Vector-Borne Pathogens in Ticks and Fleas of Client-Owned Dogs in Metro Manila, Philippines

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Abstract: *Rhipicephalus sanguineus* s.l. and *Ctenocephalides felis* are considered the most prevalent ectoparasites of dogs in the Philippines. Vector-borne pathogens (VBPs) in these ectoparasites pose health risks to humans and animals. This study aimed to confirm the morphological and molecular identity of tick and flea species parasitising dogs in Metro Manila (Philippines) and molecularly investigate the possible presence of *Bartonella* spp., *Rickettsia* spp., *Ehrlichia canis*, and *Anaplasma platys* DNA. Ticks (*n* = 58) and fleas (*n* = 52) on dogs from three veterinary clinics in Metro Manila were collected and identified morphologically and molecularly via amplification and sequencing of cytochrome c oxidase I (*cox1*). Aliquots of ectoparasite DNA underwent real-time polymerase chain reaction (qPCR) screening for VBPs. All ticks were *R. linnaei* (formerly *R. sanguineus* s.l. "tropical lineage"), while all fleas were *C. felis* from clade 6 of the tropical II cluster/"Cairns" clade known from Australia. DNA of *B. clarridgeiae* was detected in 10% of fleas. DNA of *R. felis* was detected in 10% of fleas and in 3.8% of ticks. DNA of *E. canis* and *A. platys* was not detected. This study confirmed the presence of ticks and fleas as frequent ectoparasites on dogs and VBP presence emphasises the importance of preventative actions for animal health and welfare.

Keywords: tick; veterinary; *Rhipicephalus sanguineus*; *Rhipicephalus linnaei*; flea; *Ctenocephalides felis*; dog; *Bartonella* spp.; *Rickettsia* spp.; *Ehrlichia canis*; *Anaplasma platys*

1. Introduction

Ticks and fleas are significant ectoparasites in companion animals as they carry vector-borne pathogens (VBPs) that pose a health risk to dogs and humans within the dogs' households [1,2]. Both ticks and fleas thrive in the Philippines because of the warm and humid climate. These environmental and species-specific drivers facilitate broad dog tick and flea distribution, further enabled by an increasing population of urban dogs [3,4]. As of 2020, there are approximately 10.8 million dogs in the Philippines, many of which are unowned and free-roaming street dogs [5].

The brown dog tick (*Rhipicephalus sanguineus* sensu lato) is considered the most common tick infesting dogs in the Philippines [3,6]. Genetic and biological differences divide *R. sanguineus* into tropical and temperate lineages [7]. The tropical lineage, prevalent in Asia and Australia, is now recognised as a distinct species, *Rhipicephalus linnaei*, and is suspected to be the only lineage in the Philippines [8]. The brown dog tick, *R. sanguineus* s.l., is the principal vector of *Ehrlichia canis* and *Anaplasma platys* [3,6,9]. The canine monocytic ehrlichiosis caused by *E. canis* is a potentially fatal disease of dogs with a seroprevalence as high as 33% in client-owned dogs in the Philippines [9,10]. Comparably, *A. platys* has a seroprevalence of 17% in client-owned dogs in the Philippines and causes canine infectious cyclic thrombocytopenia [3,10]. Both *E. canis* and *A. platys* are zoonotic, the former causing

human ehrlichiosis and the latter potentially causing human anaplasmosis [11,12]. Knowledge regarding tick-borne pathogens in stray unowned and/or free-roaming street dogs and their role in disease transmission is incompletely understood in the Philippines [3].

The cat flea (*Ctenocephalides felis*) is the most common flea infesting dogs in the Philippines [1,6,13]. Mitochondrial genetic differences divide *C. felis* into temperate, tropical I, tropical II, and African clusters, but to date no specimens from the Philippines have been genetically characterised [14]. Models predicting the cat flea global distribution expect the Philippines to harbor the tropical I and tropical II cluster [14]. The tropical II cat flea cluster was shown to be the dominant "Cairns" clade in Australia thriving in the tropical climates [15]. Previous studies have noted the presence of the dog flea (*Ctenocephalides canis*) and the increasing prevalence of *Ctenocephalides orientis* in East and Southeast Asia, although its' presence in the Philippines remains unconfirmed [6,14]. The cat flea is the principal vector of *Rickettsia* spp. and *Bartonella* spp. [1,6,13]. A recent survey demonstrated a *R. felis* prevalence of 27.7% in *C. felis* samples collected from dogs in the Philippines [6]. Dogs are asymptomatic reservoir hosts for *Rickettsia* species, several of which are zoonotic. For instance, *R. felis* causes flea-borne spotted fever [16]. In contrast, *Bartonella* spp. cause a broad range of clinical signs including fever and endocarditis in dogs [17]. In humans, some *Bartonella* species—such as *B. henselae* and *B. clarridgeiae*—cause cat-scratch disease [1,4]. Others such as *B. quintana* causes bacillary angiomatosis, and *B. koehlerae* causes endocarditis [17]. The seroprevalence of *Bartonella* spp. antibodies in dogs from the Philippines is 2.6% indicating a low exposure to the pathogen [1].

