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Molecular prevalence and ecoregion distribution of select tick-borne pathogens in Texas dogs

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Summary

Tick-borne diseases (TBD), caused by borrelial, rickettsial and babesial pathogens, are common across the United States and can cause severe clinical disease in susceptible hosts, such as domestic dogs. However, there are limited TBD molecular epidemiological reports for dogs in Texas, and none for the non-Lyme borrelial pathogen responsible for causing tick-borne relapsing fever (TBRF). Therefore, data to support the prevalence of TBRF in the canine population is inadequate. This study aimed to characterize the molecular prevalence of 11 causative agents responsible for three TBD groups within domestic dogs with an emphasis on pathogen distribution within Texas ecoregions. A total representative number of 1,171 whole-blood samples were collected opportunistically from two Texas veterinary diagnostic laboratories. A layerplex real-time PCR assay was utilized to screen the dog samples for all 11 pathogens simultaneously. The overall molecular infection prevalence of disease was 0.68% borrelial, 1.8% rickettsial and 0.43% babesial pathogens, for a TBD total of 2.73% across Texas. Higher molecular prevalence was observed when analysed by ecoregion distinction, including 5.78% rickettsial infections by *Ehrlichia canis* and *Anaplasma platys* in the Rolling Plains ecoregion, and an average of 1.1% *Borrelia turicatae* and 1.0% *Babesia gibsoni* across detected ecoregions. To our knowledge, our findings indicate the first molecular detection of *A. platys* in Texas, and the first report of coinfections with *E. canis* and *A. platys* in dogs of Texas. The zoonotic concerns associated with TBDs, in conjunction with dogs' implication as an effective sentinel for human disease, highlight the importance of characterizing and monitoring regions associated with active infections in dogs. Surveillance data obtained from this study may aid public health agencies in updating maps depicting high-risk areas of disease and developing preventative measures for the affected areas.

KEYWORDS

coinfection, prevalence, real-time polymerase chain reaction, Zoonoses

1 | INTRODUCTION

Due to the increased resistance of ticks to acaricides (Coles & Dryden, 2014), ease of travel, and the continuous geographical expansion of ticks (Donaldson et al., 2016; Eisen, Eisen, & Beard, 2016; Schurer, Ndao, Quewezance, Elmore, & Jenkins, 2014), dogs (*canis lupus familiaris*) are at continuous risk for tick-borne diseases (TBD) in the United States (Chomel, 2011; Fritz, 2009). The groups consisting of borrelial (*Borrelia turicatae*, *B. hermsii*, *B. parkeri*, *B. burgdorferi*), rickettsial (*Ehrlichia canis*, *E. chaffeensis*, *E. ewingii*, *Anaplasma phagocytophilum*, *Rickettsia rickettsii*), and babesial (*Babesia gibsoni*, *B. canis*) pathogens have been documented as the most common causes of TBDs in dogs (Chomel, 2011; Esteve-Gasent, Snell, Adetunji, & Piccione, 2017, Sudhakara Reddy, Sivajothi, Reddy, & Raju, 2016). Primary tick vectors responsible for transmitting these pathogens vary across disease groups and even at the pathogens' genus level, but are contained within the families of Ixodidae (hard ticks) and Argasidae (soft ticks) (Dantas-Torres, Chomel, & Otranto, 2012). Wildlife are generally considered appropriate reservoir hosts for the majority of these ticks and vectored pathogens, though dogs and humans can also act as incidental hosts and manifest disease if exposed (Dantas-Torres et al., 2012; Lopez, Krishnavahjala, Garcia, & Bermudez, 2016). Consequently, dogs are implicated as effective sentinels for human TBDs and may indicate geographical areas of increased zoonotic risk (Abdullah, Helps, Tasker, Newbury, & Wall, 2018; Esteve-Gasent et al., 2017; Mead, Goel, & Kugeler, 2011).

Few molecular prevalence studies concerning TBDs in dogs have been conducted in the U.S., including limited surveillance in dogs residing in Minnesota (Beall et al., 2008) and Oklahoma (Little et al., 2010), but none within Texas. Instead, the majority of TBD prevalence studies in the U.S. have been limited to molecular detection in humans (Harris et al., 2016; Heitman, Dahlgren, Drexler, Massung, & Behravesh, 2016), or serological analyses in dogs (Beall et al., 2012; Bowman et al., 2009; Esteve-Gasent et al., 2017; Little et al., 2010, 2014; Qurollo et al., 2014). The consensus from these reports indicated an approximate TBD seroprevalence of 2% across Texas. In addition, over the last 5 years IDEXX laboratories have serologically documented 11,406 cases of ehrlichiosis, 5,040 of anaplasmosis, and 2,705 of Lyme disease in dogs from the state of Texas (http://www.dogsandticks.com). This information alone is impressive, but does not include any data on canine babesiosis, which has been recently reported in Texas dogs (Cannon et al., 2016). Therefore, although there is seroprevalence documentation of TBDs in Texas dogs, little is known about the prevalence of actively infected dogs.

