International Journal for Parasitology: Parasites and Wildlife 19 (2022) 294–300

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

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

Prevalence and geographic distribution of *Babesia conradae* and detection of *Babesia vogeli* in free-ranging California coyotes (*Canis latrans)*

Nadia N. Javeed [a], Laura Shultz [a], Samantha Barnum [b], Janet E. Foley [a], Emir Hodzic [a,b], Emily L. Pascoe [a], Beatríz Martínez-López [a], Niamh Quinn [c], Danielle Bucklin [c], Jonathan D. Dear [a,*]

[a] Department of Medicine and Epidemiology School of Veterinary Medicine, University of California Davis, 1 Garrod Dr, Davis, CA, 95616 USA
[b] Real-Time PCR Research and Diagnostics Core Facility, School of Veterinary Medicine, University of California Davis, 1 Garrod Dr, Davis, CA 95616, USA
[c] South Coast Research and Extension Center, University of California, 1706 Irvine Blvd, Irvine CA, 92618, USA

ARTICLE INFO

Keywords:
Babesia
Babesia conradae
Babesia vogeli
Canis latrans
Hemolytic anemia

ABSTRACT

Babesia species are intraerythrocytic piroplasms that can result in disease characterized by hemolytic anemia and thrombocytopenia. Of the 5 species that are known to infect canids in the United States, *Babesia conradae* is most frequently diagnosed in California, and *Babesia vogeli* is prevalent in the US. Despite the recent re-emergence of *B. conradae*, the mechanism of transmission is not known. Coyotes (*Canis latrans*) have been a proposed reservoir of disease, and previous work has shown that dogs with known aggressive interactions with coyotes are at greater risk for infection. This study aimed to determine the prevalence of *B. conradae* in wild coyote populations in California to assess the viability of coyotes as a potential source of infection for domestic dogs. Four hundred and sixty-one splenic samples were obtained during post-mortem examination of coyote carcasses from Southern California, Fresno, and Hopland. Demographic data including age, sex, cause of death, and urbanity were collected for each coyote. DNA was extracted from samples and amplified using real-time PCR with primers specific for the *B. conradae ITS-2* gene. The *18S* gene was amplified and sequenced using conventional PCR primers specific to the *Babesia* genus from any coyotes positive for *B. conradae.* In total, 22 coyotes tested positive for *B. conradae* in Fresno (n = 15), Orange (n = 4), San Bernardino (n = 1), and Los Angeles counties (n = 1) with an overall prevalence of 4.8%. Coyotes from Fresno ($P < .01$) and rural coyotes ($P < .01$) were significantly more likely to be infected with *B. conradae*. Ten of 14 samples sequenced were 99–100% homologous to *B. conradae,* and 4 samples were 100% homologous with *B. vogeli* DNA indicating co-infection with both pathogens. This study demonstrates that coyotes can become infected and harbor *B. conradae* and *B. vogeli* and should be investigated as a possible source of infection in domestic dogs.

1. Introduction

Babesia species are intraerythrocytic piroplasms that infect a wide range of host species including canids. Five species are known to cause canine babesiosis in the United States: *Babesia vogeli*, *Babesia conradae*, *Babesia gibsoni, Babesia vulpes*, and an unnamed large *Babesia* spp. (*Babesia* 'coco') (Baneth and others 2015; Kjemtrup and others 2000; Kjemtrup and others 2006; Sikorski and others 2010). *Babesia vogeli* is the most well documented large *Babesia* species in dogs in the United States, but the pathogen has primarily been documented in the southeastern United States in dogs and coyotes (Yu and others 2020) and has not yet been documented in California. It is vectored by *Rhipicephalus sanguineus*, which is an important tick vector for many diseases that affect domestic dogs and has adapted to live in more urban environments (Dantas-Torres 2008). Disease manifestations in dogs are generally mild but infection can be fatal in immunocompromised individuals (Solano-Gallego and Baneth 2011; Solano-Gallego and others 2008; Wang and others 2018).

Babesia conradae is the most commonly documented *Babesia* species in domestic dogs in California (Dear and others 2018; Kjemtrup and Conrad 2006), and manifests as a severe hemolytic anemia and thrombocytopenia in domestic dogs that can be fatal without treatment (Conrad and others 1991). The organism was classified in 2006 and based on analysis of the 18 S rRNA gene, is genetically similar to *Babesia*

* Corresponding author.
E-mail addresses: jddear@ucdavis.edu, jddear@ucdavis.edu (J.D. Dear).

https://doi.org/10.1016/j.ijppaw.2022.11.004
Received 21 September 2022; Received in revised form 9 November 2022; Accepted 10 November 2022
Available online 16 November 2022

spp. that typically infect wildlife and humans, yet is distinct from other species of *Babesia* that infect domestic dogs (Kjemtrup et al., 2000). Since then, there have been increasing numbers of cases of *B. conradae* infections in domestic dogs (Dear and others 2018; Di Cicco and others 2012; Stayton and others 2021), but the ecology of this pathogen remains unknown. A major challenge to understanding the transmission pathways and reservoir of domestic dog infections is the lack of understanding of all susceptible host species, including wildlife. Coyotes (*Canis latrans*) are suspected to be reservoirs of *B. conradae* and in a survey in Los Angeles county 3 of 29 coyotes were seropositive for antibodies to the parasite (Yamane and others 1994). Additionally, dogs with known aggressive interactions with coyotes are at greater risk of becoming infected (Dear and others 2018), and the organism was detected via PCR in splenic tissue of coyotes killed by the dogs described in the 2018 study (unpublished data, JD Dear).