This study aimed to (i) confirm the identity of the most common tick and flea species parasitising dogs in Metro Manila, Philippines; and (ii) investigate the possible presence of canine VBP DNA. Ticks and fleas were collected from three veterinary clinics and morphological and molecular techniques were used to unequivocally identify their species. Canine VBPs (*Rickettsia* spp., *Bartonella* spp., *E. canis* and *A. platys*) were screened using real-time PCR (qPCR) assays.

2. Results

Ticks (*n* = 58) and fleas (*n* = 52) were collected from a total of 42 client-owned dogs presenting to one of three veterinary clinics in Metro Manila, Philippines. The majority of dogs (92.9%, *n* = 39) were from clinics 1 (*n* = 20) and 2 (*n* = 19), located in San Juan City, whereas clinic 3 in Quezon City provided 7.1% (*n* = 3) of dogs (Figure 1). Among the 42 dogs in the study, 50% had ticks only (*n* = 21), 42.9% had fleas only (*n* = 18), and 7.1% had both (*n* = 3). Female dogs accounted for 57.1% of the dogs sampled (*n* = 24). The age of the sampled dogs ranged from 0.2 to 13 years old with a median age of four years, however, four dogs had unknown ages. Of the sampled dogs, 45.2% were exclusively housed indoors (*n* = 19), while 40.5% were housed both indoors and outdoors (*n* = 17) and 14.3% were exclusively housed outdoors (*n* = 6) (Table 1).

All ticks were morphologically identified as unambiguous *R. sanguineus* s.l. From the total number of ticks collected, 26 specimens from 24 dogs (at least 1 tick per dog) underwent *cox1* amplification and DNA sequencing, all of which revealed high similarity (>99%) to *R. linnaei* (formerly *R. sanguineus* "tropical lineage"). In total, there were four *R. linnaei cox1* haplotypes. The most numerous (17/26) *R. linnaei cox1* haplotype was 100% identical while the remaining three were >99% identical with the reference mtDNA of *R. linnaei* (MW429381) from Australia [8].

All fleas were morphologically identified as unambiguous *C. felis.* In total, 20 flea specimens from 20 dogs (1 flea per dog) were subject to *cox1* amplification and DNA sequencing, confirming *C. felis* identity. All but one *C. felis* specimen belonged to the M_h1 haplotype (one belonged to M_h2), which is identical to haplotype h3 *sensu* Lawrence et al. [14]. There was only a single nucleotide difference between M_h1 and M_h2. Both haplotypes belonged to the *C. felis* "Cairns" clade [15].

VBPs were detected in the DNA of ticks and fleas from 5 dogs. *Bartonella* and *Rickettsia* multiplex qPCR testing of 20 *C. felis* and 26 *R. linnaei* DNA samples was performed.

Bartonella spp. DNA was detected in two fleas (10%, 2/20, 95%CI 1.57–31.3%) and *Rickettsia* spp. DNA in one tick (3.8%, 1/26, 95%CI 0–20.5%) as well as two fleas (10%, 95%CI 1.57–31.3%). Both *Rickettsia* spp. and *Bartonella* spp. DNA were detected in one flea (5%, 1/20, 95%CI 0–25.4%) (Table 2; see available data section). One other tick sample (3.8%, 1/26) had *Rickettsia* spp. C_t-values ≥36 and <40 and so was considered 'suspect' positive. All negative controls revealed no observable amplicons.

