is still no evaluation of the most effective method for collecting ectoparasites for diversity study in domesticated dogs. The current study was incorporated with the protocol for ectoparasite collection of the SEA Dog Sea project [22]. As a result, it was not surprising that TMM could collect a smaller number of ectoparasites when compared to BCM. It might collect a lower number of ectoparasites when compared to longer searching times such as 5 min, 10 min, or even 15 min, respectively. A comparison of two methods for ectoparasite collection revealed that using only three minutes helps to collect ectoparasites for diversity study in domesticated dogs with the range of low, medium, and high ectoparasite densities. Ectoparasite collection was conducted, and the ratio of male and female ticks in dogs has been compared in several studies [28]. The rate of head louse infestation in dogs was compared, including occurrence, while ectoparasite diversity [28] and the link between bacterial pathogens were previously studied [31]. Molecular and serological detection of pathogens in ticks from dogs has also actively been studied in many countries [32,33,34]. However, details concerning the time of ectoparasite search and collection procedures have rarely been mentioned in the materials and methods. The method and time of collection might be affected by the number of ectoparasites collected. In Vietnam, ectoparasite collection was conducted for 10 minutes. There were tick larvae, nymphs, and adults collected from dogs, similar to our study, and all stages of ticks were collected [15]. However, *Haemaphysalis* ticks were not found in dogs in Vietnam.

This study also showed the abundance of *R. sanguineus* ticks, as previously published [35,36]. There was also an abundance of *Haemaphysalis* spp. (villages 4 and 7) and *Dermacentor* spp. (village 6) nymphal stage collected from dogs in Saengthong sub-district, Tha Wang Pha district, Nan province, Thailand. The brown dog tick was the predominant tick species in Thailand and Southeast Asia, as described previously [36]. The findings of *Haemaphysalis* spp. and *Dermacentor* spp. nymphal stages in domesticated dogs were newly described in this study. Interestingly, *Haemaphysalis* spp. and *Dermacentor* spp. are not usually found in domesticated dogs in Thailand. *Haemaphysalis* ticks were previously found in bears and vegetation [36,37,38]. It has been carrying *Anaplasma* sp. (Rickettsiales:Ehrlichiaceae) and *Rickettsia* sp. (Rickettsiales:Rickettsiaceae) in the previous study. *Dermacentor* ticks were also previously reported in dogs, bears, and pig nests [36,37], which have also carried Rickettsiales pathogens such as *Anaplasma platys* (Rickettsiales:Ehrlichiaceae) and *Rickettsia* sp. [36,37,39]. The discovery of *Haemaphysalis* spp. and *Dermacentor* spp. ticks in domestic dogs in this study reveals the possible zoonotic transmission of Rickettsiales pathogens from wild animals to domestic dogs as well as from domestic dogs to human owners [39]. Climate change plays an important role in the expansion of tick populations as well as the transmission of tick-borne disease pathogens (TBPs) to humans and animals worldwide [41]. In Poland, a temperate zone country where *R. sanguineous* ticks are not usually found, may soon record more frequent infestations with foreign tick species [41]. Domestic dogs are a bridge between TBDs and humans as well as their peri-domestic environment [40]. A study of agriculture and hunting dog populations in a Mayan community in Mexico quickly removed ticks from hunting domestic dogs (*Canis lupus familiaris*) using tweezers, which were used for minimizing pain or discomfort, immediately after they returned from hunting, without time indication of tick sample collection [40]. In their study, nymphs and adults were collected, and their results showed that there were *Ixodes* spp. and *Amblyomma* spp. tick species found in hunting dogs and *R. sanguineous* in agriculture dogs [40], indicating the sharing of tick species from wildlife with the dogs used for risky activity like hunting. Interestingly, *Rickettsia endosymbiont* was also detected from both wildlife and domestic tick species, *Ixodes* spp. and *R. sanguineous*, respectively [40].