Little is known about the mechanism of transmission of *B. conradae*. Several studies hypothesize that dogs could be infected by direct transmission from infected coyotes similar to the blood transmission of *Babesia gibsoni* from bites among fighting dogs (Birkenheuer and others 2005; Dear and others 2018; Jefferies and others 2007), but this has not been investigated experimentally. *Babesia conradae* is suspected to be capable of vertical transmission based on a study performed in Los Angeles County in which 7 infected dogs were offspring of a single infected bitch (Di Cicco et al., 2012). Ticks, including *R. sanguineus* and *Dermacentor* spp., are competent vectors of many *Babesia* species (Donnelly and Peirce 1975; Shortt 1973; Spielman 1976; Varloud and others 2018), and *B. conradae* has been detected in *Dermacentor albipictus* in a recent study (Duncan and others 2021). However, neither *R. sanguineus* nor *Dermacentor variabilis* transmitted *B. conradae* to dogs in experimental models of infection (Yamane and others 1993).

Likewise, little is known about the geographic distribution of *B. conradae*. The pathogen was initially recognized in southern California (Conrad and others 1991), and the majority of the cases in domestic dogs have been in the Central Valley of California (Dear and others 2018), southern California (Di Cicco and others 2012), and Oklahoma (Stayton and others 2021; Thomas et al., 2015). Additionally, the protozoa was found in 2 ticks from cats in Minnesota and Colorado (Duncan and others 2021). Recent studies of canine babesiosis found a 0.01% and 0.4% incidence respectively in North American dogs tested for *Babesia* (Barash and others 2019; Birkenheuer and others 2020). However, only the latter study tested directly for *B. conradae.* Since widespread testing for *B. conradae* is not routine in dogs suspected of having vector-borne disease, its incidence might be underestimated.

Babesia conradae has the potential to be both an important infection affecting coyotes and a threat to domestic dogs, especially given increasing interactions among wild and domestic canids due to an expanding rural-urban interface (Crooks 2002; Gage and others 2008). Understanding the prevalence of *B. conradae* infection in wildlife is a critical step in understanding its epidemiology and might indicate whether it is a clinically relevant infection in coyotes. As such, the aim of this study was to determine the infection prevalence and spatial distribution of *B. conradae* PCR positive coyotes in California. A secondary aim was to use the data available to determine geographic and demographic predictors of infection.

2. Methods

2.1. Data collection

Splenic samples were collected and archived as part of previous epidemiological studies from 461 coyotes in California between June 2015 and November 2019. Coyotes from those studies were collected from depredation permits requested by California residents. Coyotes were humanely captured and euthanized by private trappers and animal control agents under license using a variety of euthanasia methods, including Belilse Foot Snares, Collarum® Live Capture canine devices, snares, and shooting, by private trappers and animal control agents or killed by vehicles. A necropsy was performed on each carcass and tissues were stored at −20 °C. Other data collected included city of death, date of death, urbanity (urban, rural, interface), sex, age class (pup, juvenile, adult), and cause of death (vehicle strike, euthanized, other). The urbanity classification for each coyote was determined based on the human population density in the area where the coyote carcass was obtained. Coyotes were defined as originating from Southern California if they originated from Imperial, Kern, Los Angeles, Orange, Riverside, San Bernardino, San Diego, Santa Barbara, San Luis Obispo, and Ventura counties. Remaining coyotes were designated by their county of origin. Age was determined by cementum annuli analysis for 177 animals at Matson's Laboratory (Manhattan, Montana) (Scrivner and others 2014). Coyote age classes were defined based on behavioral patterns and previous demographic studies (Bekoff 1977; Dumond and Villard 2000; Gese and others 1988; Harrison and others 1991): pups, aged less than 6 months old; juveniles, 6 months to 1 year old; young adults, aged 1–2 years old; adults, aged 3–5 years old; and older adults, aged 6 years or older.

2.2. Molecular analysis

DNA was extracted from the spleen tissue samples using DNeasy Blood and Tissue kits (Qiagen, Valencia, CA, USA) according to the manufacturer instructions. Splenic DNA was screened for *B. conradae* via real-time PCR (rt-PCR) targeting the *ITS-2* gene specific to *B. conradae* using a previously published protocol (Dear and others 2018). The primers and probes were tested against other species of *Babesia* to insure their specificity. Samples with a cycle threshold (CT) value of <40 and a characteristic amplification curve were considered positive.

To evaluate the homology between the *B. conradae* DNA we detected in coyotes and previously detected in domestic dogs, conventional PCR was used to amplify the 18 S rRNA gene with primers specific to the genus *Babesia* from rt-PCR positive samples using a previously published protocol (Scott and others 2020). Electrophoresis was performed on PCR products using a 1% agarose gel which were stained with GelStar nucleic acid stain (Lonza, Rockland, Maine, USA). Products were identified using ultraviolet transillumination. Amplicons of 400–500 base pairs were excised from the gel and purified for DNA sequencing using ExoSAP-IT PCR Product Cleanup Reagent (ThermoFisher Scientific, Waltham, MA, USA). Samples were then sequenced with ABI 3730 Capillary Electrophoresis Genetic Analyzers (UCDNA Sequencing Facility, Davis, CA, USA).