DNA sequencing of the three *Bartonella*-positive qPCR samples demonstrated that two 100% matched *B. clarridgeiae ssrA* (JN982716), while one had insufficient DNA quantity to be sequenced. All (*n* = 5) *Rickettsia* positive and *Rickettsia* suspect positive samples were subjected to conventional nested PCR to amplify the *ompA* and *gltA* genes. Four were successfully amplified and DNA sequence comparison to reference *R. felis* (CP000053) revealed all samples to be 100% *R. felis* at both loci [21]. The *Rickettsia* suspect positive tick sample failed to amplify using the *ompA* and *gltA* nested PCR and was therefore considered negative for *Rickettsia* spp.

Real-time PCR testing of 20 *C. felis* and 26 *R. sanguineus* DNA samples did not detect any *E. canis* or *A. platys* DNA (Table 2).
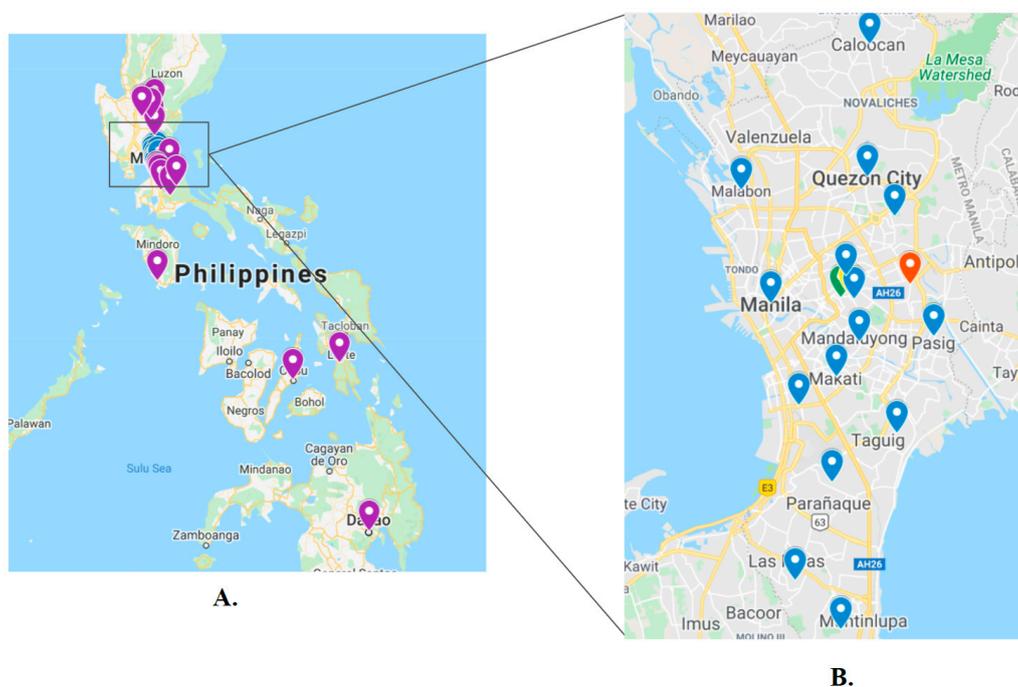

Figure 1. Map of the Philippines showing the various sample locations of previous studies investigating ectoparasites on dogs and/or vector-borne pathogens in ticks and/or fleas from dogs in (**A**) Non-Metro Manila (provincial) areas (purple) and (**B**) Metro Manila cities (blue) [10,13,18–20] (Table A1). Sample locations for this study are in San Juan City represented in yellow (clinic 1) and green (clinic 2), and Quezon City in red (clinic 3). Google Maps, Philippines (2021).

Table 1. Summary of the dog characteristics including sex, housing status, age group (years, Y), and ectoparasites. The ages of four dogs were unknown.

Demographic	Sex		Housing			Age, Y		Ectoparasite		
Clinic	M	F	Indoor	Outdoor	Both	≤4	>4	*R. linnaei*	*C. felis*	Both
Clinic 1	9	11	8	4	8	14	6	6	13	1
Clinic 2	8	11	9	2	8	8	9	12	5	2
Clinic 3	1	2	2	0	1	0	1	3	0	0
Total	18	24	19	6	17	22	16	21	18	3

Table 2. Summary of *B. clarridgeiae*, *R. felis*, *E. canis*, and *A. platys* real-time PCR results on fleas (*n* = 20) and ticks (*n* = 26) from dogs per clinic.

