Seroepidemiology of *Bartonella* Infection in Gray Foxes from Texas

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Abstract

Gray foxes (*Urocyon cinereoargenteus*) were shown to be naturally infected with *Bartonella rochalimae*, a *Bartonella* species similar to *Bartonella clarridgeiae* (*B.c.*), and *Bartonella vinsonii* subspecies *berkhoffii* (*B.v.berkhoffii*) in northern California. A serological survey was performed to investigate the presence of *Bartonella* infection in 132 gray foxes from West/Central Texas. Using an immunofluorescence antibody test directed against *B.v.berkhoffii* and *B.c.*, the antibody prevalence was 50% (66/132), with 22 (33.3%) individuals seropositive for *B.c.* only, 8 (12.2%) for *B.v.berkhoffii*, and 36 (54.5%) seroreactive for both *B.c.* and *B.v.berkhoffii*. The foxes had 3.63 more odds (95% confidence interval [CI]=1.38, 10.25) to be seropositive for *B.c.* than for *B.v.berkhoffii*. Female foxes were more likely to be seropositive for *B.c.* (odds ratio [OR]=2.90, 95% CI=1.33, 6.36) and also for both antigens (OR=2.50, 95% CI=1.06, 5.90) than males.

Key Words: *Bartonella*—Zoonosis—Serology.

Introduction

BARTONELLAE ARE GRAM-NEGATIVE, arthropod-transmitted bacteria infecting domestic animals and wildlife, with many species being zoonotic. Most *Bartonella* species exhibit a reservoir host-specificity (Chomel et al. 2009), as they are well adapted for prolonged bacteremia in their mammalian reservoir hosts. However, they can be harmful to incidental hosts as well as the reservoir species (Guptill 2010). *Bartonella vinsonii* subsp. *berkhoffii* (*B.v.berkhoffii*) and *Bartonella rochalimae* are maintained in specific wild canid populations, and are responsible for fatal and nonfatal clinical infections in domestic dogs, including endocarditis, myocarditis, granulomatous inflammatory disease, polyarthritis, cutaneous vasculitis, epistaxis, and bacteremia (Boulouis et al. 2005, Henn et al. 2007, 2009a, 2009b, Guptill 2010). In North America, previous studies, based on serological surveys, blood isolation, or polymerase chain reaction testing, have detected high rates of *B.v.berkhoffii* and *B. rochalimae* infection in free-ranging canid populations, including coyotes (*Canis latrans*) (Chang et al. 2000), gray foxes (*Urocyon cinereoargenteus*) (Henn et al. 2007, 2009b), island foxes (*Urocyon littoralis*) (Namekata et al. 2009, Schaefer et al. 2011), and raccoons (*Procyon lotor*) (Henn et al. 2009a), which are the likely wildlife reservoirs for these two *Bartonella* species or subspecies. However, these studies were restricted to popu-

lations from California. Consequently, information regarding the distribution of these *Bartonella* species outside of California should bring a better insight on their presence among gray fox populations in other parts of the United States. Gray foxes are a cosmopolitan species that interact with domestic animals and can introduce competent *Bartonella* vectors within these domestic populations, with an increased risk of *Bartonella* transmission (Henn et al. 2007, Gabriel et al. 2009). We investigated the seroprevalence of *Bartonella* infection in a convenient sample of gray foxes from central and western Texas, providing information regarding the geographic distribution patterns of *Bartonella* in addition to risk assessment for domestic dogs living in proximity to wildlife.

Materials and Methods

Sample collection

Serum (1 to 2 mL) was collected during rabies oral vaccination postdrop field surveillance of free-ranging gray foxes from 21 counties of central and western Texas in March and April 2004 as part of the Texas Oral Rabies Vaccination Program conducted by the Texas Department of State Health Services/Zoonosis Control Branch. Foxes were harvested by lethal means, and sample collection included various tissues and blood collected from heart puncture (Sidwa et al. 2005).

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Counties where foxes had been trapped were grouped into four regions based on geography (www.travelnotes.org/ustravel/texas/index.htm [accessed July 21, 2011]): Rio Grande/Permian Basin (a sedimentary basin largely contained in the western part of Texas and the southeastern part of the state of New Mexico) (Brewster [$n=1$], Glasscock [$n=3$], Midland [$n=2$], Pecos [$n=6$], Upton [$n=14$], and Terrell [$n=23$] counties), Concho valley (where the Concho river, a tributary of the Colorado river runs) (Coke [$n=21$], Kimble [$n=1$], McCulloch [$n=11$], Menard [$n=1$], Sterling [$n=6$], and Tom Green [$n=1$]), West Central (encompasses the arid and semi-arid lands in the western portion of the state of Texas: Brown [$n=5$], Coleman [$n=1$], Mitchell [$n=1$], Runnels [$n=1$], and Taylor [$n=3$]), and Middle Rio Grande/Alamo (Kerr [$n=1$], Kinney [$n=8$], Real [$n=3$], and Val Verde [$n=19$]).

Serology

Specific antibodies against *B.v.berkhoffii* and *Bartonella clarridgeiae* (*B.c.*) antigens were detected using an indirect immunofluorescence assay (IFA), as previously described (Henn et al. 2007, Namekata et al. 2009). The *B.c.* antigen is a suitable antigen marker for *B. rochalimae* detection (Namekata et al. 2009). Grading of bacillus-specific immunofluorescence was conducted on a scale of 1–4 using the subjective scoring done by the same two individuals in a double-blind manner. Averaged scores of ≥ 2 at a dilution of 1:64 were considered positive for *Bartonella* sp.-specific antibodies. Negative and positive dog control samples were included on each slide.

