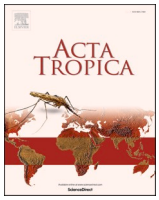
Acta Tropica 260 (2024) 107401

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica



Dog ectoparasites as sentinels for pathogenic *Rickettsia* and *Bartonella* in rural Guatemala

Yuexun Tian [a], Jose G. Juarez [b], Andrea M. Moller-Vasquez [b], María Granados-Presa [b], Francisco C. Ferreira [a], Pamela M. Pennington [b], Norma Padilla [b], Gabriel L. Hamer [a], Sarah A. Hamer [c,*]

[a] Department of Entomology, Texas A&M University, College Station, TX, USA
[b] Universidad del Valle de Guatemala, Ciudad de Guatemala, Guatemala
[c] Department of Veterinary Integrative Biosciences, College Station, Texas, USA

ARTICLE INFO

Keywords:
Dog
Ectoparasite
Guatemala
Vector-borne
Rickettsia
Bartonella

ABSTRACT

Fleas and ticks serve as vectors of multiple pathogens in the genera *Rickettsia* and *Bartonella* that cause diseases in humans and other animals. Although human rickettsiosis and bartonellosis have been reported in all countries in Central America, limited research has been conducted to investigate the natural cycles of flea- and tick-borne rickettsiosis and bartonellosis, especially in Guatemala. We evaluated dog parasites as sentinels for zoonotic disease risk in rural Guatemala by sampling ticks and fleas from dogs, which were then identified and individually screened for *Rickettsia* and *Bartonella*. A total of 77 households were surveyed and 80.5 % of them had dogs. Overall, 133 dogs were examined for fleas and ticks, of which 68.4 % had fleas and 35.3 % had ticks. A total of 433 fleas and 181 ticks were collected from the infested dogs, with an additional 33 ticks collected from house walls. Three flea species were identified: *Ctenocephalides felis* (70.0 %), *Echidnophaga gallinacea* (11.8 %), and *Pulex* sp. (17.8 %). Among the collected ticks, 97 % were identified as *Rhipicephalus sanguineus* sensu lato with the rest being *Amblyomma cajennense, A. auricularium*, and *A. ovale. Rickettsia felis* were detected in six *C. felis*, in one *Pulex* sp., and in two *R. sanguineus* sensu lato, while *Candidatus* R. senegalensis was detected in one *C. felis. Bartonella* was detected only in fleas, including three *Pulex* sp. infected with *B. vinsonii* subsp. *berkhoffii, B. henselae*, and *Bartonella* sp., respectively, and 11 *C. felis* infected with *B. henselae*. This study reports *Candidatus* R. senegalensis and *B. vinsonii* subsp. *berkhoffii* in Guatemala for the first time, and indicates the potential risk of human and dog exposure to *Rickettsia* and *Bartonella* species. These results show that dogs provide critical information relevant to managing human potential exposure to flea- and tick-borne pathogens in rural Guatemala. This approach can potentially be expanded to other regions in Central America where domestic dogs are abundant and suffer from ectoparasite infestation.

1. Background

Fleas and ticks are obligate blood-feeding ectoparasites of many animals including companion animals living in close contact with humans. Pathogens transmitted by these ectoparasites include bacteria of the genus *Rickettsia*, which are classified into five groups based on phylogenomic analyses: two spotted fever groups (e.g. *Rickettsia rickettsii, R. conorii, R. amblyommatis, R. felis*), a typhus group (e.g., *R. typhi*), a Canadensis group (*R. canadensis*), and a Bellii group (*R. bellii*) (El Karkouri et al. 2022). *Rickettsia rickettsii* is the causative agent of Rocky Mountain spotted fever (RMSF), the most severe rickettsiosis (Fang et al. 2017), with *Dermacentor variabilis* and *D. andersoni* being the main vectors in the US (Dantas-Torres 2007). *Rhipicephalus sanguineus* sensu lato is considered a driver of epidemic levels of *R. rickettsii* transmission in Mexico (Alvarez-Hernández et al. 2017) and is the tick species most commonly infected in the Americas (Ribeiro et al. 2021). Other tick species, such as *Amblyomma parvum, A. cajennense, A. aureolatum* and *A. mixtum* were also reported to harbor *R. rickettsii* (Biggs et al. 2016; Ribeiro et al. 2021). Additionally, *R. felis* has recently emerged as a human pathogen with a worldwide distribution (Angelakis et al. 2016; Legendre and Macaluso 2017; Mawuntu et al. 2020; Tsokana et al. 2022). Although the natural vectors and reservoirs of *R. felis* are not fully

* Corresponding author.
E-mail address: shamer@cvm.tamu.edu (S.A. Hamer).

https://doi.org/10.1016/j.actatropica.2024.107401
Received 5 July 2024; Received in revised form 23 August 2024; Accepted 9 September 2024
Available online 12 September 2024

known, the cat flea, *Ctenocephalides felis*, is the arthropod vector most commonly associated with *R. felis*, and cats, humans and small mammals are most commonly found to be infected by molecular methods or serology (Legendre and Macaluso 2017; Tsokana et al. 2022). *Rickettsia felis* has also been detected in other fleas and arthropods such as dog flea (*C. canis*), ticks, and mosquitoes (Tsokana et al. 2022). In the typhus group, the most common flea-borne species is *R. typhi,* causing murine typhus in humans, which is endemic to tropical and subtropical regions (Fang et al. 2017). Fleas can also transmit *Bartonella* spp., including *B. henselae* and *B. clarridgeiae*, causative agents of cat scratch disease, with cats being the reservoir host and *C. felis* being the vector (Angelakis and Raoult 2014).

In Central America, human rickettsiosis has been reported in all countries. However, the annual number of rickettsiosis cases reported is low, which may be an underestimate due to the lack of efficient diagnoses (Bermúdez and Troyo 2018) and surveillance. A recent systematic review reported that limited research has been conducted in Central America to study spotted fever group rickettsiosis, with the number of publications per country ranging from 1 in Guatemala to 27 in Costa Rica (Dye-Braumuller et al. 2022). The single publication in Guatemala reported an outbreak in 2007 where 10 out of 17 patients were confirmed or probable cases of spotted fever group rickettsiosis including two fatal cases (Eremeeva et al. 2013). Prior research on *R. felis* in Guatemala includes the detection of *R. felis* in *C. felis* fleas (Troyo et al. 2012b) and one human case of *R. felis* infection (López et al. 2022). Flea-borne *Bartonella* infection has been reported in Guatemala with both *B. henselae* and *B. clarridgeiae* detected in cats and fleas (Bai et al. 2015). Therefore, there is a gap in understanding the prevalence of flea- and tick-borne rickettsiosis and bartonellosis in Guatemala. Further, given dogs frequently spend time in both outdoor and indoor environments, they can move vectors and pathogens across this interface and their infection status may provide an indication of local zoonotic disease risk to humans that share the household. This creates conditions for the establishment and expansion of such vectors in intra- and peridomiciles, exposing humans to pathogens via contact with infective ticks and fleas.

With the increasing need for effective and efficient methods of emerging disease surveillance in low resource regions, our study aims to evaluate the potential of using dog ectoparasites as sentinels for zoonotic disease risk in a remote rural area of Guatemala. Our results can provide guidance to local public health authorities on the effectiveness of monitoring dogs for diseases that impact human health.

2. Materials and methods

2.1. Ethic statement

The study was reviewed by the Research Ethics Committee of the Center for Health Studies at UVG and was classified as 'Research not involving human subjects' (Protocol No. 270–05–2022) and was approved by the Institutional Animal Care and Use committee of Universidad del Valle de Guatemala (CEUCA – UVG) under protocol number I – 2022 (3)A. Additionally, this study was approved by the Texas A&M University's Institutional Animal Care and Use Committee (IACUC 2022–0001 CA).