Clinic	1		2		3	
Pathogen	*R. linnaei*	*C. felis*	*R. linnaei*	*C. felis*	*R. linnaei*	*C. felis*
B. clarridgeiae	0	0	0	2	0	0
R. felis	1	2	0	0	0	0
B. clarridgeiae and *R. felis*	0	0	0	1	0	0
E. canis	0	0	0	0	0	0
A. platys	0	0	0	0	0	0

3. Discussion

The majority of tick and flea samples came from the two clinics located in San Juan City. The third clinic that donated least ectoparasites is located in a more affluent area and receives more clients with a greater ability to purchase antiparasitics and other preventatives [22]. Tick infestation was reported to be 2.6% in dogs from Metro Manila, whereas tick and flea infestations in non-Metro Manila dogs were as high as 67.5% and 80%, respectively [10,23]. Factors influencing tick and flea infestation include the availability and ability to purchase antiparasitics which are likely contributing to the disparity between urban and rural dog infestation [10,23,24]. There are conflicting reports regarding age predilection to VBPs. For instance, one study reported VBPs to be more prevalent in <1-year-old dogs, whereas another reported an age range of 1–3 years [3,25]. Regardless, age predisposition has not been definitively established [26].

In this study, only the tropical brown dog tick (*R. linnaei*, formerly *R. sanguineus* s.l. "tropical lineage") was detected on dogs in Metro Manila, consistent with previous studies (Table A1) [6,13]. Molecular analysis based on *cox1* typing confirmed *R. linnaei*. This is similar to previous studies based on 16S rDNA typing of 35 *R. sanguineus* s.l samples throughout East and Southeast Asia, including nine samples from the Philippines (MN685295-MN685303) [6,10]. The tropical brown dog tick (*R. linnaei*) appears to be the most prevalent species within *R. sanguineus* s.l. distributed across East Asia, Southeast Asia, and Australia, and it is the main vector for the transmission of VBPs such as *Ehrlichia* spp., *Rickettsia* spp., and *Babesia* spp. [6,8,10]. The temperate lineage of *R. sanguineus* s.l. and *R. sanguineus* sensu stricto are found in Beijing, which has cooler climates as compared to the Philippines [6,10]. Other ticks collected from dogs in East and Southeast Asia include *Haemophysalis lognicornis* and *Haemophysalis hysterics*, however, previous tick surveys found only *R. sanguineus* s.l. on dogs in the Philippines (Table A1) [6,8,10,13,18]. The cat flea (*C. felis*) was the only flea species found on dogs in Metro Manila in this study. All *C. felis* samples belonged to M_h1 haplotype except one (M_h2). A previous survey sequenced *cox1* from one *C. felis* from the Philippines (MT027207) which was identical to the M_h1 haplotype in this study [10]. Both haplotypes (M_h1 and M_h2) belong to clade 6 of the tropical II cluster and the "Cairns" clade that was predicted to be distributed around Southeast Asia and North Australia [14,15,27]. This was to be expected as the ecological niche of the "Cairns" clade is similar to the climatic conditions of Metro Manila [15,28]. Aside from *C. felis*, previous surveys noted the presence of *C. orientis* and *C. canis*, which were not found in this study (Table A1) [6,10]. It is known that *C. orientis* is distributed across tropical Asia, including Southeast Asia [6,10,27]. In the previous studies, *C. orientis* could have been mistaken for *C. canis* as they have remarkably similar morphology [10,29].

The DNA of *B. clarridgeiae* was detected in 10% of *C. felis* samples (95% CI 1.57–31.3%), which is relatively similar to previous reports of *Bartonella* spp. prevalence in *C. felis* within East and Southeast Asia (16.5%, 95% CI 12.4–21.7%) and the Philippines (0%, 95% CI 0–9.0%) [6]. Cats are considered the main reservoir for *Bartonella* spp. as 31% of domestic cats in Metro Manila and Cebu City were seropositive for *B. clarridgeiae* and 68% were seropositive for *B. henselae* [4]. Dogs in Metro Manila were reported to have a low seroprevalence of *B. henselae* (2.6%), suggesting they are a spill-over host rather

than a true host [1]. The low detection frequency of *Bartonella* spp. in this study could also support this notion. This study failed to detect any *B. henselae*, which may be due to the low seroprevalence in dogs and the small sample size. Nevertheless, the presence of *B. clarridgeiae* in fleas is important to note as it is considered a causative agent of cat-scratch disease [30].