2.3. Phylogenetic analysis

Sequenced amplicons were compared to published *Babesia* spp. sequences using the Basic Local Alignment Search Tool search of GenBank (NCBI; http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences from GenBank for *B. conradae, B. vogeli, B. canis, B. gibsoni, B. vulpes, B. rossi, Babesia 'coco'* and *B. bovis* (the outgroup) were used to create the phylogenetic tree. Sequences were manually corrected for ambiguous bases and end-reading errors based on the trace diagram. Sequences were trimmed and aligned using the MUSCLE (Multiple Sequence Comparison by Log-Expectation) tool in MEGA (10.1.8). A maximum likelihood phylogenetic tree was created using General Time Reversible with Gamma distribution and proportion of invariable sites model based on jModeltest 2 results (2.1.10) (Darriba and others 2012; Guindon and Gascuel 2003). Bootstrap analysis was performed in 1000 replicates of the original sequence alignments.

2.4. Statistical analysis

Descriptive statistics were calculated to assess the distribution of variables using Excel (Microsoft, Redmond, WA, USA). Univariate logistic regressions were used to examine associations between

B. conradae positive coyotes and the independent variables: age class interacting with sex, cause of death, year of death, and urbanity in R (version 4.1.3 2022 R Core Team, Vienna, Austria). If data for a given variable were not available for some coyotes, these individuals were still analyzed in models testing variable for which data were available. The 'glht' function in the R package 'multcomp' was used to assess model coefficients for all pairwise combinations of the different categories of the independent variable (Hothorn and others 2008). The exponentials of the model coefficients and standard errors were used to calculate odds ratios and 95% confidence intervals. P values $< .05$ were considered significant.

3. Results

3.1. Babesia detection

Of the 461 splenic samples available for DNA extraction and analysis, 22 were PCR positive for *B. conradae* resulting in an overall prevalence of 4.8%. Eighteen of the 22 qPCR samples positive for *B. conradae* successfully amplified with conventional PCR at the 18 S gene, and 14 (63.6% of total positive) were successfully sequenced. One of these shared 100% homology with the 18 S fragment of *B. conradae* (sequence AF158702.1), 8/14 differed by a single nucleotide (99.8% homology), and 1/14 differed by 2 nucleotides (99.5% homology) (Fig. 1). Four of 14 sequenced samples were 100% homologous with *Babesia vogeli*.

3.2. Coyote demographic information

Two hundred and fifty were male, 193 were female, and the sex of 18 coyotes was unknown. Thirteen (5.2%) males and 9 females (4.7%) were positive for *B. conradae* with no apparent sex predilection ($P = .80$). Three hundred and seventy-five (81.3%) were adult, 49 (10.6%) were juveniles, 22 (4.7%) were pups, and 15 had an unknown age. Most positive coyotes were adults (n = 17) and 5 were juveniles. None of the pups were positive for *B. conradae*. Three hundred and thirty-two coyotes were euthanized, 109 were hit by vehicles and 20 had an unknown cause of death. Twenty coyotes that were PCR positive for *B. conradae* were euthanized and the remaining 2 positive coyotes were hit by vehicles. There were no significant associations between age class overall ($P = .10$), age class among sex ($P = .99$), and *B. conradae* infection.

3.3. Coyote geographic information

Coyotes were primarily recovered from southern California (n = 382), with an additional 47 from Fresno County and 11 from Hopland (Mendocino County) (Fig. 2). Twenty-one coyotes did not have a recorded location of death. Coyotes positive for *B. conradae* were found in Fresno (n = 15) and southern Californian cities (n = 7; Table 1, Fig. 1). No coyotes from Hopland were positive for *B. conradae*. The prevalence of positive coyotes in Fresno County was 31.9% (15/47) compared to the prevalence in southern California of 1.8% (7/382). There were significantly more coyotes from this county that tested positive for *B. conradae* compared to Los Angeles County (OR = 39.61, CI = 8.37–187.41, $P < .01$) and Orange County (OR = 21.22, CI – 6.46–69.69, $P < .01$). In comparison to 2017, there were significantly fewer *Babesia*-positive coyotes tested from 2016 (OR = 0.20, CI = 0.06–0.73, $P = .05$).

Of the samples analyzed, 11.7% (54/461) were classified as rural, 82.8% (382/461) were classified as urban, 1.5% (7/461) were classified as urban-rural interface, and 3.9% (n = 18) were unclassified. Most coyotes from cities in southern California were classified as urban (374/38; 97.1%); however, most positive coyotes were rural (n = 14). The remaining 8 positive coyotes were urban and none of the 7 interface coyotes were positive for *B. conradae*. Rural coyotes were significantly more likely to be *B. conradae* positive compared to urban coyotes (OR = 16.36, CI = 6.35–42.17, $P < .01$). All four coyotes that had sequencing results homologous for *B. vogeli* were from Southern Californian cities