2.2. Sampling sites

The sampling was conducted in the municipality of Comapa, Department of Jutiapa, in southeastern Guatemala (Fig. 1) from June-August 2022. There are 56 communities in Comapa with a population of >27,000, of which over 80 % live in rural areas and almost 90 % live in poverty (Juarez et al. 2018). Initially, thirty communities in Comapa were selected based on *T. dimidiata* household infestation levels and

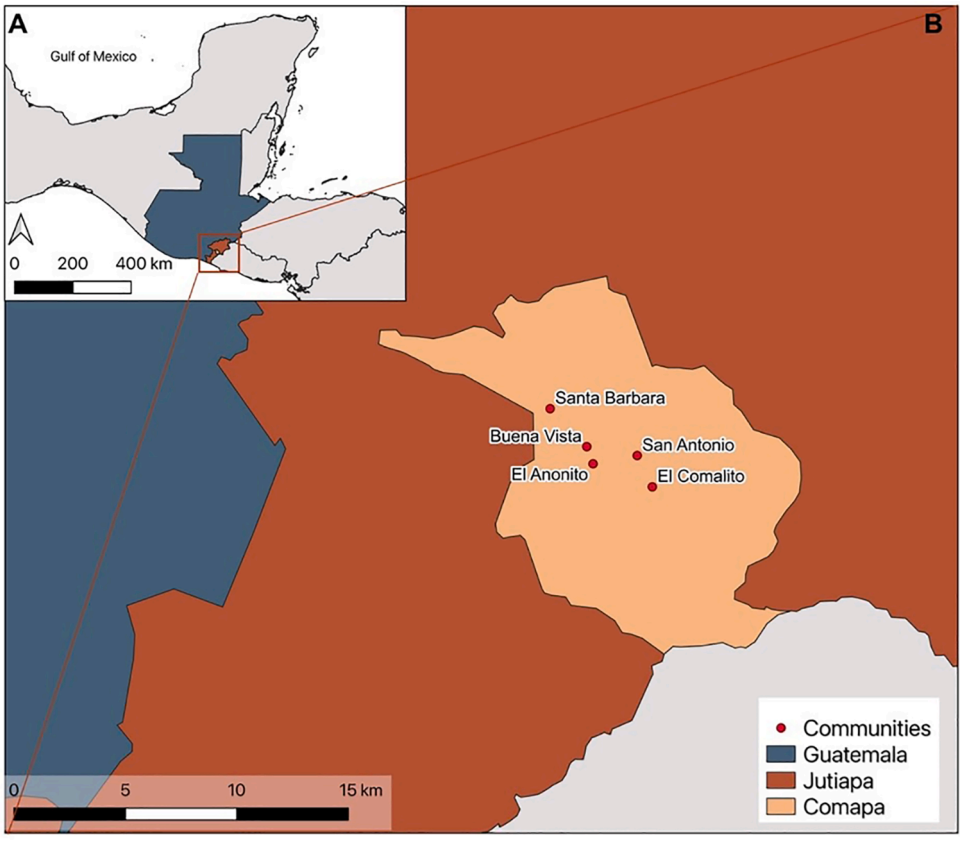


Fig. 1. A. Map of Guatemala. **B.** Map of the five communities in municipality of Comapa, Department of Jutiapa, in southeastern of Guatemala.

safety recommendations (Bustamante et al. 2014). Among these, we detected a baseline household infestation rate of >15 % in eighteen communities, which were then chosen for a Chagas disease intervention (De Urioste-Stone et al. 2015). From these eighteen communities, we randomly selected five for the current study, representative of communities with high *T. dimidiata* household infestation levels. Households surveyed were the same as the ones selected in the previous studies (24 households per community, this sampling intensity follows the guidelines of the Guatemalan Ministries of Health; (MSPAS 2012)), providing us with information regarding the presence of dogs in the household. Additionally, investigators form UVG have had extensive community engagement activities with these community members. The selected communities were Buena Vista (BV), El Anonito (EA), El Comalito (EC), San Antonio (SA), and Santa Barbara (SB).

2.3. Questionnaire

A questionnaire was designed to survey the house conditions, domiciliary and semi-domiciliary animals, vector presence, pesticide use, and awareness of vector and vector-borne disease. In this study, we focused on the frequency of house animals and ectoparasites in dogs. Therefore, based on the relevance, two variables from the survey were used to analyze their relationship with tick and flea presence and abundance in this study which are 1) Do you use something to protect your dog's health; 2) Do you use any approaches in your home to prevent or eliminate insects.

2.4. Ectoparasite collection

Dogs owned by the household residents were leashed, muzzled, and restrained with the owners' permission. Attached ticks were removed using fine-tipped forceps, and a flea comb was used to sample fleas. Households were also inspected for tick infestations by using flashlights to inspect cracks and crevices throughout the home. All collected ectoparasites were immediately stored in 70 % ethanol until further examination. Three metrics were used to measure ectoparasite infestation: abundance, defined as the number of individuals of ticks or fleas on a single dog; mean abundance, defined as the total number of individual ticks or fleas divided by the number of dogs examined; and mean intensity, defined as the total number of ticks or fleas divided by the number of dogs infected with ticks or fleas, respectively (Bush et al. 1997).

Ectoparasites were morphologically identified to species or genus using taxonomical keys (Keirans and Litwak 1989; CDC 2003). A subset of ticks and fleas were selected and subjected to a molecular identification process (see below); this subset included (i) all ticks that were not morphologically identified as *R. sanguineus* (n = 5); (ii) randomly selected ~5 % of the ticks morphologically identified as *R. sanguineus* (n = 13); (iii) ~5 % of fleas morphologically identified as *C. felis* (n = 14); (iv) ~5 % of fleas identified as *Pulex* sp. (n = 5).

2.5. DNA extraction and PCRs for arthropod identification and pathogen detection

The DNA of individual ticks and fleas was extracted using the whole body with the exception of eight ticks from which we used only half body and two fleas that were submitted as voucher specimens to the Texas A&M University Entomology Collection (TAMUIC-767). Each ectoparasite was sliced into at least four pieces using a sterile scalpel blade and subjected to DNA extraction using the E.Z.N.A. Tissue DNA Kit (Omega Bio-Tek, GA, USA) following manufacturer's instructions with overnight lysis (Salomon et al., 2022). A final elution volume of 50 uL was obtained for each sample.

Molecular identification of ectoparasites was performed via PCR with primers targeting the 12S rRNA gene for ticks and the cytochrome c oxidase subunit 1 (COI) gene for ticks and fleas. To amplify a fragment of the 12S rRNA gene, 1.5–3 μL of DNA was used in a 15 μL reaction containing 7.5 uL of FailSafe™ 2x PreMix E, 0.25 μL of FailSafe™ enzyme (Lucigen, Middleton, WI, USA), 0.5 μL of each primer (5 μM), and molecular grade water and the thermal cycle conditions described in Beati and Keirans (2001). Two pairs of COI primers were used to amplify the COI gene: LCO1490 and HCO2198 (Folmer et al. 1994); and LCO1490 and Cff_R (Lawrence et al. 2014). With LCO1490 and HCO2198 primers, the reaction consisted of 12.5 μL of FailSafe™ 2x PreMix E, 1 μL of each primer (10uM), 0.5 μL of FailSafe™ enzyme, 1 μL of DNA sample, and molecular grade water, resulting in a total volume of 25 μL. The thermal cycling condition had an initial denaturation at 94 °C for 3 min followed by 35 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s, with a final elongation at 72 °C for 8 min. Using the LCO1490 and Cff_R primers, reactions of 25 μL contained 12.5 μL of FailSafe™ 2X PreMix E, 1 μL of each primer (10 uM), 0.5 μL of FailSafe™ enzyme, 2 μL of DNA sample, and molecular grade water. The reaction condition followed the protocol from Lawrence et al. (Lawrence et al. 2014).

A quantitative PCR (qPCR) was used to detect the presence of *Rickettsia* species using primers and a probe targeting the citrate synthase protein gene (*gltA*) (Labruna et al. 2004). The reaction consisted of 12.5 μL of iTaq Universal Probes Supermix (Bio-Rad, Hercules, CA), 1.125 μL of each primer (10 uM), 0.375 μL of probe, 5 μL of DNA sample, and PCR water, resulting in a final volume of 25 μL. Positive samples from qPCR were subject to conventional PCR with primers also targeting the *glt*A gene (Kollars and Kengluecha 2001). Molecular grade water and a *Rickettsia*-positive tick sample ((Castellanos et al. 2016)) were included as negative and positive controls, respectively, and produced expected outcomes.

A conventional PCR was used to detect *Bartonella henselae*, with primers targeting the *pap31* gene (Zeaiter et al. 2002). The 25 μL reaction contained 12.5 μL of Premix E, 1.6 μL of each primer (10 μM), 0.25 μL of enzyme, 2.5 μL of DNA template, and PCR water. The reaction was started with a 3 min pre-denature at 95 °C, and followed by 44 cycles of 30 s at 95 °C, 30 s at 58 °C, 45 s at 72 °C, then finished with 7 min at 72 °C. The DNA from a *B. henselae*-positive flea (Salomon et al. 2024) was used as a positive control and molecular grade water was added as a negative control. All primers used in this study are presented in Table 1.