This survey demonstrated a low prevalence of *R. felis* DNA in ticks (3.8%, 95% CI 0–20.5%) and fleas (10%, 95% CI 1.57–31.3%), which is roughly consistent with previous reports of a 1.6% (95% CI 0–9.3%) prevalence in ticks and 27.7% in fleas (95% CI 15.6–42.6%) [6]. Despite the low prevalence, the presence of *R. felis* DNA in ticks and fleas poses a risk to humans. *Rickettsia* spp. are a group of Gram-negative, obligate intracellular bacteria whose asymptomatic reservoir hosts are dogs [16]. Specifically, *R. felis* causes symptoms similar to murine typhus and dengue in humans bitten by infected fleas or ticks [31]. Because of the presence of *R. felis* in ticks and fleas from dogs, rickettsia infections may be considered by physicians for patients with dengue-like symptoms [32]. This study reports potential co-infection of *R. felis* and *B. clarridgeiae* in one flea, which has only been previously reported in Taiwan and France [33]. Detection of *B. clarridgeiae* DNA needs to be further investigated because the positive result could have been the result of co-feeding of the two ectoparasites and thus passive transfer of the DNA from flea to tick.

No *E. canis* and *A. platys* DNA in ticks were detected, implying 0–15% prevalence assuming a 95% confidence interval. This result is expected because of a detection frequency range of 3.2% to 8.3% for *E. canis* and 0.6–0.8% for *A. platys* in ticks, as reported by previous studies (Table A1) [3,6,19,25]. Seroprevalence of *A. platys* (17%, 95% CI 11.1–25.0%) and *E. canis* in dogs are high, indicating high exposures to the pathogens [10,34]. Dogs from Metro Manila were reported to have a higher seroprevalence of *E. canis* (95.3%, 95% CI 90.9–97.9%) as compared to dogs from non-Metro Manila cities (33%, 95% CI 25.0–42.2%) [10,34]. This suggests that dogs from Metro Manila have a higher exposure to *E. canis*, despite having a low tick infestation prevalence, supporting the notion that *E. canis* infection risk is independent of tick infestation levels [23,24]. The Philippines has many free-roaming stray dogs or the street dogs whose exact numbers are not known. These dogs are neglected, likely representing a reservoir for diseases, especially tick-borne pathogens as was demonstrated for *Babesia gibsoni* [35]. These dogs may facilitate the spread and maintenance of pathogens, especially since they frequently come into close contact with outdoor owned dogs in low-income households, which increases the risk of infection and infestation of client-owned dogs [10,36].

4. Materials and Methods

4.1. Collection and Morphological Identification of Ticks and Fleas from Dogs in Metro Manila, Philippines

Metro Manila is a metropolitan area in the Philippines that has a mean temperature of 25.5 °C in the coldest months, a mean temperature of 28.3 °C in the warmest months, and annual rainfall of 965 mm to 4064 mm [28]. All ticks and fleas were collected from Metro Manila between January 2021 and March 2021 and donated by three veterinary practices: Aso, Pusa atbp. Animal Shelter and Veterinary Services Clinic in San Juan City (Clinic 1), The Pet Project Veterinary Clinic in San Juan City (Clinic 2), and Vets in Practice in Quezon City (Clinic 3). Client-owned dogs visiting the practices were examined by a veterinarian for the presence of ticks and fleas. We had no control over effort and whether all fleas and ticks were found on individual dogs or any recent application of tick and flea preventatives. Ticks were removed from the dogs via tweezers and the fleas were removed using a flea comb according to veterinary practice routine procedure. Ticks and fleas are removed and discarded routinely from animals by all veterinary practices as part of veterinary care. For our purpose, removed ticks and fleas were donated to us rather than discarded, hence animal ethics approval was not required. Samples were stored in 1.5 mL tubes with 70% ethanol at room temperature. Samples were de-identified and submitted to the Veterinary Parasitology Laboratory (VPL) at the University of Sydney along with dog age, sex, indoor and/or outdoor status, and collection date as recorded by the veterinarian;

the data were summarised descriptively, no further statistical analysis was attempted due to low sample sizes. Upon arrival in Sydney, all specimens were morphologically identified by the primary authors to the genus and species level via a stereomicroscope and morphological keys [37,38].