According to the results of this study, further research should be focused on rickettsial pathogen detection in the uncommon ticks collected from free-roaming domesticated dogs for more understanding of the current possible pathogen abundance in Tha Wang Pha district, Thailand, a domestic animals-wildlife interface area.

CONCLUSION

The fast-body search for 3 minutes is a fast, inexpensive, and effective method for the identification and study of the diversity and abundance of ectoparasite from owned dogs. In this study, the introduced method was compared to bathing and combing methods with Amitraz. The fast-body search for 3 minutes method can be used as a non-invasive technique to collect ectoparasites from domesticated dogs for further study. Sharing ectoparasites from wildlife to domestic dogs in the domestic-wildlife interface area is also reported.

ETHICAL CONSIDERATIONS

This study relates to animal restraint and ectoparasite collection, which was approved by the Kasetsart University Institutional Animal Care and Use Committee (Approval number ACKU-VTN-001).

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest with respect to this research.

REFERENCES

1. Nogueira BCF, da Silva Soares E, Mauricio Ortega Orozco A, Abreu da Fonseca L, Kanadani Campos A. Evidence that ectoparasites influence the hematological parameters of the host: a systematic review. Animal Health Research Reviews. 2023.24(1):28-39.
2. Papadogiannakis EI. Diagnostic techniques for locating ectoparasites.https://www.vin.com/apputil/content/defaultadv1.aspx?pId=11181&catId=30083&id=3852170 (accessed on June 17, 2022)
3. Jajere SM, Lawal JR, Shittu A, Waziri I, Goni MD, Fasina FO. Ectoparasites of dogs (*Canisfamiliaris*) from northeastern Nigeria: an epidemiological study. Parasitol Res. 2023.122(2):675-684.