Amplicons from conventional PCRs were examined using 1 % agarose gel electrophoresis, samples producing bands of the expected sizes were purified with ExoSAP-IT (USB Corporation, OH, USA) following the manufacturer's protocol, and were submitted to bi-directional Sanger sequencing (Eton Biosciences, San Diego, CA). Sequences were examined using UGENE (Unipro LLC, Novosibirsk, Russia) and the consensus was compared to sequences in GenBank using the Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990). Representative tick, flea, and pathogen sequences were deposited on GenBank (Accession Nos. PP940107-09; PP940828-30; PP952311-14).

2.6. Statistics

Mean intensities of fleas and ticks were calculated by dividing the total number of ectoparasite by the number of infested hosts. Logistic regression was used to explore the effect of four explanatory variables including dog number in the household, dog protection, pesticide uses, and repellent uses, with fleas and tick presence as response variables. When quasi-complete separation occurs, Firth's bias-reduced logistic regression was used instead. All analyses were conducted using R (version 4.2.2; R Foundation for Statistical Computing, Vienna).

3. Results

3.1. Household survey

Out of 77 households, 75 had domestic animals, including 62 (80.5 %) with at least one dog, 32 (41.6 %) with at least one cat, and 62 (80.5 %) with at least one chicken (Fig. 2). Sixty-two (80.5 %) households had

Table 1
Oligonucleotides used in the study for ectoparasite identification and pathogen detection and sequencing.

Gene	Primers	Size	PCR assay	Reference
12S rRNA	T1B F: AAACTAGGATTAGATACCCT T2A R: AATGAGAGCGACGGGCGATGT	360	Tick identification	(Beati and Keirans 2001)
COI	LCO1490: GGTCAACAAATCATAAAGATATTGG HCO2198: TAAACTTCAGGGTGACCAAAAAATCA	710	Tick and flea identification	(Folmer et al. 1994)
	LCO1490: GGTCAACAAATCATAAAGATATTGG Cff-R [S0368]: GAAGGGTCAAAGAATGATGT	601	Flea identification	(Lawrence et al. 2014)
Citrate Synthase	CS-5: GAGAGAAAATTATATATCCAAATGTTGAT CS-6: AGGGTCTTCGTGCATTTCTT CS-FAM: CATTGTGCCATCCAGCCTACGGT	147	*Rickettsia* screening	(Labruna et al. 2004)
	RrCS 372: TTTGTAGCTCTTCTCATCCTATGGC RrCS 989: CCCAAGTTCCTTTAATACTTCTTTGC	617	*Rickettsia* screening	(Kollars and Kengluecha 2001)
Pap31	PAPn1:TTCTAGGAGTTGAAACCGAT PAPn2: GAAACACCACCAGCAACATA	269	*Bartonella* screening	(Zeaiter et al. 2002)

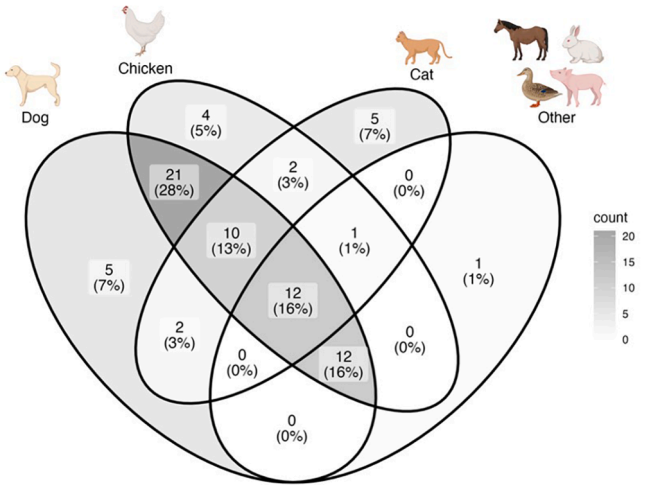


Fig. 2. Of 77 households surveyed in Comapa, Jutiapa, Guatemala, in summer 2022, 75 (97.4 %) had domestic animals. Houses frequently had multiple species of domestic animals. Values inside boxes represent the number and the proportion (in %) of households owning animals in each of four categories.

more than two species of domestic animals (Fig. 2). On average, there were 1.9 dogs/household (SE = ±0.2) and 0.6 cats/household (SE = ±0.1). Of the households with dogs sampled for ectoparasites, 80.0 % utilized products to protect dogs' health such as vaccination and shampoo; 62.0 % applied insect management approaches, such as applying pesticides and smoke (Table 2).

3.2. Ectoparasites detected

Of the 133 dogs, fleas and ticks were collected from 91 (68.4 %) and 47 (35.3 %) dogs, respectively, in which 27 (19.6 %) dogs had both fleas and ticks, and 111 dogs (83.5 %) had fleas or ticks. A total of 433 fleas and 181 ticks were collected with mean intensities of 4.8 (SE = ±0.4) and 3.9 (SE = ±0.8), respectively (Fig. 3). The flea abundance ranged from 0 to 24 on each dog with a mean abundance of 3.3 (SE = ±0.3), while the abundance of ticks on dogs ranged from 0 to 32 with a mean abundance of 1.4 (SE = ±0.4). In addition, 33 ticks were collected from house walls.

The collected fleas consisted of three species (Fig. 4). While most of the fleas (304, 70.0 %) were *Ctenocephalides felis* (cat flea), 51 (11.8 %) were *Echidnophaga gallinacea*, the sticktight flea (Fig. 4). There were 77 (17.8 %) fleas morphologically identified as *Pulex irritans*, the human flea. DNA sequencing of five of these specimens from three dogs of two households showed 96.6–97.8 % identity compared with *P. irritans* (GenBank: MH107045.1) and *P. simulans* (GenBank: OM366056.1), respectively. Because sequences obtained here had an identity of 99.4 % with *Pulex* sp. (GenBank: KM891015.1), these fleas are referred to as *Pulex* sp. in this study. Molecular barcoding confirmed the identity of 14 *C. felis*. One flea could not be identified due to extensive damage.

Table 2
Survey results of the dog health protection and insect management from the house with the dogs sampled for ticks and fleas in Comapa, Jutiapa, Guatemala, in summer 2022.

Question	Response	No. positive responses/Total (%)
Dog health protection	Use dog protection	48/60 (80.0 %)
What type of dog protection?	Vaccination	41/60 (67.2 %)
	Fumigate	4/60 (6.7 %)
	Shampoo	4/60 (6.7 %)
	Insecticide	3/60 (5.0 %)
	Deworming	2/60 (3.3 %)
	Burned oil	1/60 (1.7 %)
	Clean nest	2/60 (3.3 %)
Insect management	Use some kind of methods to manage insects	37/60 (61.7 %)
What types of insect management?	Fumigate	5/60 (8.3 %)
	Cleaning	6/60 (10.0 %)
	Commercial pesticide (Raid, Folidol, Baygon, Amitraz, Oko, Autan)	23/60 (38.3 %)
	Smoke	5/60 (8.3 %)
	Electric rackets	2/60 (3.3 %)

Of the 214 collected ticks (181 ticks from dogs and 33 ticks from house walls), the morphological identifications of 18 (8.4 %) of them were molecularly confirmed. Three tick species were identified with almost 98.0 % of them being *Rhipicephalus sanguineus* sensu lato, and the rest in genus *Amblyomma* including *A. cajennense, A. parvum*, and *A. ovale* (Fig. 4). Exclusively *R. sanguineus* sensu lato was found on house walls.

3.2.1. Rickettsia and Bartonella screenings

All ticks (n = 214) and fleas (n = 431), except the two voucher flea specimens (one *C. felis* and one *E. gallinacea*), were screened for *Rickettsia* and *Bartonella* bacteria. Ten samples were positive for *Rickettsia* including seven *C. felis* (2.3 %), one *Pulex* sp. (0.5 %), and two *R. sanguineus* sensu lato (1.0 %) from dogs in four households (Table 3). The sequences from all positive ticks and fleas matched *R. felis* (100 % identity; GenBank: CP000053; (Ogata et al. 2005)) except the sequences from one *C. felis* that matched *Candidatus* Rickettsia senegalensis with 100 % identity (GenBank: KF666472; (Mediannikov et al. 2015)). While no ticks were positive for *Bartonella* sp., 14 fleas were positive including three *Pulex* sp. infected with *B. vinsonii* subsp. *berkhoffii* (*Bvb*, 100 % identity; GenBank: CP003124; (Guy et al. 2013)), *B. henselae* (100 %

