DETECTION OF *BORRELIA* AND *EHRLICHIA* IN *RHIPICEPHALUS SANGUINEUS*

ROSA VASQUEZ-ESPINOZA AND DAVID L. BECK*
DEPARTMENT OF BIOLOGY,
TENNESSEE TECHNOLOGICAL UNIVERSITY,
COOKEVILLE, TN, USA

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CORRESPONDING AUTHOR

*David L. Beck
Assistant Professor of Biology
Department of Biology
Tennessee Technological University
Box 5063
Cookeville, TN 38505

Phone – (931) 372-3375
Fax – (931) 372-6257
Email – dbeck@tntech.edu

KEYWORDS

- Borrelia
- Ehrlichia
- *Rhipicephalus sanguineus*
- Lyme disease
- STARI

ABSTRACT

Rhipicephalus sanguineus, the brown dog tick, is endemic throughout the world wherever domestic dogs are present. It has been recently reported by some veterinarians in the city of Laredo, Texas, USA, that Lyme disease, the most common tick-borne disease in the Northern United States, is present in local domestic dogs. Fully engorged *R. sanguineus* ticks were collected and their DNA was purified. The ticks were screened to determine the prevalence of *Borrelia*, *Rickettsia* and *Ehrlichia* species. Sequences related to *Borrelia burgdorferi* in 9.8% (n=11/112), "*Candidatus* Borrelia lonestari" in 16.9% (n=19/112) and *Ehrlichia canis* in 12.5% (n=14/112) were detected by PCR. Sequencing has confirmed the presence of DNA from *Ehrlichia canis* and "*Candidatus* B. lonestari", corroborating that *Borrelia* and *Ehrlichia* are present in domestic dogs in South Texas.

INTRODUCTION

Rhipicephalus sanguineus, known as the brown dog tick, is the most widely distributed tick in the world (13). This tick is a known vector of *Ehrlichia canis* (23), the causative agent of canine ehrlichiosis (1,31). The symptoms of chronic *E. canis* infection in domestic dogs may include thrombocytopenia, anemia, weight loss, bleeding, fever, inflammation of the eye, and anoxic hepatitis (35). Acute ehrlichiosis in domestic dogs may result in loss of appetite, lethargy, shortness of breath, bruises, joint pain and depression (25). *R. sanguineus* is also thought to be a vector of *Rickettsia rickettsii* (13,22), the cause of the Rocky Mountain spotted fever (14). Although *R. sanguineus* ticks typically prefer to feed on domestic dogs, they have been reported to parasitize humans as well (20,33). The presence of a disease agent in the domestic dog population can indicate that the disease could also be present in humans (29).

B. burgdorferi sensu stricto (39) has been identified as the sole etiologic agent of Lyme disease in North America (24,38). Lyme disease is the most common vector-borne illness in the Northern United States (8) and is considered an emerging infectious disease (32). The main vectors are *Ixodes scapularis* (17,18,37) and *Ixodes pacificus* (34). The agent has also been detected at a lower incidence in *R. sanguineus* (9,21,42) and *Amblyomma inornatum* ticks (30). However, the vector potential of these ticks has not yet been characterized. It has been previously reported that *B. burgdorferi* in domestic dogs may result in arthritis, similar to

humans suffering from Lyme disease (27). Symptoms in domestic dogs from the acute form of Lyme disease may include fever, swelling, pain, lameness, lymphadenopathy and malaise (12). Acute renal failure, myocarditis, cardiac arrhythmia, peripheral edema, neurological syndrome and arthritis have been described as clinical signs found in the chronic form of Lyme disease in domestic dogs (2). A Lyme disease-like illness has also been described in the southern United States since the 1980s (41). This condition is referred to as the Southern Tick Associated Rash Illness (STARI), and is thought to be caused by "*Candidatus* B. lonestari" (40) after being bitten by *Amblyomma americanum* (3). The symptoms of "*Candidatus* B. lonestari" infection in domestic dogs have not been determined.

A veterinarian in Laredo, Texas, USA has reported that Lyme disease is present in local domestic dogs (Dr. Sandra Leyendecker, personal communication). *B. burgdorferi* has been previously detected in coyotes in Webb County (7), Texas. However, to the extent of our knowledge it has not been detected in domestic dogs. House pets, including dogs, have an increased exposure to ticks, and can serve as sentinel organisms for some diseases that occur in humans (29). Given the limited information available on vector-borne diseases in South Texas counties, there is a need for tick and pathogen surveillance in the area. This surveillance can help define areas at high risk for transmission (26) of infection to mammals, including humans. Identifying areas at high risk of transmission can increase awareness, potentially leading to the implementation of better diagnosis and prevention methods. In addition, as Laredo is the largest land-based port of entry in the United States, there is the movement of a large number of people and animals to and from this city into the rest of United States (11). For example, truck drivers frequently travel to Laredo, TX with their domestic dogs to warehouses in the city. They may have to wait a day or two before leaving to their destination. The purpose of this study was to determine the prevalence of DNA from tick-borne disease agents in domestic dogs from Laredo, Texas, by investigating the prevalence of *Borrelia*, *Ehrlichia* and *Rickettsia* species in *R. sanguineus* ticks.