4. Jamshidi S, Maazi N, Ranjbar-Bahadori S, et al., Survey of ectoparasite infestation in dogs in Tehran, Iran. Rev Bras Parasitol Vet. 2012.21(3):326-9.
5. Kumsa B, Abiy Y, Abunna F. Ectoparasites infesting dogs and cats in Bishoftu, central Oromia, Ethiopia. Vet ParasitolReg Stud Reports. 2019.15:100263.
6. Zineldar HA, Abouzeid NZ, Eisa MI, Bennour EM, Neshwy WME. Prevalence, clinical presentation, and therapeutic outcome of ectoparasitic infestations in dogs in Egypt. Open Vet J. 2023.13(12):1631-1644.
7. Ebrahimzade E, Fattahi R, Ahoo MB. Ectoparasites of Stray Dogs in Mazandaran, Gilan, and Qazvin Provinces, North and Center of Iran. J Arthropod Borne Dis. 2016.10(3):364-9.
8. Xhaxhiu D, Kusi I, Rapti D, et al. Ectoparasites of dogs and cats in Albania. Parasitol Res. 2009.105(6):1577-87.
9. Lefkaditis MA, Athanasiou LV, Ionicã AM, Koukeri SE, Panorias A, Eleftheriadis TG, Boutsini S. Ectoparasite infestations of urban stray dogs in Greece and their zoonotic potential. Trop Biomed. 2016.33(2):226-230.
10. Mosallanejad B, Alborzi A, Katvandi N. A Survey on Ectoparasite Infestations in Companion Dogs of Ahvaz District, South-west of Iran. J Arthropod Borne Dis. 2012.6(1):70-8.
11. González A, Castro Ddel C, González S. Ectoparasitic species from *Canisfamiliaris* (Linné) in Buenos Aires province, Argentina. Vet Parasitol.2004. 120(1-2):123-9.
12. Shukullari E, Rapti D, Visser M, et al. Parasites and vector-borne diseases in client-owned dogs in Albania: infestation with arthropod ectoparasites. Parasitol Res. 2017.116(1):399-407.
13. Changbunjong T, Buddhirongawatr R, Suwanpakdee S, Siengsanan J, Yongyuttawichai P, Cheewajorn K, Jangjaras J, Sangloung C, Ratanakorn P. A survey of ectoparasitic arthropods on domestic animals in Tak Province, Thailand. Southeast Asian J Trop Med Public Health. 2009.40(3):435-42.
14. Nuchjangreed C, Somprasong W. Ectoparasites species found on domestic dogs from Pattaya district, Chon Buri province, Thailand. Southeast Asian Journal of Tropical Medicine and Public Health. 2007.38: 203-207.
15. Nguyen VL, Colella V, Iatta R. et al. Ticks and associated pathogens from dogs in northern Vietnam. Parasitol Res.2019.118, 139–142.
16. Colella V, Nguyen VL, Tan DY, Lu N, Fang F, Zhijuan Y, Wang J, Liu X, Chen X, Dong J, Nurcahyo W, Hadi UK, Venturina V, Tong KBY, Tsai YL, Taweethavonsawat P, Tiwananthagorn S, Le TQ, Bui KL, Watanabe M, Rani PAMA, Annoscia G, Beugnet F, Otranto D, Halos L. Zoonotic Vector borne Pathogens and Ectoparasites of Dogs and Cats in Eastern and Southeast Asia. Emerg Infect Dis. 2020.26(6):1221-1233.
17. Marchiondo AA, Holdsworth PA, Fourie LJ, Rugg D, Hellmann K, Snyder DE, Dryden MW. World Association for the Advancement of Veterinary Parasitology. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition: guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestations on dogs and cats. Vet Parasitol. 2013.194(1):84-97.
18. Morand S. Emerging diseases, livestock expansion and biodiversity loss are positively related at global scale. BiolConserv. 2020.248:108707.
19. Ehlers J, Poppert S, Ratovonamana RY, Ganzhorn JU, Tappe D, Krüger A. Ectoparasites of endemic and domestic animals in southwest Madagascar. Acta Trop. 2019.196:83-92.
20. Dwużnik D, Mierzejewska EJ, Kowalec M, Alsarraf M, Stańczak Ł, Opalińska P, Krokowska-Paluszak M, Górecki G, Bajer A. Ectoparasites of red foxes (*Vulpesvulpes*) with a particular focus on ticks in subcutaneous tissues. Parasitology. 2020.147(12):1359-1368.
21. Morand S, McIntyre KM, Baylis M. Domesticated animals and human infectious diseases of zoonotic origins: domestication time matters. Infect Gen Evol. 2014.24:76–81.
22. Garine-Wichatitsky MD, Kritiyakan A, Artama WT, Ly S, Michaux J, et al.One Health in practice: a socio-ecological approach for the study and management of zoonotic diseases associated with free-roaming dogs in Southeast Asia (SEAdogSEA). 3rd Joint AITVM–STVM International Conference, May 2024, Montpellier, France, TropicalVeterinaryMedicine in Challenging Times: how should academic and research programs adapt?.
23. Sangvaranond A. Veterinary entomology. the 4th edition. Bangkok, Thailand. 1995.968 pages.
24. McHugh ML. Interrater reliability: the kappa statistic. Biochem Med (Zagreb). 2012.22(3): 276-82.
25. Clark, N.J., Seddon, J.M., Šlapeta, J. et al. Parasite spread at the domestic animal - wildlife interface: anthropogenic habitat use, phylogeny and body mass drive risk of cat and dog flea (*Ctenocephalides*spp.) infestation in wild mammals. Parasites Vectors.2018.11, 8 (2018).
26. Milstein MS, Shaffer CA, Suse P, Marawanaru A, Heinrich DA, Larsen PA, Wolf TM. A mixed-methods approach to understanding domestic dog health and disease transmission risk in an indigenous reserve in Guyana, South America. PLoSNegl Trop Dis.2022. 16(6):e0010469.
27. Thongsahuan S, Chethanond U, Wasiksiri S, Saechan V, Thongtako W, Musikacharoen T. Hematological profile of blood parasitic infected dogs in Southern Thailand. Vet World. 2000.13(11):2388-2394.