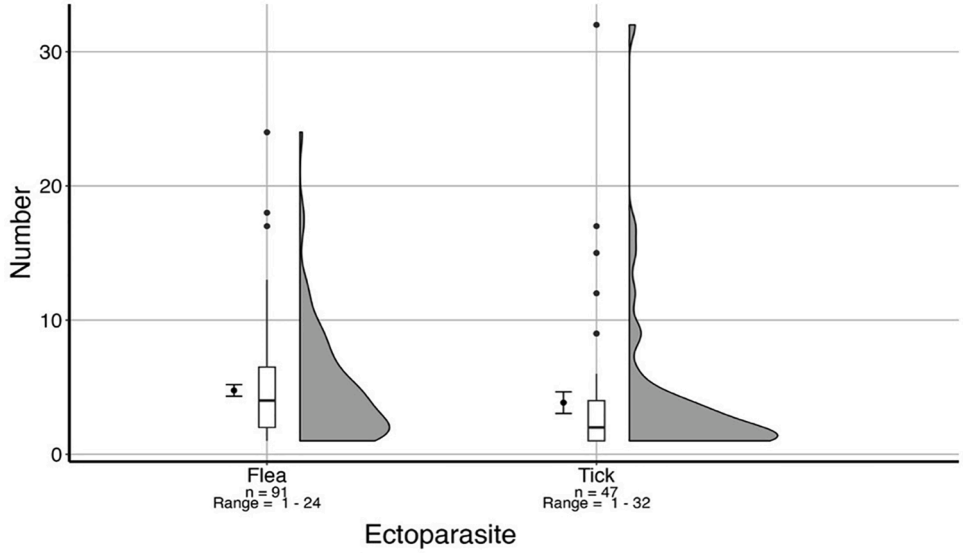


Fig. 3. Summary of fleas and ticks collected from infested dogs. The dot and whisker on the left represent the mean intensity and standard error, respectively. The box plots represent the medians, whiskers represent minimum and maximum excluding outliers, which are included as dots. The violin plots to the right represent frequency distribution of ectoparasites collected from dogs in this study.

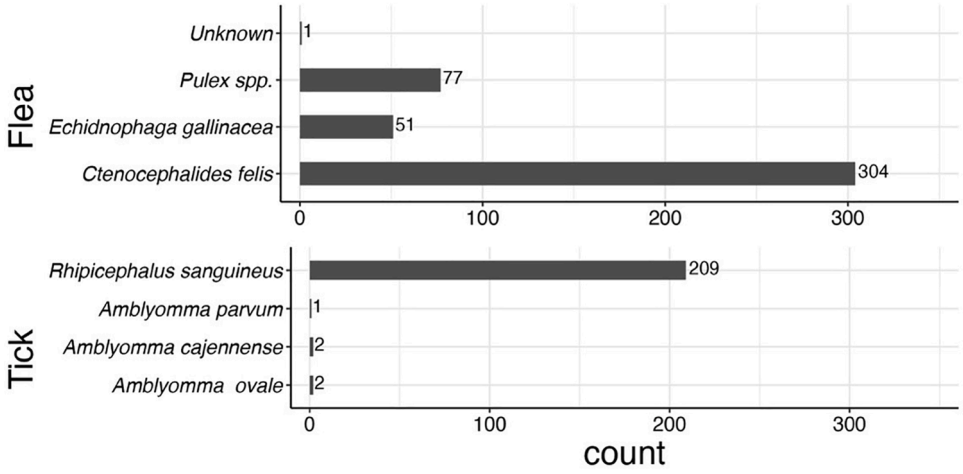


Fig. 4. Species and numbers of fleas collected from infested dogs and ticks collected from infested dogs and from house walls in Comapa, Jutiapa, Guatemala, between June and August 2022.

Table 3
Screening results of fleas and ticks removed from dogs and house walls in Comapa, Jutiapa, Guatemala, in June-August 2022, for *Rickettsia* and *Bartonella*.

Species	Number of tested	No. infected (%) with *Rickettsia* sp.		No. infected (%) with *Bartonella* sp.		
		Rickettsia felis	*Candidatus* Rickettsia senegalensis	*Bartonella vinsonii* subsp. *berkhoffii*	*Bartonella henselae*	*Bartonella* sp.
Flea						
Ctenocephalides felis	303	6 (2.0)	1 (0.3)	0	11 (3.6)	0
Echidnophaga gallinacea	51	0	0	0	0	0
Pulex spp.	76	1 (0.5)	0	1 (1.3)	1(1.3)	1(1.3)
Unknown	1	0	0	0	0	0
Total	431	7 (1.6)	1 (0.2)	1 (0.2)	12 (2.8)	1 (0.2)
Tick						
Rhipicephalus sanguineus sensu lato	209	2 (1.0)	0	0	0	0
Amblyomma ovale	2	0	0	0	0	0
Amblyomma cajennense	2	0	0	0	0	0
Amblyomma auricularium	1	0	0	0	0	0
Total	214	2 (0.9)	0	0	0	0

identity, GenBank: CP072898; (Thibau et al. 2022)), and *Bartonella* sp., respectively. We only obtained a 110 bp fragment deemed as of high quality for the latter sample, precluding us from assigning this sequence to a *Bartonella* species. An additional 11 *C. felis* (3.6 %) were infected with *B. henselae*. A dog from EC had seven fleas and two ticks, one of each were positive for *R. felis*, and a dog from SB had four fleas and one tick that were all positive for *R. felis.* Three dogs from SA had multiple fleas (3 out of 4, 6 out of 9, and 3 out of 4) positive to *B. henselae*. No co-infections were detected in ticks or fleas.

3.3. Associations between vector-control measures and ectoparasite infestation

Three questions from the survey and the number of dogs in households were selected as explanatory variables to evaluate the relationships with ectoparasite infestations. The logistic regression analysis revealed that there is no significant relationship between those variables (Table 4).

4. Discussion

This study documents that over 80 % of the households in five rural communities in Guatemala near the border with El Salvador have at least one dog, 68 % of which were infested with fleas and 35 % with ticks. The most common flea on dogs (70 % of all fleas) was the cat flea (*C. felis)*, followed by the sticktight flea (*E. gallinacea*- a common parasite of poultry) and *Pulex* spp. (which includes the human flea *P. irritans* and *P. simulans*, a parasite of carnivores, deer, and large rodents (Lewis 1972). Similarly, *C. felis* was the dominant flea species on dogs in a prior study in Guatemala (Escobar et al. 2011). Further, across the world, *C. felis* has been the dominant flea parasite on dogs, for example, in Australia (Slapeta et al. 2011), Florida (Yore et al. 2014), southern Italy (Rinaldi et al. 2007), Costa Rica (Troyo et al. 2012a), and Chile (Alcaino et al. 2002).

We found *R. sanguineus* senso lato as the dominant species on dogs in Guatemala. This is the most widespread tick species in the world, partially due to its ability to survive in both indoor and outdoor environments. Similarly, Nelson et al. (2022) and Alhassan et al. (2021) also found *R. sanguineus* senso lato as the dominant tick on dogs of Southeastern Belize and Caribbean Island of Grenada respectively. In addition to *R. sanguineus* senso lato, we also found a few *A. parvum, A. cajennense*, and *A. ovale* on the examined dogs. These three *Amblyomma* species are known to harbor disease-causing *Rickettsia* species (Londono et al. 2014; Biggs et al. 2016), with dogs being one of the common hosts (Guglielmone et al. 2003; Estrada-Peña et al. 2004; Nava et al. 2008; Murgas et al. 2013). The presence of dogs and other domestic animals in the households, such as horses and pigs, may facilitate the growth of *Amblyomma* tick population in the region.

Rickettsia felis was detected in six *C. felis*, the primary vector that has been found infected globally, including in Guatemala by Troyo et al. (2012b). These authors reported a high detection rate (54 %) in *C. felis* pools collected from the Department of Jutiapa during 2009–2010 (pools can test positive when one or more fleas in the pool is infected; in contrast, we tested fleas individually). Later, the first human case of *R. felis* in Guatemala was reported in a three-year-old boy sampled in 2017 (López et al. 2022). *Rhipicephalus sanguineus* sensu lato has also been documented to harbor *R. felis* in multiple countries such as Mexico (Peniche-Lara et al. 2015), Chile (Abarca et al. 2013), and Brazil (Gehrke et al. 2009). However, there are fewer reports of infections of *R. felis* in *Pulex* spp., which prior to our study was only reported in *Pulex irritans* from the Democratic Republic of the Congo (Sackal et al. 2008), Colombia (Ramírez-Hernández et al. 2013), and the United States (Azad et al. 1997). With multiple vector species on dogs harboring *R. felis*, there could be a risk of infections in humans given the close interactions between humans and dogs. This is important because *C. felis, Pulex* sp. and *R. sanguineus* sensu lato also feed on humans (Dantas-Torres 2010; Ferreira et al. 2020; O'Donnell and Elston 2020), showing the importance of controlling ectoparasites in companion animals as a measure to protect human health.