The aim of this study was to expand epidemiological data of TBDs in Texas dogs by evaluating the molecular prevalence of their respective causative agents with an emphasis on ecoregion distribution in Texas. Molecular screening, in contrast with serological screening, may identify active infections and indicate specific ecoregions containing sentinels of disease. Ecologists commonly delineate Texas into 10 natural ecological regions, primarily based on unique plant communities as a result of differing climate, soil and weather conditions (Gould, Hoffman, & Rechenthin, 1960). This study may reveal a unique association of TBDs within subsequent ecosystems. Furthermore, among all U.S. states Texas shares the most significant amount of land bordering Mexico. This transboundary region consists of 1,254 miles of common border per the Texas Department of Transportation (https://www.txdot.gov/inside-txdot.html). It should be noted that many zoonotic canine tick-borne pathogens are circulating within Mexico and may spillover to Texas dogs (Esteve-Gassent et al., 2014). To that end, 1,171 whole-blood dog samples were collected opportunistically from two Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL) locations (i.e. Amarillo and College Station) and screened for the presence of tick-borne pathogens.

This study was designed in order to estimate the molecular prevalence of TBDs in the general population of domestic dogs of Texas. Conducting a molecular prevalence study of TBDs may provide updated rates of active exposure and indicate specific ecoregions that may contain sentinels of disease. To our knowledge, this is the first study of its kind in Texas and can provide baseline data for future research and public health surveillance programs.

2 | MATERIALS AND METHODS

2.1 | Study area and samples

Between June 2016 and February 2018, a total number of 1,171 EDTA whole-blood samples were collected from domesticated dogs submitted to the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL). All blood samples were submitted initially to the TVMDL for complete blood count (CBC) analysis, and then transferred to the College of Veterinary Medicine & Biomedical Sciences at Texas A&M University after the 15-day legal hold period, in accordance with the Material Transfer Agreement between both institutions. No confidential information regarding the pet owners and/or veterinary clinic where the animals were evaluated was provided. No recruitment of animals for the study was performed, and the research team did no direct handling of animals.

Blood samples were collected from dogs of different ages, breed, sex, and health states, and originated from ten ecoregions of Texas: Blackland Prairie, Cross Timbers, Edwards Plateau, Gulf Prairies, High Plains, Piney Woods, Post Oak Savanah, Rolling Plains, South Texas Plains, and Trans-Pecos (Gould et al., 1960). A total of 121 samples from each ecoregion, with the exception of 82 from the Trans-Pecos ecoregion due to limited availability, were collected in order to estimate true prevalence of disease. The sample set number was calculated assuming a TBD prevalence rate of 2% (seroprevalence) in Texas at a confidence interval of 95% (Beall et al., 2012; Bowman et al., 2009; Esteve-Gasent et al., 2017; Humphry, Cameron, & Gunn, 2004; Little et al., 2010, 2014; Qurollo et al., 2014). Blood samples were collected opportunistically from two TVMDL clinical pathology departments located in College Station, TX (n = 960) and Amarillo, TX (n = 211). Figure 1 shows in grey the counties from which samples were collected and tested.