MATERIALS AND METHODS

TICK COLLECTION AND IDENTIFICATION

Fully engorged adult *R. sanguineus* ticks were collected at multiple sites in Laredo, Texas. The ticks were collected from the walls of dog kennels, or from a CO_2 trap placed in the Laredo animal shelter. Ticks that were removed while grooming dogs were also collected from animal caregivers/owners. The researchers had no contact with any animal in the study. The ticks were counted and individually examined under the microscope to identify them to the species level using a published key (10).

DNA EXTRACTION

A total of 124 *R. sanguineus* ticks (55 males and 69 females) were used for DNA extraction using the E.Z.N.A. Mollusk DNA Isolation Kits (OMEGA Bio-tek, Norcross, GA, USA). A previously reported protocol was followed and modified as previously described (30). Briefly, the tick was homogenized in 300 µl lysis buffer.

Table 1. Primers and thermal cycler settings used in this study

Primers		
Gene	Name	Sequence (5' → 3')
12S rRNA	85F	TTAAGCTTTTCAGAGGAATTTGCTC
	225R	TTTWWGCTGCACCTTGACTTAA
flaB	FlaLS	AACAGCTGAAGAGCTTGGAATG
	FlaRS	CTTTGATCACTTATCATTCTAATAGC
	BL-Fla522F	GGTACATATTCAGATGCAGACAGAGGG
	BL-Fla1182R	GCACTTGATTTGCTTGTGCAATCATAGCC
	BL-Fla662F	AACTGCTGAAGAGCTTGGAATGC
dsb	BL-Fla860R	AGCTGGTTGAACTCCTTCCTGTTGT
	Ehr-DSB-330F	GATGATGTCTGAAGATATGAAACAAAT
16S rRNA	Ehr-DSB-728R	CTGCTCGTCTATTTTACTTCTTAAAGT
	B16S-FL	GACTCGTCAAGACTGACGCTAAGTC
	B16S-R	GCACACTTAACACGTTAGCTTCGGTACTAA
	BL-16S5F	CAGTGCGTCTTAAGCATGCAAGTCAGACGG
	BL-16S486R	CTGCTGGCACGTAATTAGCCGGGG
	B16S-23S-IGSF	GTATGTTTAGTGAGGGGGGTG
	B16S-23S-IGSR	GGATCATAGCTCAGGTGGTTAG
	B16S-23S-IGSFn	AGGGGGGTGAAGTCGTAACAAG
	B16S-23S-IGSRn	GTCTGATAAACCTGAGGTCGGA
	ECAN-F	ATTTATAGCCTCTGGCTATAGGA
	HE1-F	CAATTGCTTATAACCTTTTGGTTATAAAT
rompA	HE3-R	TATAGGTACCGTCATTATCTTCCCTAT
	Rr190 70P	ATGGCGAATATTTCTCCAAAA
	Rr190 602N	AGTGCAGCATTCGCTCCCCCT

The tick was crushed for 5 minutes using a sterile microtube and pestle. After adding proteinase K, the samples were incubated at 55°C for 3h. The sample purification was then completed following the manufacturer's protocol.

POLYMERASE CHAIN REACTION (PCR)

The samples were screened using PCR for the tick 12S rRNA gene as previously described (30). Samples positive for tick rDNA (n=112) were subjected to PCR for amplification of *Borrelia*, "*Candidatus* B. lonestari", *Ehrlichia* and *Rickettsia* bacteria species (43,44).

Table 1 *(ctd.)*. Primers and thermal cycler settings used in this study

Amplicon length	PCR conditions			
Specificity	Denaturing	Annealing	Extension	Cycles
140 bp	95°C, 30sec	45°C, 30sec	72°C, 1min	40
Not Reported				
353 bp	95°C, 1min	55°C, 1min	72°C, 1min	36
Borrelia genus				
660 bp	95°C, 1min	55°C, 1min	72°C, 1min	46
"Candidatus B. lonestari"				
198 bp	95°C, 1min	55°C, 1min	72°C, 1min	36
"Candidatus B. lonestari"				
398 bp	95°C, 1min	55°C, 1min	72°C, 1min	46
Ehrlichia genus				
131 bp	95°C, 15sec	58°C, 30sec	72°C,30sec	40
Borrelia genus				
481 bp	95°C, 1min	60°C, 1min	72°C, 1min	36
"Candidatus B. lonestari"				
Variable	94°C, 30sec	56°C, 30sec	74°C, 1min	35
Borrelia genus				
Variable	94°C, 30sec	60°C, 30sec	74°C, 1min	40
Borrelia genus				
383 bp	94°C, 30sec	52°C, 30sec	72°C, 1min	36
E. canis				
383 bp	94°C, 30sec	52°C, 30sec	72°C, 1min	36
E. chaffeensis				
Ehrlichia genus	94°C, 30sec	52°C, 30sec	72°C, 1min	36
532 bp	95°C, 1min	55°C, 1min	72°C, 1min	46
Rickettsia genus				