In addition to *R. felis, Candidatus* R. senegalensis was detected from one *C. felis* sample, the first report in Guatemala. *Candidatus* R. senegalensis was first described from *C. felis* collected from Senegal in 2015 (Mediannikov et al. 2015), and was later detected in Israel (Hornok et al. 2018), Colombia (Betancourt-Ruiz et al. 2020), and in California and South Carolina, US (Reeves et al. 2005; Maina et al. 2016; Mullins et al. 2018). Although its pathogenicity in humans and other animals remains unclear (Maina et al. 2019), it has previously been detected in cat tissue (Mullins et al. 2018), and rickettsiae with similar sequences (98.5 %) were detected in human blood in Senegal (Hornok et al. 2018).

We detected two *Bartonella* species in fleas removed from dogs in Guatemala. In total, six *C. felis* (1.4 %) and one *Pulex* sp. (0.2 %) were positive for *B. henselae*, the causative agent of cat scratch disease. Bai et al. (2015) reported a higher prevalence (22.4 %) of *B. henselae* infection in *C. felis* collected from cats in Guatemala; cats are the primary reservoir for *B. henselae* whereas dogs are less likely to serve as reservoirs for *B. henselae* (Chomel et al. 2014). The detection of *B. vinsonii* subsp. *berkhoffii* (*Bvb*) in one *Pulex* sp. (0.2 %) sample is the first report of *Bvb* in Guatemala. This agent was first isolated from dogs in 1993 (BreitschwerdT et al. 1995) and can cause disease in both humans and dogs (Breitschwerdt et al. 2007; Breitschwerdt et al. 2010). In surveys conducted in Africa, Asia, South America, the seroprevalence of *Bvb* ranged from 3 % to 65 % in dogs (Chomel et al. 2006), which are likely serving as the reservoir of *Bvb* (Breitschwerdt and Kordick 2000). Wild carnivores, such as coyotes (*Canis latrans*), red fox (*Vulpes vulpes*), and raccoon (*Procyon lotor*), may also serve as reservoirs (Schaefer et al. 2011; Bai et al. 2016) where antibodies to *Bvb* were detected in coyotes with a prevalence of 7–51 % across California (Chang et al. 1999) and 71 % in Colorado (Bai et al. 2016). While the vector for *Bvb* remains unknown, *Pulex* fleas collected from dogs in Florida had been reported to harbor *Bvb* (Yore et al. 2014), similar results were also documented in Costa Rica (Rojas et al. 2015) and Italy (Greco et al. 2019). Besides *Pulex* fleas, *Bvb* was also detected in *Ctenocephalides* fleas (Tobar et al. 2020; Zarea et al. 2022), indicating a wide range of potential vectors for *Bvb* and increasing risk of exposure to *Bvb* from the interaction between humans and domestic dogs.

A total of 77 households were surveyed in this study to obtain knowledge of the domiciliary and semi-domiciliary animals, vector presence, and pesticide use. Most of the households have at least one animal, with dogs and chickens being the most common species in and around the households we visited. Poultry is common across Guatemala, being important to the family as a source of income and nutrition, especially in rural areas (Snively-Martinez and Quinlan 2019), while

Table 4
Logistic regression analysis of potential factors of affection ectoparasite infestation on dogs.

Response variables	Explanatory variables	Levels in model	Odd ratio	95 % Confidence interval	*P*-value
Flea presence	Dog number in the household		1.50	0.83–4.69	0.21
	Dog protection	No	Reference		
		Yes	0.73	0.07–4.61	0.75
	Insect management	No	Reference		
		Yes	0.27	0.03–1.41	0.13
Tick presence	Dog number in the household		1.23	0.83–1.91	0.32
	Dog protection	No	Reference		
		Yes	2.48	0.59–12.91	0.23
	Pest management	No	Reference		
		Yes	0.51	0.15–1.61	0.26

dogs are common in households partially for security reasons. The high populations of animals readily provide blood meal resources for multiple arthropod vectors, including ticks and fleas, in high numbers, increasing the contact risk between vectors and humans. Although 88 % of the households that have dogs mention the use of at least one method to protect dogs' health or manage insects, nearly 68 % and 35 % of the examined dogs were infested with fleas and ticks, respectively. These results could be attributed to multiple factors such as incorrect application of pesticides (Beck et al. 2014) and vector resistance development. Therefore, future studies evaluating the ectoparasite management approaches and resistance in the communities should be considered.

5. Conclusions

Flea and tick-borne pathogens circulate among dogs and their ectoparasites in rural Guatemala and the knowledge of these transmission cycles can inform the risk of human exposure. Further research is needed on these vector-borne disease threats and the possibility of dog ectoparasites as sentinels in neglected regions of Central America.

Funding

This study was supported by Fulbright US Scholars Program to SAH, NIH R21AI166446–01, and Texas A&M AgriLife Research.

Availability of data and materials

Parasite voucher specimens are submitted to the Texas A&M University Entomology Collection (TAMUIC-767), Gene sequences are available in Genbank with accession nos. PP940107-09; PP940828-30; PP952311-14.

Ethics approval and consent to participate

The study was reviewed by the Research Ethics Committee of the Center for Health Studies at UVG and was classified as 'Research not involving human subjects' (Protocol No. 270–05–2022) and was approved by the Institutional Animal Care and Use committee of Universidad del Valle de Guatemala (CEUCA – UVG) under protocol number I – 2022 (3)A. Additionally, this study was approved by the Texas A&M University's Institutional Animal Care and Use Committee (IACUC 2022–0001 CA).

CRediT authorship contribution statement

Yuexun Tian: Writing – review & editing, Writing – original draft, Visualization, Formal analysis. **Jose G. Juarez:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation. **Andrea M. Moller-Vasquez:** Writing – review & editing, Investigation, Data curation. **María Granados-Presa:** Writing – review & editing, Investigation, Data curation. **Francisco C. Ferreira:** Writing – review & editing, Methodology, Formal analysis. **Pamela M. Pennington:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Conceptualization. **Norma Padilla:** Writing – review & editing, Resources, Project administration, Investigation. **Gabriel L. Hamer:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Sarah A. Hamer:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

Sarah Hamer reports financial support was provided by Fulbright US Scholar Program. Gabriel Hamer reports financial support was provided by Texas A&M AgriLife Research. Gabriel Hamer reports financial support was provided by National Institutes of Health. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We appreciate the support and hospitality provided by members of the community of Comapa during household visits. We thank Henry Esquivel, Ministry of Health, Jutiapa, Guatemala, for coordinating household visits and assistance with sampling. Lisa Auckland and Isabel McAllister assisted with laboratory testing.

References

Abarca, K., López, J., Acosta-Jamett, G., Martínez-Valdebenito, C, 2013. *Rickettsia felis* in *Rhipicephalus sanguineus* from two distant Chilean cities. Vector Borne Zoonotic Dis. 13 (8), 607–609. https://doi.org/10.1089/vbz.2012.1201.

Alcaino, H.A., Gorman, T.R., Alcaino, R, 2002. Flea species from dogs in three cities of Chile. Vet. Parasitol. 105 (3), 261–265. https://doi.org/10.1016/s0304-4017(01)00626-4.

Alhassan, A., Hove, P., Sharma, B., Matthew-Belmar, V., Karasek, I., Lanza-Perea, M., Werners, A.H., Wilkerson, M.J., Ganta, R.R., 2021. Molecular detection and characterization of *Anaplasma platys* and *Ehrlichia canis* in dogs from the Caribbean. Ticks Tick Borne Dis 12 (4), 101727. https://doi.org/10.1016/j.ttbdis.2021.101727.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215 (3), 403–410.

Alvarez-Hernández, G., Roldán, J.F.G., Milan, N.S.H., Lash, R.R., Behravesh, C.B., Paddock, C.D., 2017. Rocky Mountain spotted fever in Mexico: past, present, and future. Lancet Infect. Dis. 17 (6), E189–E196. https://doi.org/10.1016/S1473-3099(17)30173-1.

Angelakis, E., Raoult, D, 2014. Pathogenicity and treatment of *Bartonella* infections. Int. J. Antimicrob. Agents 44 (1), 16–25. https://doi.org/10.1016/j.ijantimicag.2014.04.006.

Angelakis, E., Mediannikov, O., Parola, P., Raoult, D, 2016. *Rickettsia felis*: the complex journey of an emergent human pathogen. Trends Parasitol 32 (7), 554–564. https://doi.org/10.1016/j.pt.2016.04.009.