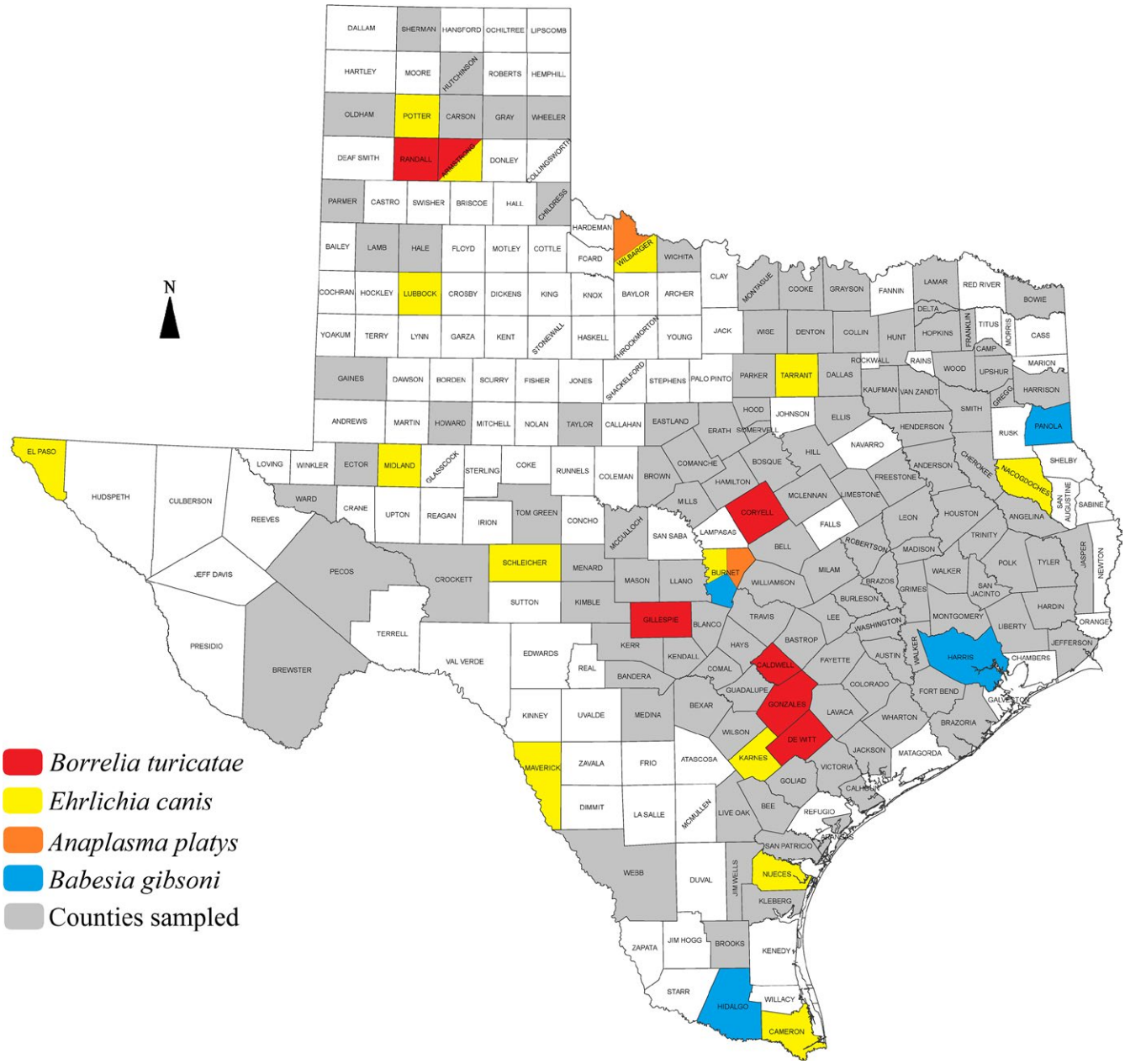

FIGURE 1 Geographic representation of study area and molecular prevalence of tick-borne pathogens in domestic dogs of Texas. Map adapted from Texas Parks and Wildlife Department courtesy © 2011

2.2 | DNA extraction and real-time polymerase chain reactions

From each animal, an aliquot of EDTA whole-blood (50 μL) was DNA purified using the MagMAX™ Nucleic Acid Isolation Kit AMB1836 (Thermo Fisher Scientific, Waltham, MA) and the KingFisher™ Flex (Thermo Fisher Scientific) automated purification system following manufacturers recommendations adopted from a previous publication (Schroeder et al., 2013). To evaluate the success of DNA extraction, a canine specific endogenous internal positive control (EIPC-K9) targeting the *MT-ND5* gene was utilized in all qPCR reactions (Modarelli, Ferro, & Esteve-Gasent, 2018).

Real-time polymerase chain reactions analysis for all 11 targets of interest were screened simultaneously utilizing a qPCR layerplex methodology (Patent application 16/130,177). In particular, the pathogens targeted with this assay include: *Borrelia burgdorferi sensu lato*, *B. turicatae*, *B hermsii* (genomic groups I and II), *B parkeri*, *Anaplasma phagocytophilum*, *Ehrlichia canis*, *E. chaffeensis*, *E. ewingii*, *Rickettsia rickettsii* and *Babesia* spp. The layerplex qPCR was performed using an Applied Biosystems® 7500 Fast Real-Time PCR System (Thermo Fisher Scientific), following primer/probe concentrations and

thermocycler conditions established in the patent disclosure. Samples with a quantification cycle (Cq) ≤ 38 were considered positive and confirmed through conventional PCR and Sanger sequencing.