The PCR mixture was a 25 μL reaction volume containing 0.25 μL of GoTaq polymerase (Promega, Madison, WI), 1X GoTaq Buffer, 160 ng/μL bovine serum albumin, 1.0 mM $MgCl_2$, 200 μM of each dNTP, 2 pmol primers, and 5μL of template (1 μL for nested reactions). Amplifications were performed on a Bio-Rad MyCycler thermal cycler (Bio-Rad, Carlsbad, CA) (30). Different thermal cycler settings, as indicated in Table 1, were used for different primers due to different optimal annealing temperatures. An initial denaturation step (95°C) of 5 min. and a final extension step (72°C) of 5 min. were used.

Table 2. Detection of bacterial DNA in adult *R. sanguineus* ticks by PCR

	B. burgdorferi-like species	"*Candidatus* B. lonestari"	*E. canis*	*Rickettsia*	*B. burgdorferi*-like species +"*Candidatus* B. lonestari"	*E. canis* + *B. burgdorferi*-like species
Male	12.2% (6/49)	16.3% (8/49)	8.2% (4/49)	0% (0/49)	2.0%(1/49)	4.1% (2/49)
Female	7.9% (5/63)	17.5%(11/63)	15.9% (10/63)	0% (0/63)	1.6%(1/63)	0% (0/63)
Total	9.8% (11/112)	16.9% (19/112)	12.5% (14/112)	0% (0/112)	1.8% (2/112)	1.8% (2/112)
2005	9/85	16/85	11/85	0/85	2/85	2/85
2006	1/7	1/7	0/7	0/7	0/7	0/7
2009	1/11	2/11	1/11	0/11	0/11	0/11
2010	0/4	0/4	1/4	0/4	0/4	0/4
2011	0/5	0/5	1/5	0/5	0/5	0/5
Total	11 of 112	19 of 112	14 of 112	0 of 112	2 of 112	2 of 112

Nested PCR procedures were performed for *Borrelia* and "*Candidatus* B. lonestari" using 1µl from the initial reaction as a template. Amplification of target sequences was performed in a Bio-Rad MyCycler thermal cycler (Bio-Rad, Carlsbad, CA) with several denaturing, annealing, and extension times and temperatures (Table 1). For each PCR assay, 5µL of sterile distilled water was used instead of template DNA as a negative control. The positive controls, when used, were added using separate hoods and pipettors to reduce the risk of cross contamination.

VISUALIZATION AND SEQUENCING OF PCR PRODUCTS

Five microliters of each PCR reaction was subjected to gel electrophoresis, using 2% agarose gels stained with ethidium bromide in 0.5X TBE (45 mM Tris, 45 mM boric acid, 1 mM disodium ethylene diamine tetraacetic acid) with 0.00005% ethidium bromide. The gels were run at 100 V for 40min.. After electrophoresis, the gels were examined under UV light. Positive samples were purified using SpinPrep PCR Clean-UP Kits (Novagen, La Jolla CA, USA) following the manufacturer's protocols. Each purified PCR product was sent for Sanger sequencing at Eurofins (Alabama, USA) or MCLab (San Francisco, CA) using the primers used for PCR.

NUCLEOTIDE SEQUENCE ACCESSION NUMBERS

The DNA sequences were visualized using Finch TV, Geospiza, Inc software version 1.5.0 and compared to reported sequences in the NCBI GenBank using BLAST. The assigned GenBank accession numbers are: KR183798-KR183823.

PHYLOGENETIC ANALYSIS

Initial alignments for *Borrelia* and *Ehrlichia* genes were executed using the MUltiple Sequence Comparison by Log-Expectation (MUSCLE) (16) as performed by the European Bioinformatics Institute's