Azad, A.F., Radulovic, S., Higgins, J.A., Noden, B.H., Troyer, J.M., 1997. Flea-borne rickettsioses: ecologic considerations. Emerg. Infect. Dis. 3 (3), 319.

Bai, Y., Gilbert, A., Fox, K., Osikowicz, L., Kosoy, M, 2016. *Bartonella Rochalimae* and *B. Vinsonii* Subsp. *berkhoffii* in Wild carnivores from Colorado, USA. J. Wildl. Dis. 52 (4), 844–849. https://doi.org/10.7589/2016-01-015.

Bai, Y., Rizzo, M.F., Alvarez, D., Moran, D., Peruski, L.F., Kosoy, M, 2015. Coexistence of *Bartonella henselae* and *B. clarridgeiae* in populations of cats and their fleas in Guatemala. J. Vector Ecol. 40 (2), 327–332. https://doi.org/10.1111/jvec.12171.

Beati, L., Keirans, J.E., 2001. Analysis of the systematic relationships among ticks of the genera *Rhipicephalus* and *Boophilus* (Acari: ixodidae) based on mitochondrial 12S ribosomal DNA gene sequences and morphological characters. J. Parasitol. 87 (1), 32–48. https://doi.org/10.1645/0022-3395(2001)087[0032:Aotsra]2.0.Co;2.

Beck, S., Schreiber, C., Schein, E., Krücken, J., Baldermann, C., Pachnicke, S., von Samson-Himmelstjerna, G., Kohn, B., 2014. Tick infestation and prophylaxis of dogs in northeastern Germany: a prospective study. Ticks Tick Borne Dis 5 (3), 336–342. https://doi.org/10.1016/j.ttbdis.2013.12.009.

Bermúdez, C.S.E., Troyo, A., 2018. A review of the genus *Rickettsia* in Central America. Res. Rep. Trop. Med. 29 (9), 103–112. https://doi.org/10.2147/RRTM.S160951.

Betancourt-Ruiz, P., Martínez-Díaz, H.-C., Gil-Mora, J., Ospina, C., Olaya-M, L-A, Benavides, E., Bolaños, E., Cuervo, C., Blanton, L., Hidalgo, M, 2020. *Candidatus* Rickettsia senegalensis in cat fleas (Siphonaptera: pulicidae) collected from dogs and cats in Cauca, Colombia. J. Med. Entomol. 57 (2), 382–387. https://doi.org/10.1093/jme/tjz177.

Biggs, H.M., Behravesh, C.B., Bradley, K.K., Dahlgren, F.S., Drexler, N.A., Dumler, J.S., Folk, S.M., Kato, C.Y., Lash, R.R., Levin, M.L., Massung, R.F., Nadelman, R.B., Nicholson, W.L., Paddock, C.D., Pritt, B.S., Traeger, M.S., 2016. Diagnosis and management of tickborne Rrckettsial diseases: rocky Mountain spotted fever and other spotted fever group rickettsioses, ehrlichioses, and anaplasmosis - United States. Morb. Mortal. Weekly Rep. 65 (2), 1–44.

Breitschwerdt, E.B., Kordick, D.L., 2000. *Bartonella* infection in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. Clin. Microbiol. Rev. 13 (3), 428–438. https://doi.org/10.1128/CMR.13.3.428.

BreitschwerdT, E.B., Kordick, D.L., Malarkey, D.E., Keene, B., Hadfield, T.L., Wilson, K, 1995. Endocarditis in a dog due to infection with a novel *Bartonella* subspecies. J. Clin. Microbiol. 33 (1), 154–160. https://doi.org/10.1128/jcm.33.1.154-160.1995.

Breitschwerdt, E.B., Maggi, R.G., Duncan, A.W., Nicholson, W.L., Hegarty, B.C., Woods, C.W., 2007. *Bartonella* species in blood of immunocompetent persons with animal and arthropod contact. Emerg. Infect. Dis. 13 (6), 938–941. https://doi.org/10.3201/eid1306.061337.

Breitschwerdt, E.B., Maggi, R.G., Lantos, P.M., Woods, C.W., Hegarty, B.C., Bradley, J. M., 2010. *Bartonella vinsonii* subsp. *berkhoffii* and *Bartonella henselae* bacteremia in a father and daughter with neurological disease. Parasit Vectors 3 (1), 29. https://doi.org/10.1186/1756-3305-3-29.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. 83 (4), 575–583. https://doi.org/10.2307/3284227.

Bustamante, D.M., De Urioste-Stone, S.M., Juarez, J.G., Pennington, P.M., 2014. Ecological, social and biological risk factors for continued *Trypanosoma cruzi* transmission by *Triatoma dimidiata* in Guatemala. PLoS ONE 9 (8), e104599. https://doi.org/10.1371/journal.pone.0104599.

Castellanos, A.A., Medeiros, M.C., Hamer, G.L., Morrow, M.E., Eubanks, M.D., Teel, P.D., Hamer, S.A., Light, J.E., 2016. Decreased small mammal and on-host tick abundance in association with invasive red imported fire ants (*Solenopsis invicta*). Biol. Lett. 12 (9) https://doi.org/10.1098/rsbl.2016.0463.

CDC, 2003. Pictorial Keys to Arthropods, Reptiles, Birds, and Mammals of Public Health Significance. Department of Health & Human Services, Centers for Disease Control and Prevention.

Chang, C., Yamamoto, K., Chomel, B.B., Kasten, R.W., Simpson, D.C., Smith, C.R., Kramer, V.L., 1999. Seroepidemiology of *Bartonella vinsonii* subsp. *berkhoffii* infection in California coyotes, 1994-1998. Emerg. Infect. Dis. 5 (5), 711–715. https://doi.org/10.3201/eid0505.990514.

Chomel, B.B., Boulouis, H.J., Maruyama, S., Breitschwerdt, E.B., 2006. *Bartonella* spp. in pets and effect on human health. Emerg. Infect. Dis. 12 (3), 389–394. https://doi.org/10.3201/eid1203.050931.

Chomel, B.B., Ermel, R.W., Kasten, R.W., Henn, J.B., Fleischman, D.A., CC, Chang, 2014. Experimental infection of dogs with various *Bartonella* species or subspecies isolated from their natural reservoir. Vet. Microbiol. 168 (1), 169–176. https://doi.org/10.1016/j.vetmic.2013.11.009.

Dantas-Torres, F., 2007. Rocky Mountain spotted fever. Lancet Infect. Dis. 7 (11), 724–732. https://doi.org/10.1016/S1473-3099(07)70261-X.

Dantas-Torres, F., 2010. Biology and ecology of the brown dog tick, *Rhipicephalus sanguineus*. Parasit Vectors 3. https://doi.org/10.1186/1756-3305-3-26.

De Urioste-Stone, S.M., Pennington, P.M., Pellecer, E., Aguilar, T.M., Samayoa, G., Perdomo, H.D., Enriquez, H., Juarez, J.G., 2015. Development of a community-based intervention for the control of Chagas disease based on peridomestic animal management: an eco-bio-social perspective. Trans. R. Soc. Trop. Med. Hyg. 109 (2), 159–167. https://doi.org/10.1093/trstmh/tru202.

Dye-Braumuller, K.C., Rodríguez Aquino, M.S., Self, S.C., Kanyangarara, M., Nolan, M.S, 2022. Spotted fever group rickettsioses in Central America: the research and public health disparity among socioeconomic lines. Insects 13 (8), 674. https://doi.org/10.3390/insects13080674.

El Karkouri, K., Ghigo, E., Raoult, D., Fournier, P.E., 2022. Genomic evolution and adaptation of arthropod-associated *Rickettsia*. Sci. Rep. 12 (1), 3807. https://doi.org/10.1038/s41598-022-07725-z.

Eremeeva, M.E., Berganza, E., Suarez, G., Gobern, L., Dueger, E., Castillo, L., Reyes, L., Wikswo, M.E., Abramowicz, K.F., Dasch, G.A., 2013. Investigation of an outbreak of rickettsial febrile illness in Guatemala, 2007. Int. J. Infect. Dis. 17 (5), e304–e311. https://doi.org/10.1016/j.ijid.2012.11.011.

Escobar, L., Álvarez, D., Villatoro, F., Morán, D., Estévez, A, 2011. Two new flea records from Guatemala: *pulex simulans* and *Echidnophaga gallinacea* (Siphonaptera: pulicidae), and their host-parasite relationship. J Parasitol Vector Biol 3 (3), 40–43.

Estrada-Peña, A., Guglielmone, A.A., Mangold, A.J., 2004. The distribution and ecological'preferences' of the tick *Amblyomma cajennense* (Acari: ixodidae), an ectoparasite of humans and other mammals in the Americas. Ann. Trop. Med. Parasitol. 98 (3), 283–292. https://doi.org/10.1179/000349804225003316.