2.3 | DNA sequencing and sequence analysis

All positive and suspect results from layerplex qPCR analysis were compared with those obtained by conventional PCR. Conventional PCR protocols for the detection of the *16S rRNA* gene of *Ehrlichia* and *Anaplasma* species (Wen et al., 1997), the *16S rRNA-23S rRNA* intergenic spacer sequence (IGS) of *Borrelia* species (Bunikis et al., 2004), and the *18S rRNA* of *Babesia* species were utilized as described previously (Davitkov et al., 2015). Positive (genomic) controls and negative controls (water) were included in all PCR assays. All attained DNA amplicons were then Sanger sequenced in both directions to obtain a consensus sequence (Eurofins Scientific, Louisville, KY). Consensus sequences were then evaluated with CLC Main Workbench (CLCbio, Aarhus, Denmark) and compared with published sequences on the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST). All DNA extractions and PCR reactions were prepared and performed under veterinary diagnostic laboratory conditions (i.e. biosafety level 2, biosafety cabinets, good laboratory practices) to avoid potential cross-contamination among tested samples.

3 | RESULTS

3.1 | Summary of study area and dog samples

The 1,171 canine whole blood samples originated from dogs residing in 55.9% (142/254) of the total counties in Texas, between June 2016 and February 2018. Due to the large geographic size and population dispersion within counties in Texas, the study area was separated into rural and urban counties per designations set by the Texas Department of State Health Services, which bases distinctions on population census reports (https://www.dshs.texas.gov/chs/hprc/counties.shtm). In that respect, samples originated from 36.4% (426/1171) rural counties, and 63.6% (745/1171) urban counties. Within this distinction, samples collected for this study accounted for 45.9% (79/172) and 76.8% (63/82) of all counties within either rural or urban settings, respectively. Sample coverage of each representative county within the 10 ecoregions of Texas ranged as follows: Piney Woods 68.0% (17/25), Gulf Parries 76.5% (13/17), Post Oak Savannah 89.3% (25/28), Blackland Prairies 73.7% (14/19), Cross Timbers 75.0% (21/28), South Texas Plains 48.0% (12/25), Edwards Plateau 51.9% (14/27), Rolling Plains 23.8% (10/42), High Plains 38.7% (12/31) and Trans-Pecos 33.3% (4/12). The average age of sampled dogs was 7.8 years (range 8 weeks–20 years). The sex ratio of our sample set was 41.8% male, 52.3% female and 5.9% unreported.

Of the samples tested, a total of 2.73% (32/1,171) dogs across Texas had one or more tick-borne pathogen DNA detected by layerplex qPCR analysis and confirmed by subsequent conventional PCR and Sanger sequencing. Infections identified included 1.62% (19/1,171) *Ehrlichia canis*, 0.17% (2/1,171) *Anaplasma platys*, 0.68% (8/1,171) *Borrelia turicatae* and 0.42% (5/1171) *Babesia gibsoni*. The two dogs infected with *A. platys* were also found to be coinfected with *E. canis*. Furthermore, molecular prevalence rates of each tick-borne pathogen varied across each ecoregion as depicted in Table 1. Additional tick-borne pathogens screened in the sample set, including *Ehrlichia chaffeensis*, *E. ewingii*, *Rickettsia rickettsii*, *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, *B. hermsii*, *B. parkeri* and *Babesia canis*, were not detected.

TABLE 1 Molecular prevalence of tick-borne pathogens in domestic dogs across Texas ecoregions, ranging East to West

Ecoregion	*Ehrlichia canis*	*Anaplasma platys*	*Borrelia turicatae*	*Babesia gibsoni*
Piney Woods	0.82% (1/121)	ND	ND	0.82% (1/121)
Gulf Parries	2.48% (3/121)	ND	ND	0.82% (1/121)
Post Oak Savannah	ND	ND	1.65% (2/121)	ND
Blackland Prairies	ND	ND	0.82% (1/121)	ND
Cross Timbers	1.65% (2/121)	0.82% (1/121)	1.65% (2/121)	1.65% (2/121)
South Texas Plains	2.48% (3/121)	ND	ND	0.82% (1/121)
Edwards Plateau	0.82% (1/121)	ND	0.82% (1/121)	ND
Rolling Plains	4.96% (6/121)	0.82% (1/121)	0.82% (1/121)	ND
High Plains	1.65% (2/121)	ND	0.82% (1/121)	ND
Trans-Pecos	1.22% (1/82)	ND	ND	ND
Total	1.62% (19/1,171)	0.17% (2/1,171)	0.68% (8/1,171)	0.43% (5/1,171)

ND: not detected.