28. Caminade C, Marie Mclntyre K, Jones AE. Impact of recent and future climate change on vector-borne diseases. Ann.N.Y.Acad.Sci. 2019.1436, 157-173.
29. Costa-Junior LM, Rembeck K, Mendonça FL, Azevedo SC, Passos LM, Ribeiro MF. 2012. Occurrence of ectoparasites on dogs in rural regions of the state of Minas Gerais, Brazil. Rev Bras Parasitol Vet. 21(3):237-42.
30. PavlovicI, JovcevskiS, RogozarskiD, CsordasF, MitrovicN, MijatovicI ,MarcicD , Ilic Z, CirkovicD, SeklerM, JovcevskiS, RisticM. Biodiversity of Ticks and Fleas of Dogs in the Western Balkans – Preliminary Examinations. Bulletin UASVM Veterinary Medicine 2016. 73(2).
31. Nguyen HM, Theppannga W, Vongphayloth K, Douangngeun B, Blacksell SD, Robinson MT. Screening of ectoparasites from domesticated dogs for bacterial pathogens in Vientiane, Lao PDR. Zoonoses Public Health. 2020.67(8):862-868.
32. Silva CBD, Santos HA, Navarrete MG, Ribeiro CCDU, Gonzalez BC, Zaldivar MF, Pires MS, Peckle M, Costa RLD, Vitari GLV, Massard CL. Molecular detection and characterization of *Anaplasmaplatys* in dogs and ticks in Cuba. Ticks Tick Borne Dis. 2016.7(5):938-944.
33. Latrofa MS, Dantas-Torres F, Giannelli A, Otranto D. Molecular detection of tick-borne pathogens in *Rhipicephalussanguineus* group ticks. Ticks Tick Borne Dis. 2014.5(6):943-6.
34. Çetinkaya H, Matur E, Akyazi İ, Ekiz EE, Aydin L, Toparlak M. Serological and molecular investigation of *Ehrlichia* spp. and *Anaplasma* spp. in ticks and blood of dogs, in the Thrace Region of Turkey. Ticks Tick Borne Dis. 2016.7(5):706-714.
35. Petney TN, Kolonin GV, Robbins RG. Southeast Asian ticks (Acari: Ixodida): a historical perspective. Parasitol Res. 2007.101 (Suppl 2): S201–S205.
36. Ahantarig A, Trinachartvanit W, Milne JR. Tick-borne pathogens and diseases of animals and humans in Thailand. Southeast Asian J Trop Med Public Health. 2008.39:6, 1015-1032.
37. Parola P, Cornet J, Sanogo Y, et al. Detection of *Ehrlichia*spp, *Anaplasma*spp, *Rickettsia*spp, and other eubacteria in ticks from the Thai-Myanmar border and Vietnam. J ClinMicrobiol. 2003.41: 1600-8.
38. Hirunkanokpun S, Kittayapong P, Cornet JP, Gonzalez JP. Molecular evidence for novel tick-associated spotted fever group Rickettsiae from Thailand. J Med Entomol.2003.40: 230-7.
39. Saengsawang P, Kaewmongkol G, Phoosangwalthong P, Chimnoi W, Inpankaew T. Detection of zoonotic *Bartonella* species in ticks and fleas parasitizing free-ranging cats and dogs residing in temples of Bangkok, Thailand. Vet ParasitolReg Stud Reports. 2021.25:100612.
40. Dzul-Rosado KR, Arroyo-Solís KA, Torres-Monroy AJ, Arias-León JJ, Peniche-Lara GF, Puerto-Manzano FI, Landa-Flores MG, Del Mazo-López JC, Salceda-Sánchez B. Tick-associated diseases identified from hunting dogs during the COVID-19 pandemic in a Mayan community in Yucatan, Mexico. Open Vet J. 2023.13(6):794-800.
41. Kocoń A, Nowak-Chmura M, Asman M, Kłyś M. Review of ticks attacking domestic dogs and cats, and their epidemiological role in the transmission of tick-borne pathogens in Poland. Ann Agric Environ Med. 2023.30(1):22-30.