Fang, R., Blanton, L.S., Walker, D.H., 2017. Rickettsiae as emerging infectious agents. Clin. Lab. Med. 37 (2), 383–400. https://doi.org/10.1016/j.cll.2017.01.009.

Ferreira, F.C., Fonseca, D.M., Hamilton, G., Price, D, 2020. Metagenomic analysis of human-biting cat fleas in urban northeastern United States of America reveals an emerging zoonotic pathogen. Sci. Rep. 10 (1), 15611. https://doi.org/10.1038/s41598-020-72956-x.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3 (5), 294–299.

Gehrke, F., Gazeta, G., Souza, E., Ribeiro, A., Martelli, M., Schumaker, T.T.S., 2009. *Rickettsia rickettsii, Rickettsia felis* and *Rickettsia* sp. TwKM03 infecting *Rhipicephalus sanguineus* and *Ctenocephalides felis* collected from dogs in a Brazilian spotted fever focus in the State of Rio De Janeiro/Brazil. Clin. Microbiol. Infect. 15, 267–268. https://doi.org/10.1111/j.1469-0691.2008.02229.x.

Greco, G., DANTAS, TORRES F, Zarea, A., Lia, R.P., Buonavoglia, C., Tarallo, V.D., Tempesta, M., Otranto, D, 2019. *Bartonella rochalimae* and *Bartonella vinsonii* subsp. *berkhoffii* co-infection in *Pulex irritans* from dogs, Italy. In: 9th International Congres on Bartonella as Emerging Pathogen 9.

Guglielmone, A.A., Estrada-Pena, A., Mangold, A.J., Barros-Battesti, D.M., Labruna, M.B., Martins, J.R., Venzal, J.M., Arzua, M., Keirans, J.E., 2003. *Amblyomma aureolatum* (Pallas, 1772) and *Amblyomma ovale* Koch, 1844 (Acari: ixodidae): hosts, distribution and 16S rDNA sequences. Vet. Parasitol. 113 (3–4), 273–288. https://doi.org/10.1016/s0304-4017(03)00083-9.

Guy, L., Nystedt, B., Toft, C., Zaremba-Niedzwiedzka, K., Berglund, E.C., Granberg, F., Naslund, K., Eriksson, A.S., Andersson, S.G, 2013. A gene transfer agent and a dynamic repertoire of secretion systems hold the keys to the explosive radiation of the emerging pathogen *Bartonella*. PLoS Genet 9 (3), e1003393. https://doi.org/10.1371/journal.pgen.1003393.

Hornok, S., Baneth, G., Grima, A., Takács, N., Kontschán, J., Meli, M.L., Suter, V., Salant, H., Farkas, R., Hofmann-Lehmann, R., 2018. Molecular investigations of cat fleas (*Ctenocephalides felis*) provide the first evidence of *Rickettsia felis* in Malta and *Candidatus* Rickettsia senegalensis in Israel. N. M. N. I. 25, 3–6. https://doi.org/10.1016/j.nmni.2018.05.001.

Juarez, J.G., Pennington, P.M., Bryan, J.P., Klein, R.E., Beard, C.B., Berganza, E., Rizzo, N., Cordon-Rosales, C, 2018. A decade of vector control activities: progress and limitations of Chagas disease prevention in a region of Guatemala with persistent *Triatoma dimidiata* infestation. PLoS Negl. Trop. Dis. 12 (11), e0006896 https://doi.org/10.1371/journal.pntd.0006896.

Keirans, J.E., Litwak, T.R., 1989. Pictorial key to the adults of hard ticks, family Ixodidae (Ixodida: ixodoidea), east of the Mississippi River. J. Med. Entomol. 26 (5), 435–448. https://doi.org/10.1093/jmedent/26.5.435.

Kollars, T.M., Kengluecha, A, 2001. Spotted fever group *Rickettsia* in *Dermacentor variabilis* (Acari: ixodidae) infesting raccoons (Carnivora: procyonidae) and opossums (Marsupialia: didelphimorphidae) in Tennessee. J. Med. Entomol. 38 (4), 601–602. https://doi.org/10.1603/0022-2585-38.4.601.

Labruna, M.B., Whitworth, T., Horta, M.C., Bouyer, D.H., McBride, J.W., Pinter, A., Popov, V., Gennari, S.M., Walker, D.H., 2004. *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the State of São Paulo, Brazil, where Brazilian spotted fever is endemic. J. Clin. Microbiol. 42 (1), 90–98. https://doi.org/10.1128/Jcm.42.1.90-98.2004.

Lawrence, A.L., Brown, G.K., Peters, B., Spielman, D.S., Morin-Adeline, V., Slapeta, J., 2014. High phylogenetic diversity of the cat flea (*Ctenocephalides felis*) at two mitochondrial DNA markers. Med. Vet. Entomol. 28 (3), 330–336. https://doi.org/10.1111/mve.12051.

Legendre, K.P., Macaluso, K.R., 2017. *Rickettsia felis*: a review of transmission mechanisms of an emerging pathogen. Trop. Med. Infect. Dis. 2 (4), 64. https://doi.org/10.3390/tropicalmed2040064.

Lewis, R.E., 1972. Notes on the geographical distribution and host preferences in the order Siphonaptera. 1. Pulicidae. J. Med. Entomol. 9 (6), 511–520. https://doi.org/10.1093/jmedent/9.6.511.

Londono, A.F., Diaz, F.J., Valbuena, G., Gazi, M., Labruna, M.B., Hidalgo, M., Mattar, S., Contreras, V., Rodas, J.D., 2014. Infection of *Amblyomma ovale* by *Rickettsia* sp. strain Atlantic rainforest, Colombia. Ticks Tick Borne Dis 5 (6), 672–675. https://doi.org/10.1016/j.ttbdis.2014.04.018.

López, B., Jaramillo, J., Armstrong, P.A., Lopez, M.R., Gleaton, A.N., Chung, I.H., Kato, C.Y., Reyes, L., Sagastume, M., McCracken10, J.P., 2022. First human case of *Rickettsia felis* reported in Guatemala. Alerta 5 (2), 159–160. https://doi.org/10.5377/alerta.v5i2.14502.

Maina, A.N., Jiang, J., Luce-Fedrow, A., John St., H.K., Farris, C.M., Richards, A.L, 2019. Worldwide presence and features of flea-borne *Rickettsia asembonensis*. Front. Vet. Sci. 5, 334. https://doi.org/10.3389/fvets.2018.00334.

Maina, A.N., Fogarty, C., Krueger, L., Macaluso, K.R., Odhiambo, A., Nguyen, K., Farris, C.M., Luce-Fedrow, A., Bennett, S., Jiang, J., 2016. Rickettsial infections among *Ctenocephalides felis* and host animals during a flea-borne rickettsioses outbreak in Orange County. California. PLoS One 11 (8), e0160604. https://doi.org/10.1371/journal.pone.0160604.

Mawuntu, A.H.P., Johar, E., Anggraeni, R., Feliana, F., Bernadus, J.B.B., Safari, D., Yudhaputri, F.A., Dhenni, R., Dewi, Y.P., Kato, C., Powers, A.M., Rosenberg, R., Soebandrio, A., Myint, K.S.A., 2020. *Rickettsia felis* identified in two fatal cases of acute meningoencephalitis. PLoS Negl. Trop. Dis. 14 (2), e0007893 https://doi.org/10.1371/journal.pntd.0007893.

Mediannikov O., Aubadie-Ladrix M., and Raoult D. 2015. *Candidatus* 'Rickettsia senegalensis' in cat fleas in Senegal. N. M. N. I. 3(24–28. doi:10.1016/j.nmni.2014.10.005.

MSPAS, 2012. Manual Operativo de Vigilancia y Control Entomológico de la Enfermedad de Chagas. Ministerio de Salud Publica y Asistencia Social, Guatemala, p. 36.

Mullins, K.E., Maina, A.N., Krueger, L., Jiang, J., Cummings, R., Drusys, A., Williams, G., Dhillon, M., Richards, A.L., 2018. Rickettsial infections among cats and cat fleas in Riverside County, California. Am. J. Trop. Med. Hyg. 99 (2), 291. https://doi.org/10.4269/ajtmh.17-0706.

Murgas, I.L., Castro, A.M., Bermudez, S.E., 2013. Current status of *Amblyomma ovale* (Acari: ixodidae) in Panama. Ticks Tick Borne Dis 4 (1–2), 164–166. https://doi.org/10.1016/j.ttbdis.2012.09.002.