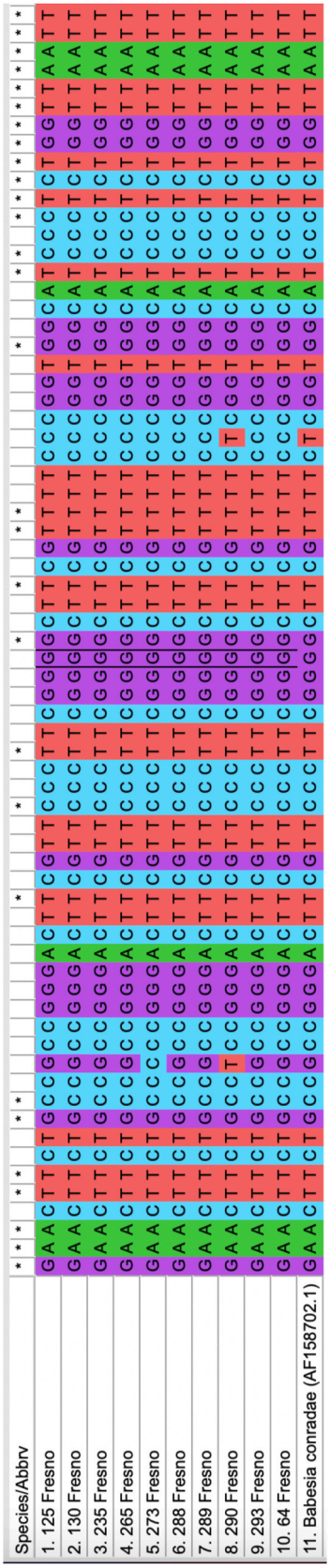

Fig. 1. Base pair differences in a 70 base pair region of the *18S* gene from *Babesia conradae* DNA sequences isolated from California coyotes (*Canis latrans*) splenic samples collected between 2015 and 2019 compared to published sequence available in GenBank.

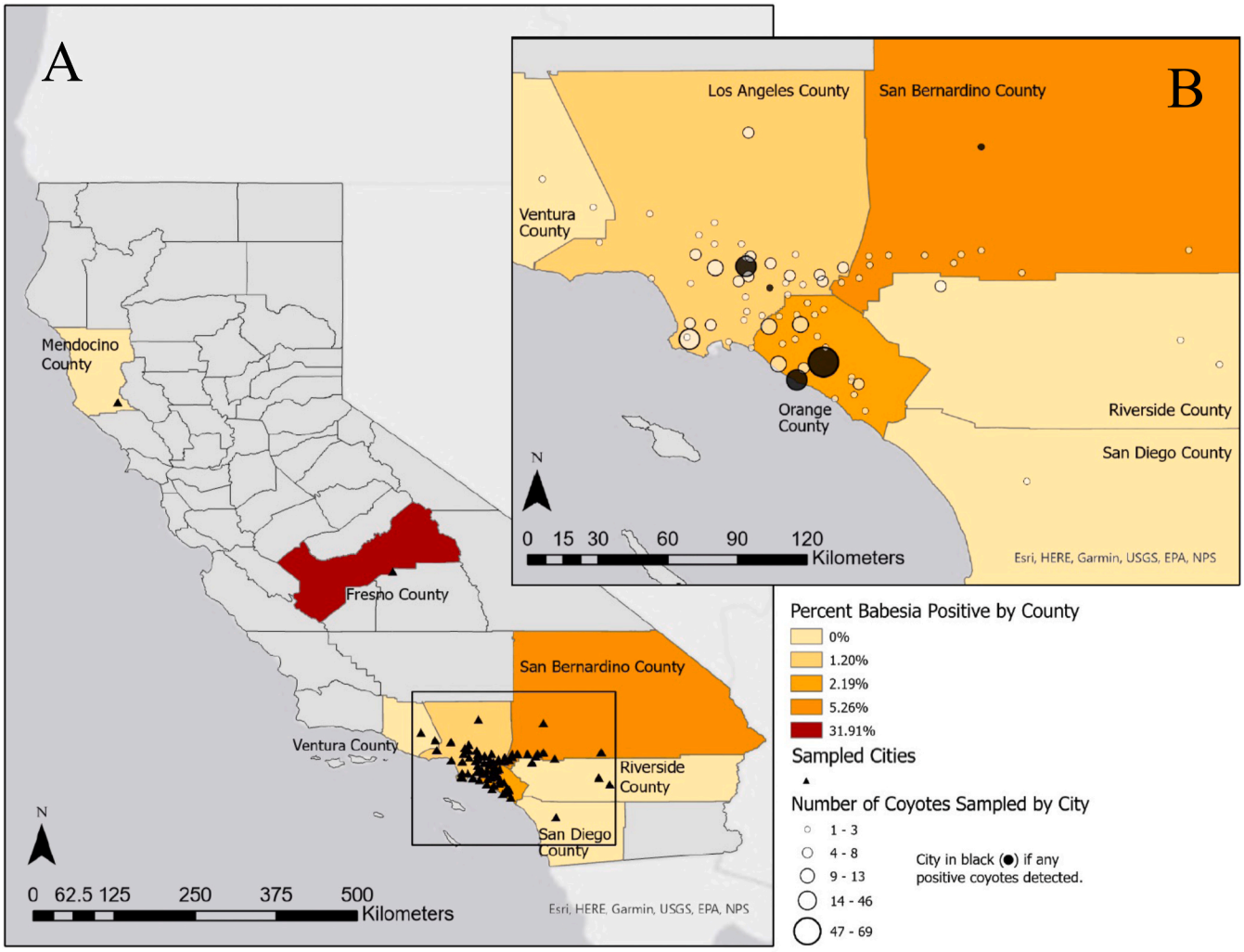

Fig. 2. A) PCR positivity (indicated by color) of coyotes (*Canis latrans*) carcasses recovered (▲) in each county between 2015 and 2019. B) Map of southern California including Los Angeles, Orange, Ventura, San Bernardino, Riverside, and San Diego counties showing *B. conradae* PCR positivity (indicated by color) in each city where coyote carcasses were recovered. The number of coyotes sampled at each location is indicated by the size of the circle.

(Fig. 3): a juvenile female and an adult male from Irvine, a juvenile male from Whitter, and an adult male from Newport Beach.

4. Discussion

4.1. *Babesia conradae*

The present study testing 461 splenic samples using molecular techniques has established that California coyotes can be infected with *B. conradae* and *B. vogeli.* To our knowledge, this is the first documentation of infection of *B. conradae* in free-ranging coyotes. The overall prevalence of this infection found was 4.8%. Although the true prevalence of *B. conradae* is not known in domestic dogs, our results are much higher than the prevalence of infection reported in domestic dogs (ranging from 0.01 to 2.6%) (Barash and others 2019; Yamane and others 1994).