3.2 | Rickettsial molecular findings

DNA from rickettsial pathogens were detected in a total of 21 (1.79%; 19 *Ehrlichia canis* and 2 *Anaplasma platys*) across Texas. A higher molecular prevalence of *E. canis* infected dogs were detected in the Rolling plains ecoregion (4.96%), followed by a uniform prevalence of 0.82%–2.48% across all other ecoregions except for the Post Oak Savannah and the Blackland Prairies, where *E. canis* DNA was not detected (Table 1, Figure 1). The mean age of dogs infected with *E. canis* was 6.3 years, (12 weeks–12.5 years), and no predilection of breed or sex was found. In this study, molecular detection was highest in September (*n* = 6), followed by February (*n* = 4), but were also detected in January, April, June, July, October and December. Two dogs, aged 7 and 8, were found coinfected with *E. canis* and *A. platys*. Both dogs originated from different central ecoregions (Table 1, Figure 1) but were detected during the month of February. Moreover, 36.8% (including both coinfected dogs) and 63.2% of the dogs were detected in rural and urban counties, respectively. The CBC analysis revealed that 94.1% of the infected dogs were thrombocytopenic (platelets below reference interval of 200–500 K/μL), 52.9% were anaemic (hematocrit below reference interval 32%–50%), and 47.1% presented with both anaemia and thrombocytopenia. When clinical history was available, the most common clinical signs and findings included lethargy (58%), inappetence (43%) and known exposure to ticks (43%). All *E. canis* and *A. platys* samples identified from Texas dogs were uploaded into GenBank® (Tables S1 and S2) and revealed 99%–100% identity to *E. canis* and *A. platys* sequences already published. Of note, a single *E. canis* and *A. platys* coinfected dog was responsible for the 99% identity in both pathogen sequences, due to a single nucleotide polymorphism (SNP) present within each gene region in respect to published sequences. Additional sequence significance was not observed.

3.3 | Borrelial molecular findings

A total of 8 (0.68%) *Borrelia turicatae* infections were detected at a molecular prevalence of 0.82%–1.65% across six ecoregions (Table 1, Figure 1). Further depicted in Figure 1 were two collections of counties in which infections were detected; one northern group maintaining a prevalence at 0.82%, and another located in central Texas ranging from 0.82%–1.65%. The mean age of dogs infected with *B. turicatae* was 7.3 years, (2–10 years), and no predilection of breed or sex was indicated. Infections were detected in serial months ranging from February to August, with one to two detections per month. 37.5% and 62.5% of the infected dogs were detected in rural and urban counties, respectively. CBC analysis available for 6 of the dogs revealed that 100% were thrombocytopenic (platelets below reference interval of 200–500 K/μL), 16.7% anaemic (hematocrit below reference interval 32%–50%), and only one presented as both. Clinical history was not available for a majority of infected dogs, but spirochetemia was observed in 80% of the dogs by blood smear review. Sequence analysis of all 8 *B. turicatae* samples purified from Texas dogs indicated 99% identity with *B. turicatae* strain BTE5EL (CP015629) isolated from a Texas human (Christensen et al., 2017; Kingry et al., 2016). Various SNPs were observed in each sample, and a phylogenetic tree was generated (Figure 2) alongside two additional *B. turicatae* isolates from Texas (i.e. *Ornithodoros turicata*, CP000049; and human, CP015629). Interestingly, the phylogenetic tree revealed four distinct clusters, though no specific grouping pattern at the ecoregion level was noted. As depicted in Figure 2, one cluster contained only the tick isolate, two clusters contained seven of the dog samples across multiple county origins, and a final cluster comprised of one single dog sample alongside the human isolate. All *B. turicatae* sequences obtained from dogs were uploaded into GenBank® (Table S3).

3.4 | Babesial molecular findings

A total of 5 (0.43%) *Babesia gibsoni* infections were detected at a molecular prevalence of 0.82%–1.65% in 4 eastern ecoregions (Table 1, Figure 1). The mean age of dogs infected with *B. gibsoni* was 5.2 years, (1.5–10 years), and though no predilection of sex was found, 80% of the dogs were reported as pit bull-type dogs. Infections were distributed in January, February, March and November. Moreover, 60% and 40% of the infected dogs were detected in rural and urban counties, respectively. The CBC analysis available for all of the dogs revealed that 100% were thrombocytopenic (platelets below reference interval of 200–500 K/μL), 80% anaemic (hematocrit below reference interval 32%–50%), and all but one presented as both. Although clinical history was not available for the infected dogs, small intraerythrocytic *Babesia* spp. parasites were observed in 80% of the dogs by blood smear review. All *B. gibsoni* samples purified from Texas dogs were uploaded into GenBank® (Table S4) and revealed 100% identity to *B. gibsoni* sequences published in GenBank®.