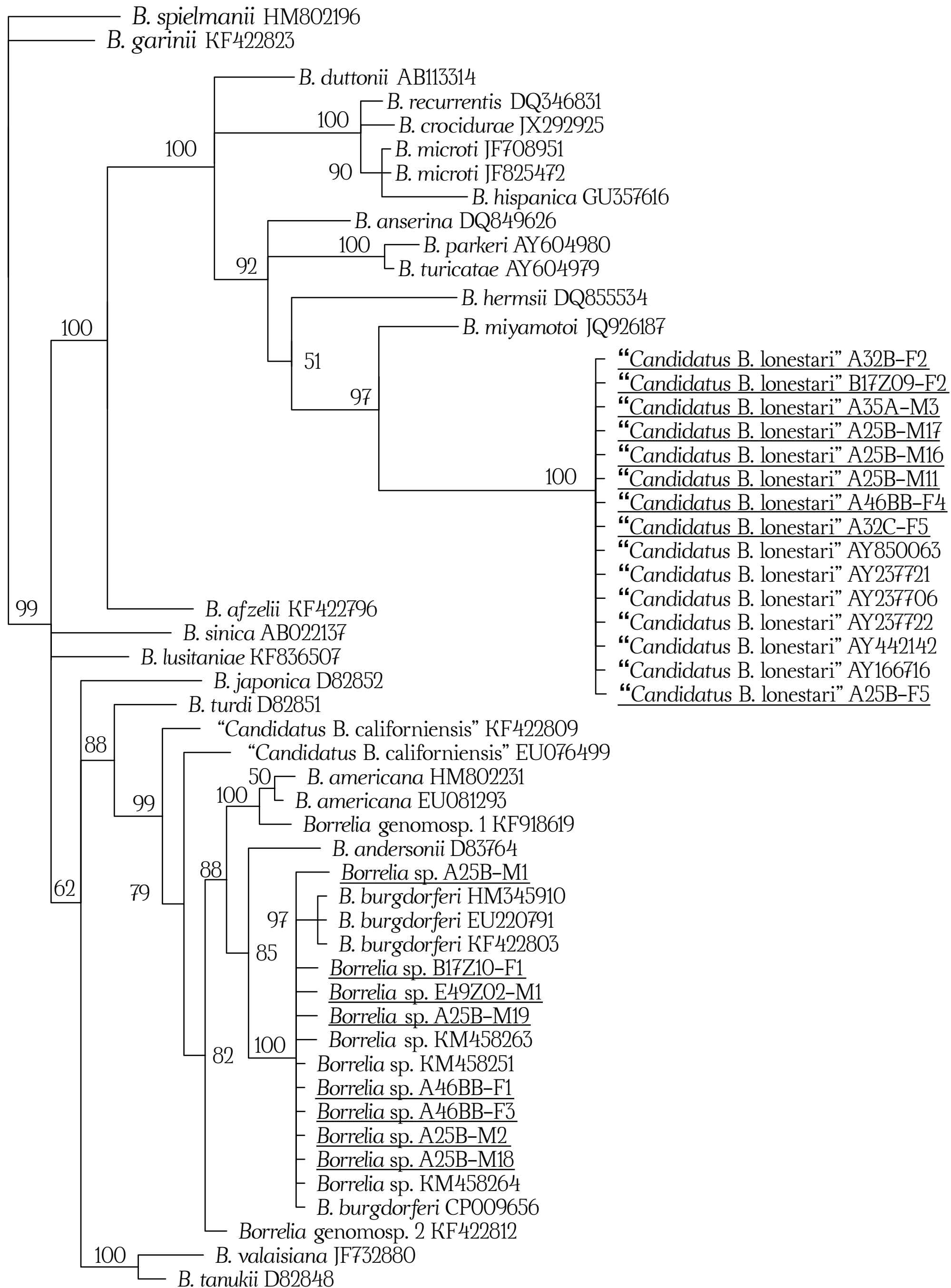
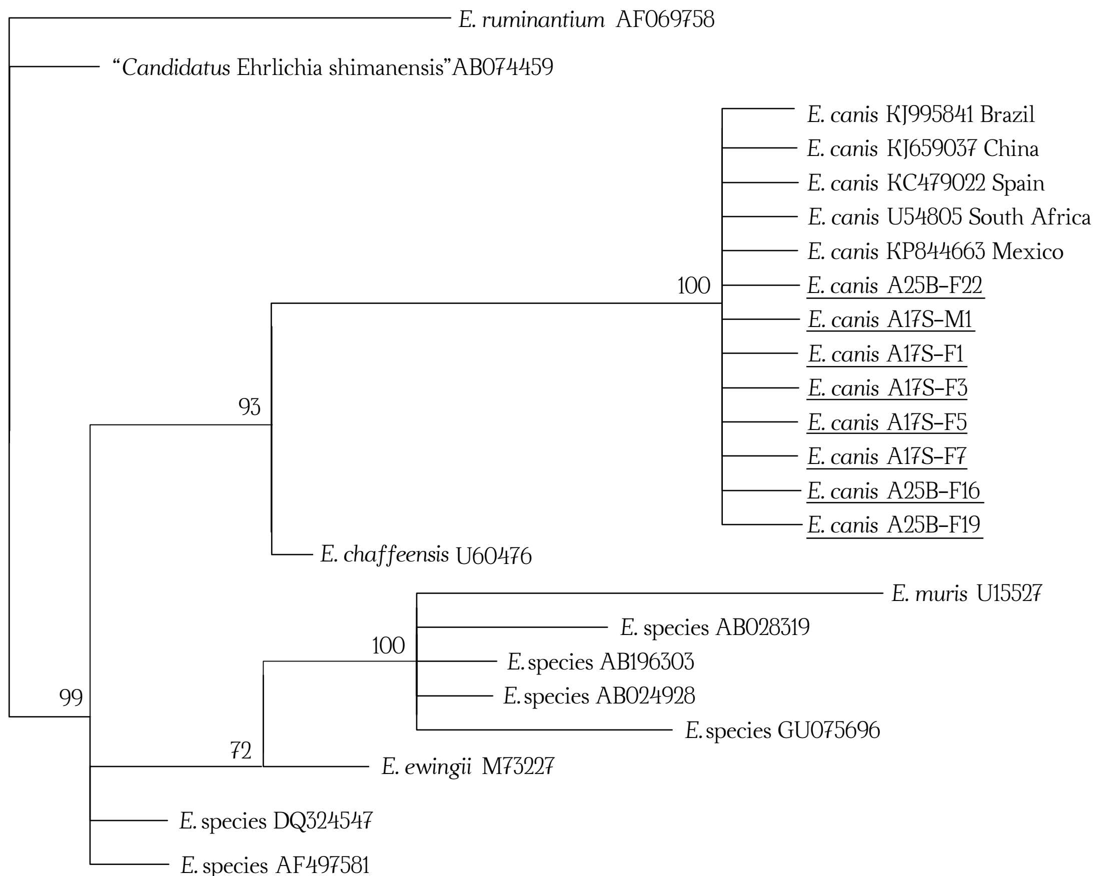