This new evidence of naturally occurring infection in free-ranging coyotes is the first step to investigating coyotes as a potential reservoir of canine babesiosis for domestic dogs. However, the infection prevalence, in conjunction with previous evidence that dogs with aggressive interaction with coyotes are at greater risk of becoming infected with *B. conradae* (Dear and others 2018), supports the hypothesis that coyotes could be a source of infection for domestic dog populations. The few, consistent nucleotide differences in the coyote sequences compared to those from domestic dogs may be explained by differences in geographic region or different strains. In this study, only samples from Fresno were successfully sequenced. This is a limitation as there might be regional differences within the genome that were not identified. Sequencing larger portions of the genome and samples from more infected coyotes in more areas could help inform the extent of transmission between dogs and coyotes. Similarly, comparing the geographic distribution between dogs and coyotes was not possible in the present study. However, previous cases of *B. conradae* infection have been reported in the same areas that positive coyotes were identified in this study (Conrad and others 1991; Dear and others 2018; Yamane and others 1994). The previously reported prevalence in domestic dogs suggests that exposure to and infection with this protozoa is uncommon in domestic dogs but should be considered in animals with consistent clinical signs, potential exposure to wildlife and history of living in California.

If coyotes experience a similar clinical disease as dogs when infected, this pathogen has the potential to have a major impact on coyote populations. *Babesia conradae* tends to be more pathogenic than large *Babesia* spp. infecting canids. Infected dogs experience a profound anemia and thrombocytopenia, which can progress to hepatitis, glomerulonephritis, and protein losing nephropathy if left untreated (Wozniak and others 1997). Clinical signs can be severe and include lethargy, vomiting, pyrexia, and weight loss (Conrad and others 1991; Dear and others 2018). Although disease from *B. conradae* has not been

Table 1
Summary of coyotes (*Canis latrans*) collected between 2015 and 2019 in California testing PCR positive for *Babesia conradae* by city. If data for a given variable were not available for some coyotes, they were excluded from that column of the table.

Location	N	Urbanity			Age Class			Method of Death	
		Rural (%)	Urban (%)	Interface (%)	Pups (%)	Juveniles (%)	Adult (%)	Euthanized (%)	Vehicle Strike (%)
Fresno County			1						0
Positive	15	14	3	0	0	3	12	15	0
Negative	31	25	4	3	0	4	27	31	0
Total	46	39		3	0	7	39	46	
Irvine (city									
Positive	3	0	3	0	0	1	2	1	2
Negative	66	0	66	0	0	2	63	3	59
Total	69	0	69	0	0	3	65	4	61
Apple Valley (city)									
Positive	1	0	1	0	0	0	1	1	0
Negative	0	0	0	0	0	0	0	0	0
Total	1	0	0	0	0	0	1	1	0
Monterey Park (city)									
Positive	1	0	1	0	0	0	1	1	0
Negative	25	0	25	0	1	0	24	24	1
Total	26	0	26	0	1	0	25	25	1
Newport Beach (city)									
Positive	1	0	1	0	0	0	1	1	0
Negative	34	0	34	0	1	7	26	27	7
Total	35	0	35	0	1	7	27	28	7
Whitter (city)									
Positive	1	0	1	0	0	1	0	1	0
Negative	2	0	2	0	0	0	2	1	1
Total	3	0	3	0	0	1	2	2	1
Positive	22	14	8	0	0	5	17	0	2
Negative	439	40	374	7	22	44	358	312	107
Total	461	54	382	7	22	49	375	322	109

studied in coyotes, infection with other closely related species of *Babesia* produces similar lesions in coyotes as those found in dogs (Evers and others 2003; Roher and others 1985). It is also possible that, if a reservoir species, the organism may be better adapted to persistent, and perhaps less severe clinical infection in order to maintain infection in the population. Regardless, more investigation of the disease history of *B. conradae* in this newly detected host species is required to determine the extent to which it impacts coyote health.

Coyotes found in a rural setting and from Fresno County were at increased risk for infection with *B. conradae*. However, these two variables were closely associated in this study, with nearly all the coyotes found in Fresno County from rural areas and nearly all the coyotes from southern California from urban areas. This might be influenced by regional coyote density or vector density, but further investigation into the mechanisms of transmission of *B. conradae* is needed to speculate the cause of these findings. As no pups were found to be infected, further research into vertical transmission is needed to determine if this is a possible route of infection as has been suggested in other *Babesia* species.

4.2. *Babesia vogeli*

This is the first documentation of *B. vogeli* infection in California coyotes—only one other study reported *B. vogeli* in free-ranging coyotes in areas of southern Texas (Yu and others 2020). Although determining the prevalence of *B. vogeli* was not an aim of our study, 4 of the 14 (28.6%) sequenced samples were 100% homologous with *B. vogeli* which is higher than has been previously documented in coyote populations (9%) (Yu and others 2020). However, it is impossible to know how this compares to the actual PCR prevalence of *B. vogeli* in coyotes because only a small subset of our samples was sequenced. *Babesia vogeli* has been found to infect dogs in the United States (Barash and others 2019; Birkenheuer and others 2005), and has been primarily diagnosed in southeastern states. Given the number of infected coyotes in this study, *B. vogeli* may be more ubiquitous in the western United States than previous thought.