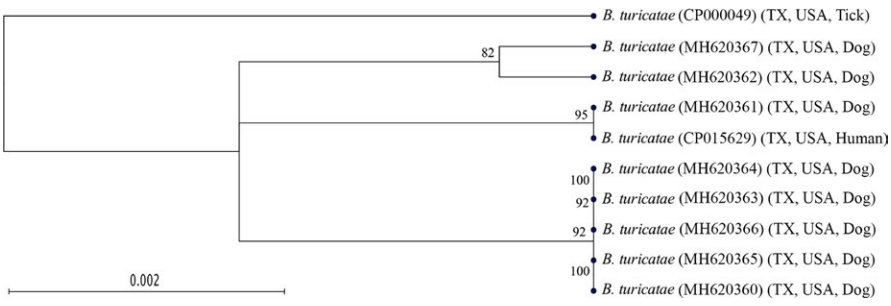

FIGURE 2 Phylogenetic tree of *16S-23S rRNA* intergenic spacer gene sequence alignments for *Borrelia turicatae* purified from dogs (this study), tick (*Ornithodoros turicata*) and a human in Texas

4 | DISCUSSION

Tick-borne diseases of domestic dogs are caused by numerous pathogens belonging to multiple genera. Typical diseases found in Texas include borreliosis, ehrlichiosis, anaplasmosis, rickettsiosis and babesiosis. While past TBDs prevalence investigations of dogs in Texas have focused on seroprevalence studies, and therefore potentially detecting past exposure and not active infections, this study aimed to characterize the molecular prevalence of active infection(s) in dogs by directly detecting the pathogen(s) in blood samples. To our knowledge, this is the first molecular prevalence study of tick-borne pathogens in domestic dogs in Texas, and the first molecular report of *A. platys* in Texas and coinfection of *E. canis* and *A. platys* in Texas dogs.

In the present study, the molecular prevalence of TBDs across Texas dogs ranged from 0.68% for borreliosis, 1.60% for ehrlichiosis, 0.17% for anaplasmosis, 0.00% for rickettsiosis and 0.43% for babesiosis. As expected, these percentages are slightly lower than reported seroprevalence data for Texas as this data represents a current record of active infections by molecular analysis and not past or recent exposure detected by serological tools (e.g. antibody detection). When prevalence was analysed by each ecoregion, a higher prevalence was found in specific regions that more closely resembles past seroprevalence data. The differences in prevalence among ecoregions may be attributed to the diverse topography, climate, and habitat features across Texas; characterized by west arid deserts, eastern swamps, southern subtropical and a temperate north. The state of Texas is also home to 91 mountain peaks with a majority located in far west Texas, contrasted by vast cave systems and canyons clustered in central and north Texas, respectively.

In respect to rickettsial infections, the highest prevalence was observed in the north-central Rolling Plains ecoregion at 5.78% (6 *E. canis* and 1 *A. platys* infections), followed by the South Texas Plains at 2.48% (3 *E. canis* infections). It is interesting to note that a majority of infections were detected in the Rolling Plains despite having the least sample coverage from representative counties when compared to coverage in other ecoregions. While these findings may potentially be inflated due to limited ecoregion coverage, the data also suggests that a higher prevalence may be determined if more samples from other counties within the ecoregion were available for collection. Future studies aimed at characterizing *E. canis* infections in Texas dogs should include collections in this ecoregion.

It is also worth noting that *E. canis* infections were detected in 80% of the ecoregions of Texas, indicating the pathogens ability to colonize dogs in numerous habitats. The ability for *E. canis* to be detected across Texas can be credited to its primary tick vector, *Rhipicephalus sanguineus* (brown dog tick), which is known as a hardy tick species found on dogs within either rural or urban settings, and can remain active in a variety of climates (Dantas-Torres et al., 2012). The brown dog tick is well known for its ability to dwell within homes and parasitize urban dogs, which is further supported by the 63.2% of *E. canis* infected dogs detected by this study residing within urban counties throughout most of the year. The brown dog tick has also been implicated as a primary vector for *A. platys*, supporting the potential for further coinfections in Texas dogs (Ramos et al., 2014). It is important to note that while *A. platys* was detected in this study, the molecular layerplex assay utilized for screening samples does not detect any other *Anaplasma* species besides *A. phagocytophilum* (Patent application 16/130,177). Both *A. platys* infections were incidentally detected through confirmation testing with conventional PCR analysis. Therefore, the potential for additional dogs to be actively infected with *A. platys* in Texas should be realized and further investigated.

In this study no other rickettsial pathogens (i.e. *Ehrlichia ewingii*, *E. chaffeensis*, *Anaplasma phagocytophilum*, and *Rickettsia rickettsii*) were detected despite past studies indicating serological evidence of exposure in the study area. Possible considerations for the discrepancies include inadequate sample size or coverage of ecoregions for molecular detection, previous exposure without active infection, and false positive serologic results. Another potential reason for prevalence inconsistencies between studies may be due to increased cross-reactivity or limited specificity featured by serological methods utilized for seroprevalence investigations for closely related species currently or previously circulating in infected dogs (Modarelli, Borst, Piccione, & Esteve-Gasent, 2019).