Fig. 1. Baysian inference consensus tree inferred from *flaB* of *Borrelia* species. Node support is indicated by the posterior probabilities at the node. The name of the species is followed by the GenBank accession number. A25B-F5, A25B-M1, A25B-M2, A25B-M11, A25B-M16, A25B-M17, A25B-M18, A25B-M19, A32B-F2, A32C-F5, A35A-M3, A46BB-F1, A46BB-F3, A46BB-F4, B17Z09-F2, B17Z10-F1 and E49Z02-M1 represent amplicons from *R. sanguineus* and are underlined. The scale bar indicates the mean number of nucleotide substitutions per site.

Fig. 2. Baysian inference consensus tree inferred from 16S rDNA of *Erhlichia* species. Node support is indicated by the posterior probabilities at the node. The name of the species is followed by the GenBank accession number. A17S-M1, A17S-F1, A17S-F3, A17S-F5, A17S-F7, A25B-F16, A25B-F19 and A25B-F22 represent amplicons from R. sanguineus and are underlined. The scale bar indicates the mean number of nucleotide substitutions per site.



MUSCLE server (http://www.ebi.ac.uk/Tools/muscle/). Default settings were used, with posterior manual adjustments if needed. Bayesian inference phylogenetic analyses were performed using MrBayes v.3.2.5 (36) using two runs, for 10,000,000 generations each, using eight chains and a temperature coefficient of 0.1, and trees sampled every 5,000 generations. Determination of the appropriate model for each genus was completed via jModelTest 2 (15): GTR + I + Γ for *Borrelia* and *Ehrlichia*. The gamma distribution included six categories for all models obtained. After analysis was completed, the first 25% of trees from each run were discarded as burnin. The consensus trees were observed in FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

RESULTS

PCR DETECTION OF *BORRELIA* AND *EHRLICHIA*

Of the 112 positive samples for tick rDNA, 44 ticks (18 males and 26 females) were positive for *B. burgdorferi*-like species, "*Candidatus* B. lonestari" or *Ehrlichia* bacteria species (Table 2). "*Candidatus* B. lonestari", the most commonly detected tick-borne pathogen, was detected in 16.9% (n=19, 16.3% of males (8/49) and 17.5% (11/63) of females) of all ticks.

B. burgdorferi-like species were detected in 9.8% (n=11, 12.2% (6/49) of males and 7.9% (5/63) of females) of all ticks. *E. canis* was detected in 12.5% (n= 14, 8.2% (4/49) of males and 15.9% (10/63) of females) of all samples. Two male ticks (4.1%, n=2 of 49) were positive for both *E. canis* and *B. burgdorferi*-like species, indicating a 1.8% co-infection rate (2 of 112) of all ticks. One male and one female tick (male: 2.0%, n=1 of 49; female: 1.6%, n=1 of 63) were positive for both "*Candidatus* B. lonestari" and *B. burgdorferi*-like species, indicating a 1.8% co-infection rate (2 of 112) of all ticks. No tick was positive for the spotted fever group rickettsial *rompA* gene.

PHYLOGENETIC ANALYSIS

Samples with strong bands by gel electrophoresis were prepared for sequencing. Sequencing confirmed the PCR results. A Bayesian inference tree including these sequences and other published sequences are shown in Figs. 1 and 2. Phylogenetic analysis showed that "*Candidatus* B. lonestari" amplicons were in the same clade as known sequences of "*Candidatus* B. lonestari" and *B. burgdorferi*-like species amplicons clustered with *B. burgdorferi* species complex (Fig. 1). "*Candidatus* B. lonestari" sequences were all identical except A25B-F5, which was polymorphic at one position. Three of the *B. burgdorferi*-like species sequences (B17Z10-F1, A25B-M1 and E49Z02-M1) were different at one position. *Ehrlichia* amplicons were in the same clade as known sequences of *E. canis* (Fig. 2).

DISCUSSION

The ticks used for this study were fully engorged. Thus, the detection of pathogen DNA either represents the most recent blood meal or potentially a prior infection of the tick. We detected the presence of *B. burgdorferi*-like species, "*Candidatus* B. lonestari" and *E. canis* DNA in *R. sanguineus* ticks from Laredo, Texas. Our study does not address the issue of vector competency of *R. sanguineus* ticks in regard to *B. burgdorferi*-like species and "*Candidatus* B. lonestari".