Statistical tests

Fisher exact test was performed to calculate any difference in prevalence by geographical region, using Epi-Info version 3.5.1.

Results

Sixty-six (50%) of the 132 gray foxes tested had reciprocal IFA titers of ≥ 64 for either *Bartonella* spp., including 22 (33.3%) individuals positive for *B.c.* only, 8 (12.2%) for *B.v. berkhoffii*, and 36 (54.5%) for both antigens. Overall, 58 (88%) foxes were seropositive for *B.c.* and 44 (66.6%) for *B.v.berkhoffii*. Therefore, the foxes had 3.63 more odds (95% confidence interval [CI]=1.38, 10.25) to be seropositive for *B.c.* than for *B.v.berkhoffii*. All foxes were classified as adults (≥ 1 year old) at the time of capture and males accounted for 60.6% (80/132) of the animals. Among the male foxes, 27 (33.7%) were seropositive for *B.c.* and 23 (28.7%) for *B.v.berkhoffii*. Thirty-one (59.6%) females were seropositive for *B.c.* and 21 (40.4%) were seropositive for *B.v.berkhoffii*. Female foxes were also more frequently seropositive for both antigens (38.5%) than male foxes (20%). Female foxes were more likely to be seropositive for *B.c.* (odds ratio [OR]=2.90, 95% CI=1.33, 6.36) and for both antigens (OR=2.50, 95% CI=1.06, 5.90) than male foxes. However, such a statistically significant difference was not detected for *B.v.berkhoffii* (OR=1.68; 95% CI=0.75, 3.73).

Seroprevalence for the four geographical regions was, respectively, 53% ($n=49$) for the Rio Grande/Permian Basin, 51.2% ($n=41$) for Concho valley, 27.3% ($n=11$) for West

FIG. 1. Map of Texas based on geographical features. (Color images available at: www.liebertonline.com/vbz).

Central Texas, and 54.8% ($n=31$) for the Middle Rio Grande/Alamo Area. No difference in prevalence was detected between these geographic regions.

Discussion

We report the first detection of *Bartonella* infection in gray foxes from Texas. Our data suggest that *Bartonella* infection is likely to be widespread among gray fox populations in North America, as it was detected in half of the tested animals. The overall seroprevalence was lower than the 89% ($n=53$) observed in gray foxes from Northern California (Henn et al. 2007) and the 62.7% prevalence reported in island foxes from Santa Rosa Island (Schaefer et al. 2011), but higher than the overall percentage reported in island foxes from four of the Channel Islands (Namekata et al. 2009). A larger percentage of foxes were seropositive for *B.c.* than for *B.v.berkhoffii*. Despite the limitation of this serological study and the possibility of some cross-reactivity between antigens, such data are suggestive that gray foxes in Texas could also be a major reservoir for *B. rochalimae*, as observed in California (Henn et al. 2007) and are less commonly infected with *B.v.berkhoffii*. Further studies will be necessary to confirm this observation based on bacterial isolation or detection by molecular techniques. The higher antibody prevalence in female foxes observed in our study may be an artifact related to convenience sampling or the limited sample size of animals tested. Such an observation will need to be validated through further studies. By comparison, no sex differences were observed in island foxes (female=36/133 [27%], male=32/130 [24.6%]) (Namekata et al. 2009) or in coyotes (female=155/407 [38%], male=152/455 [33%]) from California (Chang et al. 2000). Unfortunately, no information regarding ectoparasite prevalence among these Texan foxes was available. However, it was reported that 10% of fox tails collected for bounties in Texas had ectoparasites on them, mainly fleas (*Pulex irritans*), but also ticks (Eads and Menzies 1950). The cause of the observed difference in seroprevalence between California and Texas could not be determined. Possible factors include lower numbers of competent vectors, lower fox population density in Texas, dryer climatic conditions not as favorable for ectoparasite survival, as well as differing geographic distributions of *Bartonella* spp. between Northern California and Texas. Interpretation of serological results may be complicated by antigen cross-reactivity, as *B.c.* is known to cross react with antibodies specific to other *Bartonella* spp., including *B. rochalimae* and possibly *B.v.berkhoffii*. Given the low number of samples that were only seroreactive to *B.v.berkhoffii* (10.5%), it is plausible to suggest that the antibodies specific to *B.v.berkhoffii* exhibited some cross-reactivity to the *B.c.* antigen or that individuals may have experienced co-infection or sequential infection by both *Bartonella* species. In the wild, gray foxes may live up to 6 to 10 years and therefore will have plenty of opportunities to be exposed to ectoparasites and the pathogens they carry with them during their lifespan (www.nsrl.ttu.edu/tmot1/uroccine.htm [accessed August 30, 2011]).

Bartonellae are emerging zoonotic agents, including *B. rochalimae* and *B.v.berkhoffii*. The detection of infection by these pathogens in gray foxes supports the role of wildlife as a widespread reservoir of zoonotic agents in many parts of the United States, including Texas.

Acknowledgments

The authors would like to thank Eric Fonken for providing contact with the Texas Department of State Health Services, Zoonosis Control Branch. Funding for this research project was partially provided by the Morris Animal Foundation (Grant no. D07Z0-011).

Disclosure Statement

No competing financial interests exist.

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