4.2. Molecular Characterisation of Ticks and Gleas at Cytochrome C Oxidase Subunit I (cox1)

Between one to three ticks and/or fleas representing at least one of each identified species per dog were selected for DNA isolation at VPL. A small incision was made to the body of each tick and flea using single-use sterile scalpel blades and dried in a heat block at 60 °C for 1 h [39]. Total genomic DNA was isolated using Monarch® Genomic DNA Purification Kit (New England Biolabs, Australia). A blank isolation with no flea/tick DNA was included to control for cross-contamination (negative extraction control, NEC). DNA was eluted into 75 µL of elution buffer and stored at −20 °C.

Extracted tick and flea DNA samples were subjected to conventional polymerase chain reaction (PCR) targeting cytochrome c oxidase subunit I (*cox1*) using MyTaq Red Mix (BioLine), with 2 µL (1–5 ng/µL) DNA, and nuclease-free water as previously described [14,39,40]. All reactions were run with their respective NECs and sterile PCR water in place of DNA acted as a non-target control (NTC). Amplicons were verified via agarose gel electrophoresis to visualise the bands stained with GelRed® (Botium, Fremont, CA, USA). Amplicons of *cox1* were bi-directionally sequenced (Macrogen Ltd., Seoul, Korea) and visually inspected by eye using CLC Main Workbench 21 (CLC bio, Qiagen, Australia). Newly obtained tick *cox1* were compared to *Rhipicephalus* spp. complete mitochondrial DNA reference sequences (MW429381-MW429383) [8]. Newly obtained flea *cox1* were compared to *Ctenocephalides* spp. reference *cox1* haplotypes (h1-h90) *sensu* Lawrence et al. [14].

4.3. Molecular Detection of Vector-Borne Pathogens in Ticks and Fleas

An aliquot of extracted tick and flea DNA was submitted to The Elizabeth Macarthur Agricultural Institute (EMAI) Laboratory (NSW Department of Primary Industries and Environment), Menangle, New South Wales) for *Ehrlichia canis* DNA and *Anaplasma platys* DNA diagnostic evaluation using real-time PCR following OIE protocols and assays [41,42].

Flea DNA underwent further screening at VPL at the University of Sydney using a multiplex TaqMan qPCR targeting the *Rickettsia* spp. and *Bartonella* spp. genes *gltA* (citrate synthase) and *ssrA* (transfer-messenger RNA), respectively [21,43,44]. The reactions were performed in duplicate using the CFX96 Touch™ Real-Time PCR Detection System (BioRad, Australia) and contained Luna® Universal Probe qPCR Master Mix (New England BioLabs, Omnico, Australia) as described [21]. Results were considered positive if duplicates yielded C_t values < 36. Results were considered suspect positive if one or more duplicates yielded C_t values ≥ 36 and samples were considered negative if neither duplicate crossed the threshold (C_t > 40). Positive *Bartonella* spp. results were sent to Macrogen for sequencing (Macrogen Ltd., Seoul, South Korea) and compared to reference *Bartonella* spp. sequences. Samples considered either positive or suspect positive for *Rickettsia* spp. (Ct value < 38) were further characterised using a pair of conventional nested PCRs targeting the outer membrane protein A (*ompA*) gene and *gltA* [21,45]. PCR products were sequenced at Macrogen Inc. (Seoul, Korea), assembled using CLC Main Workbench 21 (CLC bio, Qiagen, Australia), inspected manually by eye and compared to reference *Rickettsia* spp. sequences, i.e., *R. felis* (CP000053) [21].

5. Conclusions

This study confirms that the tropical brown dog tick (*R. linnaei*) and the cat flea (*C. felis*) are the most common tick and flea species parasitising dogs in the Manila Metro area in the Philippines. The canine VBPs *R. felis* and *B. clarridgeiae* were confirmed by demonstration of their DNA in ectoparasites collected from dogs in Manila Metro. Fleas and ticks remain significant pathogens for urban owned dogs in Metro Manila implying that prevention

of ectoparasites is imperative for both the welfare of animals, as well as for the possible prevention of vector-borne disease and transmission.

Appendix A

Table A1. Summary of the previous studies showing the number of dogs examined, species and quantity of ticks and fleas if stated, vector-borne pathogens (VBPs) detected with associated prevalence, and location in the Philippines.