Nava, S., Mangold, A.J., Guglielmone, A.A., 2008. Aspects of the life cycle of *Amblyomma parvum* (Acari: ixodidae) under natural conditions. Vet. Parasitol. 156 (3–4), 270–276. https://doi.org/10.1016/j.vetpar.2008.05.029.

Nelson, S.P., Bourke, B.P., Badr, R., Tarpey, J., Caicedo-Quiroga, L., Leiva, D., Pott, M., Cruz, A., Chao, C.-C., Achee, N.L, 2022. Ticks (Acari: ixodidae) and associated pathogens collected from domestic animals and vegetation in Stann Creek District, Southeastern Belize, Central America. J. Med. Entomol. 59 (5), 1749. https://doi.org/10.1093/jme/tjac112.

O'Donnell, M., Elston, D.M., 2020. What's eating you? human flea (*Pulex irritans*). Cutis 106 (5), 233–235. https://doi.org/10.12788/cutis.0107.

Ogata, H., Renesto, P., Audic, S., Robert, C., Blanc, G., Fournier, P.E., Parinello, H., Claverie, J.M., Raoult, D, 2005. The genome sequence of *Rickettsia felis* identifies the first putative conjugative plasmid in an obligate intracellular parasite. PLoS Biol 3 (8), e248. https://doi.org/10.1371/journal.pbio.0030248.

Peniche-Lara, G., Jimenez-Delgadillo, B., Dzul-Rosado, K, 2015. *Rickettsia rickettsii* and *Rickettsia felis* infection in *Rhipicephalus sanguineus* ticks and *Ctenocephalides felis* fleas co-existing in a small city in Yucatan, Mexico. J. Vector Ecol. 40 (2), 422–424. https://doi.org/10.1111/jvec.12185.

Ramírez-Hernández, A., Montoya, V., Martínez, A., Pérez, J.E., Mercado, M., De La Ossa, A., Vélez, C., Estrada, G., Correa, M.I., Duque, L, 2013. Molecular detection of *Rickettsia felis* in different flea species from Caldas, Colombia. Am. J. Trop. Med. Hyg. 89 (3), 453. https://doi.org/10.4269/ajtmh.12-0698.

Reeves, W.K., Nelder, M.P., Korecki, J.A., 2005. *Bartonella* and *Rickettsia* in fleas and lice from mammals in South Carolina, USA. J. Vector Ecol. 30 (2), 310.

Ribeiro, C.M., Carvalho, J.L.B., Bastos, P.A.S., Katagiri, S., Batalha, E.Y., Okano, W., Costa, V.M.D, 2021. Prevalence of *Rickettsia rickettsii* in ticks: systematic review and

meta-analysis. Vector Borne Zoonotic Dis. 21 (8), 557–565. https://doi.org/10.1089/vbz.2021.0004.

Rinaldi, L., Spera, G., Musella, V., Carbone, S., Veneziano, V., Iori, A., Cringoli, G, 2007. A survey of fleas on dogs in southern Italy. Vet. Parasitol. 148 (3–4), 375–378. https://doi.org/10.1016/j.vetpar.2007.06.036.

Rojas, N., Troyo, A., Castillo, D., Gutierrez, R., Harrus, S, 2015. Molecular detection of *Bartonella* species in fleas collected from dogs and cats from Costa Rica. Vector Borne Zoonotic Dis. 15 (10), 630–632. https://doi.org/10.1089/vbz.2015.1799.

Sackal, C., Laudisoit, A., Kosoy, M., Massung, R., Eremeeva, M.E., Karpathy, S.E., Van Wyk, K., Gabitzsch, E., Zeidner, N.S., 2008. *Bartonella* spp. and *Rickettsia felis* in fleas, Democratic Republic of Congo. Emerg. Infect. Dis. 14 (12), 1972. https://doi.org/10.3201/eid1412.080610.

Salomon, J., Fernandez Santos, N.A., Zecca, I.B., Estrada-Franco, J.G., Davila, E., Hamer, G.L., Rodriguez Perez, M.A., Hamer, S.A., 2022. Brown dog tick (Rhipicephalus sanguineus Sensu Lato) infection with endosymbiont and human pathogenic Rickettsia spp., in Northeastern Mexico. Int. J. Environ. Res. Public Health 19 (10), 6249. https://doi.org/10.3390/ijerph19106249.

Salomon, J., Leeke, E., Montemayor, H., Durden, C., Auckland, L., Balasubramanian, S., Hamer, G.L., Hamer, S., 2024. On-host flea phenology and flea-borne pathogen surveillance among mammalian wildlife of the pineywoods of East Texas. J. Vector Ecol. In press. https://doi.org/10.52707/1081-1710-49.2.R39.

Schaefer, J.D., Kasten, R.W., Coonan, T.J., Clifford, D.L., Chomel, B.B., 2011. Isolation or detection of *Bartonella vinsonii* subspecies *berkhoffii* and *Bartonella rochalimae* in the endangered island foxes (*Urocyon littoralis*). Vet. Microbiol. 154 (1–2), 135–139. https://doi.org/10.1016/j.vetmic.2011.06.031.

Slapeta, J., King, J., McDonell, D., Malik, R., Homer, D., Hannan, P., Emery, D, 2011. The cat flea (*Ctenocephalides f. felis*) is the dominant flea on domestic dogs and cats in Australian veterinary practices. Vet. Parasitol. 180 (3–4), 383–388. https://doi.org/10.1016/j.vetpar.2011.03.035.

Snively-Martinez, A., Quinlan, M.B, 2019. Family poultry systems on the Southern Pacific Coast of Guatemala: gender, livelihoods, and food security. J Ethnobiol 39 (4), 493–509. https://doi.org/10.2993/0278-0771-39.4.493.

Thibau, A., Hipp, K., Vaca, D.J., Chowdhury, S., Malmström, J., Saragliadis, A., Ballhorn, W., Linke, D., Kempf, V.A., 2022. Long-read sequencing reveals genetic adaptation of *Bartonella adhesin* A among different *Bartonella henselae* isolates. Front. Microbiol. 13, 838267 https://doi.org/10.3389/fmicb.2022.838267.

Tobar, B.Z., Lapsley, W.D., Swain, W.L., Jaffe, D.A., Setien, A.A., Galvez-Romero, G., Obregon-Morales, C., Olave-Leyva, J.I., Chomel, B.B., 2020. *Bartonella* in dogs and fleas from Tulancingo, Hidalgo, Mexico. Med. Vet. Entomol 34 (3), 302–308. https://doi.org/10.1111/mve.12438.

Troyo, A., Calderon-Arguedas, O., Alvarado, G., Vargas-Castro, L.E., Avendano, A, 2012a. Ectoparasites of dogs in home environments on the Caribbean slope of Costa Rica. Rev. Bras. Parasitol. Vet. 21 (2), 179–183. https://doi.org/10.1590/s1984-29612012000200021.

Troyo, A., Álvarez, D., Taylor, L., Abdalla, G., Calderón-Arguedas, Ó., Zambrano, M.L., Dasch, G.A., Lindblade, K., Hun, L., ME, Eremeeva, 2012b. *Rickettsia felis* in *Ctenocephalides felis* from Guatemala and Costa Rica. Am. J. Trop. Med. Hyg. 86 (6), 1054. https://doi.org/10.4269/ajtmh.2012.11-0742.

Tsokana, C.N., Kapna, I., Valiakos, G., 2022. Current data on *Rickettsia felis* occurrence in vectors, human and animal hosts in europe: a scoping review. Microorganisms 10 (12). https://doi.org/10.3390/microorganisms10122491.

Yore, K., DiGangi, B., Brewer, M., Balakrishnan, N., Breitschwerdt, E.B., Lappin, M, 2014. Flea species infesting dogs in Florida and *Bartonella* spp. prevalence rates. Vet. Parasitol. 199 (3–4), 225–229. https://doi.org/10.1016/j.vetpar.2013.10.017.

Zarea, A.A.K., Bezerra-Santos, M.A., Nguyen, V.L., Colella, V., Dantas-Torres, F., Halos, L., Beugnet, F., Tempesta, M., Otranto, D., Greco, G., 2022. Occurrence and bacterial loads of *Bartonella* and haemotropic *Mycoplasma* species in privately owned cats and dogs and their fleas from East and Southeast Asia. Zoonoses Public Health 69 (6), 704–720. https://doi.org/10.1111/zph.12959.

Zeaiter, Z., Fournier, P.E., Raoult, D, 2002. Genomic variation of *Bartonella henselae* strains detected in lymph nodes of patients with cat scratch disease. J. Clin. Microbiol. 40 (3), 1023–1030. https://doi.org/10.1128/JCM.40.3.1023-1030.2002.