The detection of pathogens in canines can indicate a potential risk for infection of humans (28,29). We have detected *B. burgdorferi*-like species DNA in 9.8% of ticks collected from the local animal shelter, as well as ticks submitted by pet caregivers. The main vector for *B. burgdorferi* in the Northeastern United States is *I. scapularis* (17,18,37). This tick is present throughout much of Texas and Northern Mexico. *B. burgdorferi* was previously detected in 45% of tested *I. scapularis* ticks (19). However, in Webb County TX, no *I. scapularis* were identified in the combined collection of over 70,000 ticks (5). *B. burgdorferi*-like

species have been previously reported in *A. inornatum* from Webb County (30) and *A. mixtum* from Northeastern Mexico (21). We also detected "*Candidatus* B. lonestari", which is thought to be the cause of STARI (40), in 16.9% of ticks. Cohen *et al.* (1990) reported a 5.5% seroprevalence for *Borrelia* in domestic dogs from Texas. However, of the dogs in their study 0 of 5 dogs that came from Webb County were seropositive (9). Likewise, Bowman *et al.* (2009) reported *B. burgdorferi* in Central and Northern Texas, but did not have any results for South Texas. This is the first report of *Borrelia* from *R. sanguineus* ticks and domestic dogs in Webb County, Texas.

R. sanguineus is the only known vector of *E. canis* (23), and is widely distributed throughout the United States (6). We detected *E. canis* in 12.5% of ticks. However, all of the ticks that were positive for *E. canis* were collected from the Laredo animal shelter. Many pet owners acquire their pet from the animal shelter. These dogs are at a high risk for acquiring *E. canis* at this location. However, domestic dogs in the city of Laredo appear to be at a low risk for acquiring *E. canis*. The previously reported seroprevalence for *E. canis* (2.0%) was higher in Texas than in much of the United States (4). We did not detect *E. chaffeensis* and *E. ewingii* in *R. sanguineus*, even though they are present in the area (30). We also did not detect any spotted fever group *Rickettsia*. This would suggest that any spotted fever group *Rickettsia* are either absent from the area or were present at a very low prevalence.

Further research on *R. sanguineus* distribution and the prevalence of *B. burgdorferi*-like species, "*Candidatus* B. lonestari" and *Ehrlichia* species in South Texas is needed. Further research will help elucidate if *R. sanguineus* is a vector of a *B. burgdorferi*-like species. This additional research would allow for better and more accurate diagnosis of tick-borne illnesses, ultimately leading to better treatment and health care for domestic dogs and humans. This research supports the observation of Dr. Sandra Leyendecker and suggests that domestic dogs should be screened for Lyme disease if they present with appropriate symptoms.

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REFERENCES

1. Aguiar, D. M., Cavalcante, G. T., Pinter, A., Gennari, S. M., Camargo, L. M. A., & Labruna, M. B. 2007. Prevalence of *Ehrlichia canis* (Rickettsiales: Anaplasmataceae) in dogs and *Rhipicephalus sanguineus* (Acari: Ixodidae) ticks from Brazil. *J. Med. Entomol.* 44: 126-132. doi:10.1093/jmedent/41.5.126
2. Appel, M. J., Allan, S., Jacobson, R. H., Lauderdale, T. L., Chang, Y. F., Shin, S. J., Thomford, J. W., Todhunter, R. J., & Summers, B. A. 1993. Experimental Lyme disease in dogs produces arthritis and persistent infection. *J. Infect. Dis.* 167: 651-654.

3. Bacon, R. M., Gilmore, R. D., Quintana, M., Piesman, J., & Johnson, B. J. 2003. DNA evidence of *Borrelia lonestari* in *Amblyomma americanum* (Acari: Ixodidae) in southeast Missouri. *J. Med. Entomol.* 40: 590-592. doi:10.1603/0022-2585-40.4.590

4. Beall, M. J., Alleman, A. R., Breitschwerdt, E. B., Cohn, L. A., Couto, C. G., Dryden, M. W., Guptill, L.C., Iazbik, C., Kania, S. A., Lathan, P., Little, S. E., Roy, A., Sayler, K. A., Stillman, B. A., Welles, E. G., Wolfson, W., & Yabsley, M. J. 2012. Seroprevalence *of Ehrlichia canis, Ehrlichia chaffeensis* and *Ehrlichia ewingii* in dogs in North America. *Parasit. Vectors.* 5: 29.

5. Beck, D.L., Zavala, J., Montalvo, E.O., & Quintana, F.G. 2011. Meteorological Indicators for *Amblyomma cajennense* Population Dynamics in the Tamaulipan Biotic Province in Texas. *J. Vector Ecol.* 36: 135-146.