Babesia vogeli generally causes less severe disease relative to other *Babesia* spp. that infect dogs—animals that show clinical signs in natural occurring infections are often puppies, have concomitant disease, or are immunosuppressed (Solano-Gallego and others 2008). Clinical signs, if present, include fever, lethargy, and anorexia, but occasionally the disease can be lethal (Solano-Gallego and others 2008). As for *B. conradae*, the effect of infection on coyote health has not been investigated.

4.3. *Geographical distribution*

In our data, *B. conradae* and *B. vogeli* appear to be regionally distinct. The difference between the prevalence of *B. conradae* in southern California (1.8%) cities and Fresno County (31.9%) could also be explained by environmental conditions or geographic distribution of a possible vector. Similarly, *B. vogeli* was only sequenced from coyotes from cities in southern California, where coyotes were almost exclusively recovered in urban areas, consistent with the habitat of *R. sanguineus*, in contrast with rural locations in Fresno County. Although we had sampled areas from the north, central, and southern regions of California, our study was limited in geographic scope, making state-wide generalizations regarding the distribution of these pathogens difficult. Even so, these data provide an important baseline for future research into disease ecology of *B. conradae* including transmission mechanisms and a potential vector.

Our findings also show evidence of coyotes coinfected with *B. vogeli* and *B. conradae*. This may suggest that these protozoa might share a vector but more data is needed to investigate this potential. No such statistical significance was established in domestic dogs between *B. conradae* positivity and infection with other tick-borne diseases (Dear and others 2018), nor have ticks been often reported on infected dogs (Conrad and others 1991; Dear and others 2018; Yamane and others 1994). Prior studies did not successfully induce experimental transmission using *Rh. sanguineus* and *D. variabilis*; however, the organism was found in the salivary glands of *Rh. sanguineus* (Yamane and others 1993). Further investigations regarding a possible tick vector of *B. conradae* should be pursued.

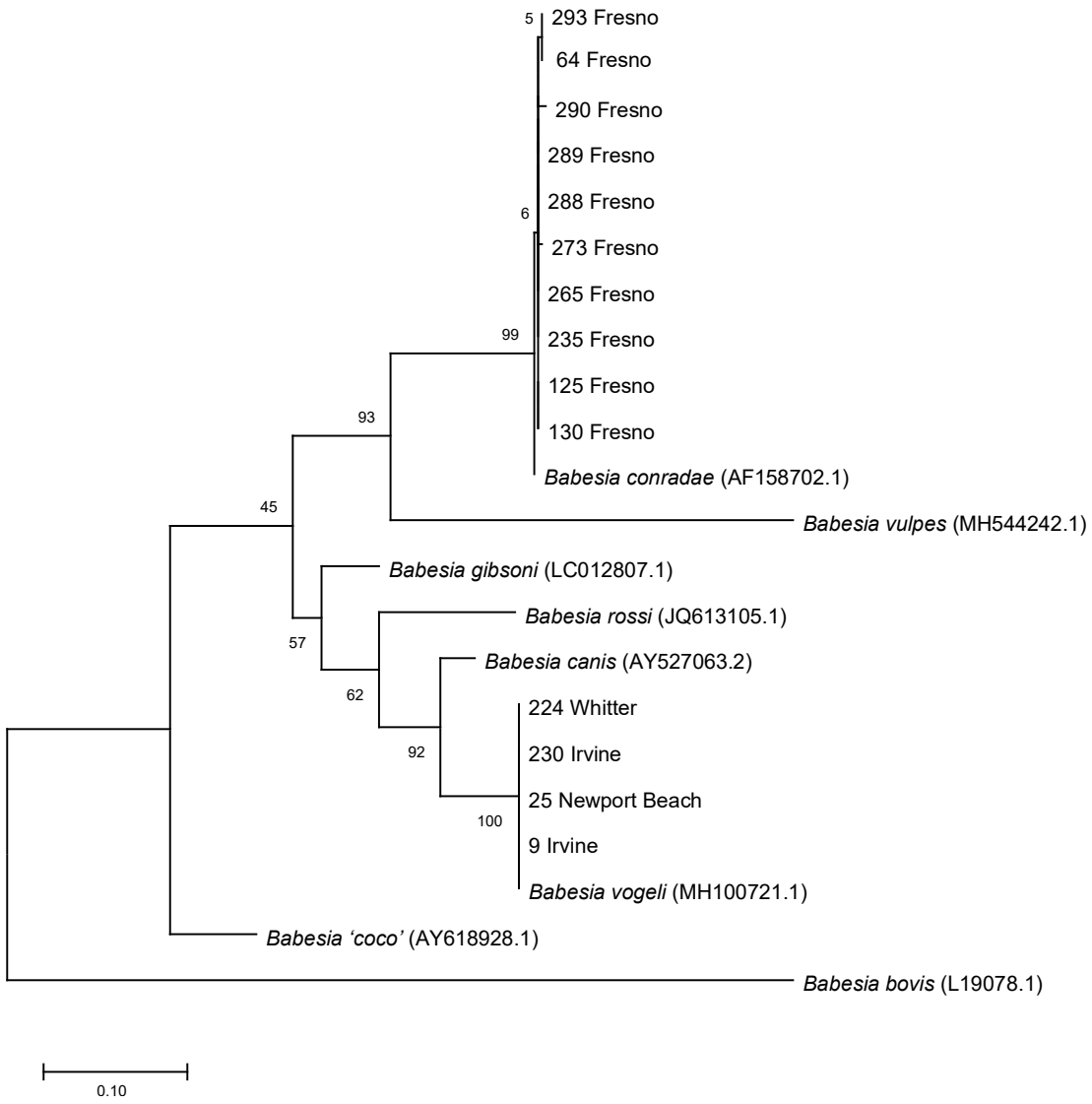

Fig. 3. Maximum likelihood phylogenetic tree of *Babesia* positive coyotes (*Canis latrans*) collected in California from 2015 to 2019 with 7 different published reference sequences from other *Babesia* species for comparison. Scale bar represents percent of genetic variation along tree branches. Labels include coyote ID and location found. Alphanumeric values in parenthesis denote published GenBank sequence. Clades in <60% of bootstraps are collapsed.