Infections by the borrelial pathogen, *Borrelia turicatae*, were limited at a total molecular prevalence of 0.68% across Texas, and was the only borrelial pathogen detected. Within Texas, 60% of ecoregions indicated molecular exposure ranging from 0.68%–1.65%, with a majority of detection occurring only in north or central Texas. Interestingly, data from this study indicated two groups of counties where *B. turicatae* infections were found. The first group was identified in northwest Texas with two dog infections. Though detection prevalence was limited, this group resembled counties within the same ecoregions described previously in a case report of three spirochetemic dogs in north Texas diagnosed with TBRF due to infection with *B. turicatae* (Whitney, Schwan, Sultemeier, McDonald, & Brillhart, 2007). Furthermore, the county locations of the three case report dogs reside in the same two ecoregions that contain the northern group of *B. turicatae* infected dogs indicated in the present study (i.e. High Plains, and Rolling Plains). The second group, located in central Texas, contained six infected dogs within five counties across four ecoregions (i.e. Post Oak Savannah, Blackland Prairies, Cross Timbers, Edwards Plateau). Two additional case reports of five dogs (Piccione et al., 2016) and one human (Bissett et al., 2018) also coincide with our findings by indicating infections with *B. turicatae* centred in the same aforementioned ecoregions. The preliminary observation of geographical grouping, or clustering, of samples from case reports and surveillance sample sets may be explained by the ecology of its primary soft tick vector, *Ornithodoros turicata*, which has traditionally been associated with the cave system of central Texas, therefore corroborating the central cluster observed in this current study and prior case studies (Dworkin, Schwan, Anderson, & Borchardt, 2008).

The northern cluster, defined as including infected dogs from past case reports and this study, was identified in the High Plains

and Rolling Plains ecoregions, of which the topography does not typically include cave systems. However, both ecoregions contain vast canyons, cliffs, and tunnels, which leads us to suspect that this landscape, despite the lack of caves, may provide a competent habitat for *O. turicata* ticks to thrive and transmit *B. turicatae* to susceptible hosts. It is important to note, that a soft tick species (i.e. *Carios kelleyi*) ecologically similar to *O. turicata* has been collected from bats emerging from cave systems in Texas (Donaldson et al., 2016). Donaldson and colleagues suggest that bats may facilitate the dissemination of *O. turicata* ticks given the regular cave locality and opportunistic feeding nature of the ticks. Texas Parks and Wildlife report 12 major sites where bats roost in Texas, including nine caves/bridges in central Texas ecoregions (i.e. Edwards Plateau, Blackland Prairies), one tunnel system in north Texas (i.e. Rolling Plains) and two bridges in east Texas (i.e. Gulf Prairies). Interestingly, bat roosting sites coincide with the two *B. turicatae* clustering locations identified in this study, specifically in the central Edwards Plateau and the northern Rolling Plains ecoregions. A similar association has been observed in respect to *B. hermsii*, where it is hypothesized that infected migratory birds may contribute to the geographic distribution of the pathogen (Schwan, Raffel, Schrumpf, & Porcella, 2007). Therefore, the potential for bats to play a role in the dispersion of *B. turicatae* should be further explored.

It is also predicted that *O. turicata* ticks are sensitive to specific environmental conditions that restrict its spread within additional U.S. states that span between the established locations of Texas and Florida (Donaldson et al., 2016). Briefly, this intrastate region has been described to feature elevated temperatures during the driest quarter of the year and low temperatures during the wettest quarter as compared to average readings across the county, which may impede the ticks ability to colonize the area (Lopez et al., 2016). Therefore, the same environmental variables may be viewed within the vastly different Texas ecoregions, resulting in the geographical clustering observed within this study and both case reports.

Nevertheless, it should be noted that the grouping may be due to sampling bias around both TVMDL facility locations. Sequence analysis of the *16S-23S rRNA* IGS gene region amplified from all 8 *B. turicatae* samples were aligned and evaluated for SNP groupings in order to identify potential evolutionary support for the two geographical locations. While SNPs were observed across all samples of *B. turicatae*, and independent clusters were formed within the constructed phylogenetic tree, no remarkable patterns among clusters were noted. Thus, there is no current evidence that the two groups are genetically distinct.

Of the three tick-borne relapsing fever species screened for in this study (i.e. *B. turicatae*, *B. parkeri*, *B. hermsii*) only *B. turicatae* was expected to be circulating in Texas dogs (Lopez et al., 2016). However, due to limited past prevalence studies of TBRF in Texas, and recent predictions of additional soft tick species migrating south towards Texas (i.e. *Ornithodoros hermsi*, *O. parkeri*), we included surveillance testing for the respective *B. hermsii* and *B. parkeri* pathogens in our analysis (Sage, Johnson, Teglas, Nieto, & Schwan, 2017). Findings from this study support the conclusions of past investigations and emphasize, that currently, the only TBRF species that has been detected in Texas is *B. turicatae*. The lack of *B. burgdorferi sensu lato* detected infections may be due to utilizing blood as a sole sample type, as well as the use of single aliquot of blood (50 μL), and should not be viewed as supporting evidence for lack of Texas dog exposure to the pathogen (Primus et al., 2018).