6. Bowman, D., Little, S. E., Lorentzen, L., Shields, J., Sullivan, M. P., & Carlin, E. P. 2009. Prevalence and geographic distribution of *Dirofilaria immitis, Borrelia burgdorferi, Ehrlichia canis,* and *Anaplasma phagocytophilum* in dogs in the United States: results of a national clinic-based serologic survey. *Vet. Parasitol.* 160: 138-148.

7. Burgess, E. C., & Windberg, L. A. 1989. *Borrelia* sp. infection in coyotes, black-tailed jack rabbits and desert cottontails in southern Texas. *J. Wildlife Dis.* 25: 47-51.

8. Centers for Disease Control and Prevention. 2014. Summary of notifiable diseases, United States, 2012. *MMWR Morb. Mortal. Wkly. Rep.*, 61 (No. 53): 25.

9. Cohen, N. D., Carter, C. N., Thomas Jr, M. A., Angulo, A. B., & Eugster, A. K. 1990. Clinical and epizootiologic characteristics of dogs seropositive for *Borrelia burgdorferi* in Texas: 110 cases (1988). *J. Am. Vet. Med. Assoc.* 197: 893-898. PMID:2228777

10. Cooley, R.A. 1946. The genera *Boophilus, Rhipicephalus* and *Haemaphysalis* (Ixodidae) of the New World. Natl. Inst. Health Bull. 187:1-54.

11. Curtis, J. R. 1993. Central Business Districts of The Two Laredos. *Geographical Review.* 83: 54-65

12. Dambach, D. M., Smith, C. A., Lewis, R. M., & Van Winkle, T. J. 1997. Morphologic, immunohistochemical, and ultrastructural characterization of a distinctive renal lesion in dogs putatively associated with *Borrelia burgdorferi* infection: 49 cases (1987-1992). *Vet. Pathol. Online.* 34: 85-96.

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

14. Dantas-Torres, F., Chomel, B. B., & Otranto, D. 2012. Ticks and tick-borne diseases: a One Health perspective. *Trends Parasitol.* 28: 437-446. doi:10.1016/j.pt.2012.07.003

15. Darriba, D., Taboada, G.L., Doalla, R. & Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods.* 9: 772.

16. Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32: 1792-1797

17. Falco, R. C. & Fish, D. 1988. Ticks Parasitizing Humans in a Lyme Disease Endemic Area of Southern New York State. *Am. J. Epidemiol.* 128: 1146-1152.

18. Felz, M. W., Durden, L. A., & Oliver Jr, J. H. 1996. Ticks Parasitizing Humans in Georgia and South Carolina. *J. Parasitol.* 505-508.

19. Feria-Arroyo, T. P., Castro-Arellano, I., Gordillo-Perez, G., Cavazos, A. L., Vargas-Sandoval, M., Grover, A., Torres, J., Medina, R., Perez de León, A. A. & Esteve-Gassent, M. D. 2014. Implications of climate change on the distribution of the tick vector *Ixodes scapularis* and risk for Lyme disease in the Texas-Mexico transboundary region. *Parasit. Vectors.* 7:1, 199.

20. Goddard, J. 1989. Focus of Human Parasitism by the Brown Dog Tick, *Rhipicephalus sanguineus* (Acari: Ixodidae). *J. Med. Entomol.* 26: 628-631. doi:10.1093/jmedent/26.6.628

21. Gordillo-Pérez, G., Vargas, M., Solórzano-Santos, F., Rivera, A., Polaco, O. J., Alvarado, L. & Torres, J. 2009. Demonstration of *Borrelia burgdorferi* sensu stricto infection in ticks from the northeast of Mexico. *Clin. Microbiol. Infect.* 15: 496-498. doi:10.1111/j.1469-0691.2009.02776.x

22. Gray, J., Dantas-Torres, F., Estrada-Peña, A., & Levin, M. 2013. Systematics and ecology of the brown dog tick, *Rhipicephalus sanguineus. Ticks Tick-Borne Dis.* 4: 171-180. doi:10.1016/j.ttbdis.2012.12.003

23. Groves, M. G., Dennis, G. L., Amyx, H. L., & Huxsoll, D. L. 1975. Transmission of Ehrlichia canis to dogs by ticks (*Rhipicephalus sanguineus*). *Am. J. Vet. Res.* 36:7, 937-940. PMID:1147359

24. Heymann, W. R., & Ellis, D. L. 2012. *Borrelia burgdorferi* infections in the United States. *J. Clin. Aesthetic Derm.* 5: 18-28. PMCID: PMC3424593

25. Hildebrandt, P. K., Huxsoll, D. L., Walker, J. S., Nims, R. M., Taylor, R., & Andrews, M. 1973. Pathology of canine ehrlichiosis (tropical canine pancytopenia). *Am. J. Vet. Res.* 34: 1309-1320.

26. Jongejan, F., & Uilenberg, G. 2004. The Global Importance of Ticks. *Parasitology.* 129: S3-S14. doi:10.1017/S0031182004005967

27. Kornblatt, A. N., Urband, P. H., & Steere, A. C. 1985. Arthritis caused by Borrelia burgdorferi in dogs. *J. Am. Vet. Med. Assoc.* 186: 960-964.