5. Conclusion

Our study suggests that coyotes should be investigated as a possible source of infection of *B. conradae* in domestic dogs. Despite the finding that coyotes can be infected, the mechanism of transmission of *B. conradae* remains unknown. Given this evidence of infections in coyotes, targeted investigations into the transmission and ecology of *B. conradae* in wildlife and domestic animals will help elucidate the disease ecology of *B. conradae*. Additionally, *B. vogeli* was identified for the first time in California coyotes and suggests that *B. vogeli* might be more regionally expansive in the United States than previously recognized. Both *B. vogeli* and *B. conradae* have the potential to be clinically important in coyotes and contribute to population effects on the species.

Declaration of competing interest

None.

Acknowledgements

This project was supported by the Judith McBean Foundation and the Student Training in Advanced Research (STAR) Program through Boehringer-Ingelheim Animal Health. JED, LS, and ELP acknowledge support from the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases, funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516). The authors thank Natasha Hunt and Cara Wademan for their assistance during the project.

References

Baneth, G., Florin-Christensen, M., Cardoso, L., Schnittger, L., 2015. Reclassification of Theileria annae as Babesia vulpes sp nov. Parasites Vectors 8.

Barash, N.R., Thomas, B., Birkenheuer, A.J., Breitschwerdt, E.B., Lemler, E., Qurollo, B. A., 2019. Prevalence of *Babesia* spp. and clinical characteristics of *Babesia vulpes* infections in North American dogs. J. Vet. Intern. Med. 33 (5), 2075–2081.

Bekoff, M., 1977. *Canis latrans*. Mamm. Species (79), 1–9.

Birkenheuer, A.J., Buch, J., Beall, M.J., Braff, J., Chandrashekar, R., 2020. Global distribution of canine Babesia species identified by a commercial diagnostic laboratory. Vet Parasitol Reg Stud 22.

Birkenheuer, A.J., Correa, M.T., Levy, M.G., Breitschwerdt, E.B., 2005. Geographic distribution of babesiosis among dogs in the United States and association with dog bites: 150 cases (2000-2003). J. Am. Vet. Med. Assoc. 227 (6), 942–947.