In respect to babesial infections, only *Babesia gibsoni* was detected and indicated limited molecular prevalence from 0.82%–1.65% across four eastern ecoregions (i.e. Cross Timbers, Gulf Prairies, Piney Woods, South Texas Plains). Molecular prevalence of *Babesia gibsoni* within Texas has been established in the past, though it was limited to a single analysis of dogs rescued from dog fighting rings, and no indication of specific prevalence within the state was available (Cannon et al., 2016). As expected, a breed specific association was observed in the present study with 80% of the *B. gibsoni* infections occurring in pit bull-type dogs. In addition to a breed specific genetic predisposition for *B. gibsoni* to infect pit bull-type dogs, these breeds are unfortunately more likely to encounter the infection through direct blood transmission from bites, or unsterile ear/tail cropping/docking commonly associated with dog fighting rings (Cannon et al., 2016). Data from the current study should serve as a reminder in conjunction with findings from Cannon et al. to properly screen susceptible dogs for potential infections and carrier states, and promptly administer appropriate treatment.

The layerplex qPCR assay utilized for this study detects pan-*Babesia* species, though only *B. canis vogeli* was expected to potentially indicate prevalence alongside *B. gibsoni* due to suggestions of a shared tick vector, the brown dog tick (Jongejan et al., 2018). However, it is important to note that it is currently unknown which babesiosis causing pathogens are most prevalent within Texas dogs. Additional *Babesia* species such as *B. conradae* are expected to re-emerge within the dog population, though, the geographic distribution of the pathogen is also unknown and the screening assay used in this study does not detect this specific species of *Babesia* (Di Cicco et al., 2012). Future studies aiming to characterize babesial infections within Texas dogs should include *B. conradae* in their analysis.

Limitations of the study include potential sampling bias due to dog samples originating only from two TVMDL locations in contrast to active sample collections within the study areas. Furthermore, samples were randomly selected from an archived pool of opportunistically collected TVMDL cases, and were only available from counties with established TVMDL clients. Ecoregions sampled in this study are represented by counties containing submitting veterinarians, and in the case of more rural areas, may not accurately reflect the origin of the dog sample. Sample analysis from the Trans-Pecos ecoregion, consisting of 83.3% rural counties, was severely restricted due to limited submitted samples, resulting in an incomplete sample set. Finally, it is important to note that whole-blood samples

are not ideal for detecting *Borrelia burgdorferi* and *Rickettsia rickettsii* due to limited blood-borne circulation, and therefore findings from this investigation may differ from true prevalence of the pathogens in the area. However, studies have documented a low percentage of detection of the pathogens by PCR in dog and human blood samples (Kidd et al., 2008; Primus et al., 2018). In addition, this study based the 121 dogs desired per ecoregion sample size on prior seroprevalence investigations due to a lack of available molecular prevalence data. Findings presented here may not be representative of true prevalence of the study area, but should be used as a baseline for future investigations.

Despite these limitations, this study highlights the significance of molecular surveillance screening in order to characterize areas where active infections occur. Furthermore, it is important to note the zoonotic implication of *B. turicatae* (Christensen et al., 2017), *E. canis* (Ojeda-Chi et al., 2019), and *A. platys* (Maggi, Mascarelli, Havenga, Naidoo, & Breitschwerdt, 2013) detected in this study. As Texas supports competent tick vectors for all pathogen species detected in this study (e.g. *Ornithodoros turicata* and *Rhipicephalus sanguineus*), and dogs may represent effective sentinels for human TBDs, the zoonotic transmission potential of the diseases should be considered in ecoregions indicating an increased molecular prevalence.

In conclusion, the present study provided an estimation of molecular prevalence of various TBDs in the general population of domestic dogs of Texas. Our findings indicate that dogs are experiencing clinical infections with several pathogens, many of which have zoonotic implications. When analysing the state of Texas, the overall prevalence was relatively low (2.73%) but was in accordance with previous seroprevalence studies. When prevalence analysis was applied to the ecoregion level, pathogen prevalence was found to be less diluted (up to 5.78%), indicating the influence of ecological factors on pathogen prevalence in an area, and highlighting specific regions of increased risk to public health. Future studies aiming to further characterize TBDs in Texas should consult the ecoregion findings established in this preliminary report when designing new molecular surveillance investigations in order to provide a more accurate molecular prevalence study.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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