Authors	Number of Dogs Examined	Ticks/Fleas on Dogs (no#)	VBP's (Prevalence)			Location
			Molecular Analysis		Serology-based	
			Blood PCR	Ectoparasite PCR		
Baron and Ruaes [18]	16	*R. sanguineus* s.l (44) *C. felis* (96)	n/a	n/a	n/a	Davao City
Bartolome-Cruz [23]	97	*R. sanguineus* s.l (2498)	n/a	n/a	n/a	Quezon City, Pasay City, Laguna City
Bartolome-Cruz [25]	953	*R. sanguineus* s.l (52)	n/a	*Babesia* spp. (2.08%), *Hepatozoon* spp. (2.08%), *Ehrlichia* spp. (8.33%)	n/a	Quezon City, Pasay City, Laguna City
Baticados and Baticados [34]	169	*R. sanguineus* s.l	n/a	n/a	*E. canis* (95.3%)	Malabon, Quezon City, Manila, San Juan City, Pasig City, Makati City, Mandaluyong City, Taguig City, Parañaque City, Muntinlupa City, Las Pinas, Caloocan, Rizal
Colella, Nguyen, Tan, Lu, Fang, Zhijuan, Wang, Liu, Chen, Dong, Nurcahyo, Hadi, Venturina, Tong, Tsai, Taweethavonsawat, Tiwananthagorn, Le, Bui, Watanabe, Rani, Annoscia, Beugnet, Otranto, and Halos [10]	120	*R. linniae, C. felis, C. canis, C. orientis*	*H. canis* (9.7%), *B. gibsoni* (0%)	n/a	*E. canis* (33.0%), *A. platys* (17.0%), *D. immitis* (29.8%)	San Jose City (Occidental Mindoro), Cabanatuan and Muñoz (Nueva Ecija)

Table A1. *Cont.*

Authors	Number of Dogs Examined	Ticks/Fleas on Dogs (no#)	VBP's (Prevalence)			Location
Corales, Viloria, Venturina, and Mingala [19]	70	n/a	*E. canis* (2.85%), *Babesia* spp. (7.14%), *A. platys* (0%)	n/a	n/a	San Jose City, Muñoz, Talavera, Guimba, Sta. Rosa, Cabanatuan City, Gapan City (Nueva Ecija)
Galay, Manalo, Dolores, Aguilar, Sandalo, Cruz, Divina, Andoh, Masatani, and Tanaka [3]	248	*R. sanguineus* s.l (157)	*E. canis* (19.8%), *A. platys* (6.0%), *Rickettsia* spp. (2.4%), *B. vogeli* (6.8%), *H. canis* (2.4%)	*E. canis* (3.2%), *A. platys* (0.6%), *B. vogeli* (0.6%), *H. canis* (0.6%), *Rickettsia* spp. (0%)	n/a	Pasay, Taguig City, Parañaque City, Las Piñas, Muntinlupa City, Laguna (San Pedro, Biñan, Santa Rosa, Calamba, Los Baños, San Pablo, Pagsanjan)
Nguyen, Colella, Greco, Fang, Nurcahyo, Hadi, Venturina, Tong, Tsai, Taweethavonsawat, Tiwananthagorn, Tangtrongsup, Le, Bui, Do, Watanabe, Rani, Dantas-Torres, Halos, Beugnet, and Otranto [6]	120	*R. linniae* (63), *C. felis* (47), *C. orientis* (10)	n/a	*R. sanguineus* s.l: *R. felis* (1.6%), *H. canis* (15.9%), *A. platys* (0%), *E. canis* (0%), *R. asembonensis* (0%), *Babesia vogeli* (0%) *C. felis*: *R. felis* (27.7%), *R. asembonensis* (2.1%), *Bartonella* spp. (0%) *C. orientis*: *R. asembonensis* (80%), *R. felis* (0%), *Bartonella* spp. (0%)	n/a	Philippines
Portugaliza and Bagot [13]	Not specified	*C. felis* (70), *Pulex irritans* (1), *R. sanguineus* s.l (93)	n/a	n/a	n/a	Baybay (Leyte)
Singer, Loya, Lapsley, Tobar, Carlos, Carlos, Carlos, Adao, Rivera, Jaffe, Mazet, and Chomel [1]	116	n/a	*B. henselae* (11.2%)	n/a	*B. henselae* (2.6%)	Makati City, Parañaque City
Ybañez, Julian, and Carlos [9]	68	n/a	n/a	n/a	*E. canis* (86.7%)	Makati City
Ybanez, Ybanez, Arnado, Belarmino, Malingin, Cabilete, Amores, Talle, Liu, and Xuan [20]	100	n/a	*Ehrlichia/Anaplasma* spp. (10%), *Babesisa* spp. (18%)	n/a	n/a	Cebu

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