Conrad, P., Thomford, J., Yamane, I., Whiting, J., Bosma, L., Uno, T., Holshuh, H.J., Shelly, S., 1991. Hemolytic anemia caused by *Babesia gibsoni* infection in dogs. J. Am. Vet. Med. Assoc. 199 (5), 601–605.
Crooks, K.R., 2002. Relative sensitivities of mammalian carnivores to habitat fragmentation. Conserv. Biol. 16 (2), 488–502.
Dantas-Torres, F., 2008. The brown dog tick, Rhipicephalus sanguineus (Latreille, 1806) (Acari : ixodidae): from taxonomy to control. Vet. Parasitol. 152 (3–4), 173–185.
Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9 (8), 772-772.
Dear, J.D., Owens, S.D., Lindsay, L.L., Biondo, A.W., Chomel, B.B., Marcondes, M., Sykes, J.E., 2018. *Babesia conradae* infection in coyote hunting dogs infected with multiple blood-borne pathogens. J. Vet. Intern. Med. 32 (5), 1609–1617.
Di Cicco, M.F., Downey, M.E., Beeler, E., Marr, H., Cyrog, P., Kidd, L., Diniz, P., Cohn, L. A., Birkenheuer, A.J., 2012. Re-emergence of *Babesia conradae* and effective treatment of infected dogs with atovaquone and azithromycin. Vet. Parasitol. 187 (1–2), 23–27.
Donnelly, J., Peirce, M.A., 1975. Experiments on the transmission of *Babesia divergens* to cattle by the tick *Ixodes ricinus*. Int. J. Parasitol. 5 (3), 363–367.
Dumond, M., Villard, M.A., 2000. Demography and body condition of coyotes (Canis latrans) in eastern New Brunswick. Can. J. Zool. 78 (3), 399–406.
Duncan, K.T., Grant, A., Johnson, B., Sundstrom, K.D., Saleh, M.N., Little, S.E., 2021. Identification of Rickettsia spp. and Babesia conradae in dermacentor spp. collected from dogs and cats across the United States. Vector Borne Zoonotic Dis. 21 (12), 911–920.
Evers, H.V., Kocan, A.A., Reichard, M.V., Meinkoth, J.H., 2003. Experimental *Babesia gibsoni* infection in coyotes (*Canis latrans*). J. Wildl. Dis. 39 (4), 904–908.
Gage, K.L., Burkot, T.R., Eisen, R.J., Hayes, E.B., 2008. Climate and vectorborne diseases. Am. J. Prev. Med. 35 (5), 436–450.
Gese, E.M., Rongstad, O.J., Mytton, W.R., 1988. Home range and habitat use of coyotes in southeastern Colorado. J. Wildl. Manag. 52 (4), 640–646.
Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52 (5), 696–704.
Harrison, D.J., Harrison, J.A., Odonoghue, M., 1991. Predispersal movements of coyote (*Canis latrans)* pups in eastern Maine. J. Mammal. 72 (4), 756–763.
Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biom. J. 50 (3), 346–363.
Jefferies, R., Ryan, U.M., Jardine, J., Broughton, D.K., Robertson, I.D., Irwin, P.J., 2007. Blood, Bull Terriers and Babesiosis: further evidence for direct transmission of *Babesia gibsoni* in dogs. Aust. Vet. J. 85 (11), 459–463.
Kjemtrup, A.M., Conrad, P.A., 2006. A review of the small canine piroplasms from California: *Babesia conradae* in the literature. Vet. Parasitol. 138 (1–2), 112–117.
Kjemtrup, A.M., Kocan, A.A., Whitworth, L., Meinkoth, J., Birkenheuer, A.J., Cummings, J., Boudreaux, M.K., Stockham, S.L., Irizarry-Rovira, A., Conrad, P.A., 2000. There are at least three genetically distinct small piroplasms from dogs. Int. J. Parasitol. 30 (14), 1501–1505.
Kjemtrup, A.M., Wainwright, K., Miller, M., Penzhorn, B.L., Carreno, R.A., 2006. *Babesia conradae*, sp Nov., a small canine *Babesia* identified in California. Vet. Parasitol. 138 (1–2), 103–111.
Roher, D.P., Anderson, J.F., Nielsen, S.W., 1985. Experimental babesiosis in coyotes and coydogs. Am. J. Vet. Res. 46 (1), 256–262.
Scott, J.D., Pascoe, E.L., Sajid, M.S., Foley, J.E., 2020. Detection of Babesia odocoilei in ixodes scapularis ticks collected from songbirds in ontario and quebec, Canada. Pathogens 9 (10).
Scrivner, J.H., Johnson, C.A., Sego, C.A., 2014. Use of cementum annuli and eye-lens weight for aging coyotes. Wildl. Soc. Bull. 38 (4), 874–877.
Shortt, H.E., 1973. *Babesia canis*: the life cycle and laboratory maintenance in its arthropod and mammalian hosts. Int. J. Parasitol. 3 (2), 119–148.
Sikorski, L.E., Birkenheuer, A.J., Holowaychuk, M.K., McCleary-Wheeler, A.L., Davis, J. M., Littman, M.P., 2010. Babesiosis caused by a large Babesia species in 7 immunocompromised dogs. J. Vet. Intern. Med. 24 (1), 127–131.
Solano-Gallego, L., Baneth, G., 2011. Babesiosis in dogs and cats-Expanding parasitological and clinical spectra. Vet. Parasitol. 181 (1), 48–60.
Solano-Gallego, L., Trotta, M., Carli, E., Carcy, B., Caldin, M., Furlanello, T., 2008. Babesia canis canis and Babesia canis vogeli clinicopathological findings and DNA detection by means of PCR-RFLP in blood from Italian dogs suspected of tick-borne disease. Vet. Parasitol. 157 (3–4), 211–221.
Spielman, A., 1976. Human babesiosis on Nantucket Island: transmission by nymphal *Ixodes* ticks. Am. J. Trop. Med. Hyg. 25 (6), 784–787.
Stayton, E., Lineberry, M., Thomas, J., Bass, T., Allen, K., Chandrashekar, R., Yost, G., Reichard, M., Miller, C., 2021. Emergence of Babesia conradae infection in coyote-hunting Greyhounds in Oklahoma, USA. Parasites Vectors 14 (1).
Thomas, J.E., Chandrashekar, R., Leutenegger, C.M., Yost, G., Reichard, M.V., 2015. Infection of *Babesia Conradae* in Hunting Greyhounds from Oklahoma. Paper presented at the ACVIM Forum, Indianapolis.
Varloud, M., Liebenberg, J., Fourie, J., 2018. Early *Babesia canis* transmission in dogs within 24 h and 8 h of infestation with infected pre-activated male *Dermacentor reticulatus* ticks. Parasites Vectors 11.
Wang, J.W., Zhang, J.L., Kelly, P., Zheng, X.F., Li, M., You, J.F., Huang, K., Qiu, H.X., Wang, Y.Y., Zhang, R., et al., 2018. First description of the pathogenicity of *Babesia vogeli* in experimentally infected dogs. Vet. Parasitol. 253, 1–7.
Wozniak, E.J., Barr, B.C., Thomford, J.W., Yamane, I., McDonough, S.P., Moore, P.F., Naydan, D., Robinson, T.W., Conrad, P.A., 1997. Clinical, anatomic, and immunopathologic characterization of *Babesia gibsoni* infection in the domestic dog (*Canis familiaris*). J. Parasitol. 83 (4), 692–699.
Yamane, I., Gardner, I.A., Ryan, C.P., Levy, M., Urrico, J., Conrad, P.A., 1994. Serosurvey of *Babesia canis, Babesia gibsoni,* and *Ehrlichia canis* in pound dogs in California, USA. Prev. Vet. Med. 18 (4), 293–304.
Yamane, I., Gardner, I.A., Telford, S.R., Elward, T., Hair, J.A., Conrad, P.A., 1993. Vector competence of *Rhipicephalus sanguine* and *Dermacentor variables* for American isolates of *Babesia gibsoni*. Exp. Appl. Acarol. 17 (12), 913–919.
Yu, S.R.N., Modarelli, J., Tomecek, J.M., French, J.T., Hilton, C., Esteve-Gasent, M.D., 2020. Prevalence of common tick-borne pathogens in white-tailed deer and coyotes in south Texas. Int. J. Parasitol.: Parasites Wildl 11, 129–135.