28. Lemon, S. M., Sparling, P. F., Hamburg, M. A., Relman, D. A., Choffnes, E. R., Mack, A., & Sparling, F. 2008. Vector-borne diseases: understanding the environmental, human health, and ecological connections. Workshop summary. National Academies Press. ISBN:0-309-10897-7

29. Mead P., Goel R. & Kugeler K. 2011. Canine Serology as Adjunct to Human Lyme Disease Surveillance. *Emerg Infect Dis.* 17: 1710-1712. doi: http://dx.doi.org/10.3201/1709.110210

30. Medlin, J.S., Cohen, J.I. & Beck, D.L. 2015. Vector potential and population dynamics for *Amblyomma inornatum*. *Ticks Tick-Born Dis.* 6:463-472. doi:10.1016/j.ttbdis.2015.03.014

31. Ndip, L. M., Ndip, R. N., Ndive, V. E., Awuh, J. A., Walker, D. H., & McBride, J. W. 2007. Ehrlichia species in *Rhipicephalus sanguineus* ticks in Cameroon. *Vectorborne Zoonotic Dis.* 7: 221-227. doi:10.1089/vbz.2006.0608

32. Parola, P., & Raoult, D. 2001. Ticks and Tickborne Bacterial Diseases in Humans: an Emerging Infectious Threat. *Clin. Infect. Dis.* 32: 897-928. doi: 10.1086/319347

33. Perez, M., Bodor, M., Zhang, C., Xiong, Q., & Rikihisa, Y. 2006. Human infection with Ehrlichia canis accompanied by clinical signs in Venezuela. *Ann. Ny. Acad. Sci.* 1078: 110-117. doi/10.1196/annals.1374.016

34. Piesman, J. & Sinsky, R. J. 1988. Ability of *Ixodes scapularis*, *Dermacentor variabilis*, and *Amblyomma americanum* (Acari: Ixodidae) to acquire, maintain, and transmit Lyme disease spirochetes (*Borrelia burgdorferi*). *J. Med. Entomol.* 25: 336-339. doi:10.1093/jmedent/25.5.336

35. Reardon, M. J., & Pierce, K. R. 1981. Acute experimental canine ehrlichiosis I. Sequential reaction of the hemic and lymphoreticular systems. *Vet. Pathol. Online.* 18: 48-61.

36. Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinform.* 19: 1572-1574

37. Stafford, K. C., Cartter, M. L., Magnarelli, L. A., Ertel, S. H., & Mshar, P. A. 1998. Temporal Correlations between Tick Abundance and Prevalence of Ticks Infected with *Borrelia burgdorferi* and Increasing Incidence of Lyme Disease. *J. Clin. Microbiol.* 36: 1240-1244.

38. Steere, A. C., Coburn, J., & Glickstein, L. 2004. The emergence of Lyme disease. *J. Clin. Inv.* 113: 1093-1101. doi:10.1172/JC121681

39. Steere, A. C., & Sikand, V. K. 2003. The Presenting Manifestations of Lyme disease and the Outcomes of Treatment. *New England J. Med.* 348: 2472-2474. doi:10.1056/NEJM200306123482423

40. Stromdahl, E. Y., Williamson, P. C., Kollars, T. M., Evans, S. R., Barry, R. K., Vince, M. A., & Dobbs, N. A. 2003. Evidence of *Borrelia lonestari* DNA in *Amblyomma americanum* (Acari: Ixodidae) Removed From Humans. *J. Clin. Microbiol.* 41: 5557-5562. doi: 10.1128/JCM.41.12.5557-5562.2003

41. Varela, A. S., Luttrell, M. P., Howerth, E. W., Moore, V. A., Davidson, W. R., Stallknecht, D. E., & Little, S. E. 2004. First Culture Isolation of *Borrelia lonestari*, Putative Agent of Southern Tick-Associated Rash Illness. *J. Clin. Microbiol.* 42: 1163-1169. doi: 10.1128/JCM.42.3.1163-1169.2004

42. Whitney, M. S., Schwan, T. G., Sultemeier, K. B., McDonald, P. S., & Brillhart, M. N. 2007. Spirochetemia caused by *Borrelia turicatae* infection in 3 dogs in Texas. *Vet. Clin. Path.* 36: 212-216. doi: 10.1111/j.1939-165X.2007.tb00213.x

43. Wilhelmsson, P., Fryland, L., Börjesson, S., Nordgren, J., Bergström, S., Ernerudh, J., Forsberg, P., & Lindgren, P.E. 2010. Prevalence and diversity of *Borrelia* species in ticks that have bitten humans in Sweden. *J. Clin. Microbiol.* 48: 4169-4176. doi: 10.1128/JCM.01061-10

44. Williamson, P. C., Billingsley, P. M., Teltow, G. J., Seals, J. P., Turnbough, M. A., & Atkinson, S. F. 2010. *Borrelia*, *Ehrlichia*, and *Rickettsia* in ticks removed from persons, Texas, USA. *Emerg. Infect. Dis.* 16:3, 441-446. doi: 10.3201/eid1603.091333