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ECOLOGY OF *ANAPLASMA PHAGOCYTOPHILUM* INFECTION IN GRAY FOXES (*UROCYON CINEREOARGENTEUS*) IN NORTHWESTERN CALIFORNIA

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ABSTRACT: Although granulocytic anaplasmosis, caused by infection of *Anaplasma phagocytophilum*, is an emerging human and domestic animal disease, the ecology and natural history of the parasite is not well understood. Gray foxes (*Urocyon cinereoargenteus*) are relatively common, occasionally peri-urban mesocarnivores whose geographic distribution overlaps the reported distribution of granulocytic anaplasmosis in humans and domestic animals in North America. We evaluated the potential of foxes as hosts and reservoirs of *A. phagocytophilum* in both urban and backcountry habitats of the Hoopa Valley Indian Reservation, Humboldt County, California, USA. We trapped 54 individual foxes and had 16 recaptures for a total of 70 fox samples between June 2003 and October 2004 in delineated urban and backcountry zones. We collected 296 adult and 145 nymphal ticks from the 70 captured foxes including 193 *Ixodes pacificus*, 149 *Ixodes texanus*, 98 *Dermacentor variabilis*, and one *Dermacentor occidentalis*. There were seasonal differences in tick intensities, with most *I. pacificus* adults occurring in winter and spring ($P < 0.001$), most *I. texanus* nymphs in spring ($P = 0.03$), and most *D. variabilis* adults in spring and summer ($P = 0.01$). Thirty-six (51%) of the 70 fox sera had antibodies against *A. phagocytophilum*, with a higher ($P = 0.24$) prevalence in backcountry foxes (16 of 23) than in urban-zone foxes (12 of 31). Six (9%) of 70 fox samples were polymerase chain reaction–positive for *A. phagocytophilum*. Twenty-eight (31%) of 90 domestic dogs sampled from vaccine clinics within the study area were seropositive for *A. phagocytophilum*. There was a significant difference in prevalence between dogs and backcountry foxes (70%), but no differences were found between dogs and urban foxes (39%). We propose that gray foxes are a good sentinel species for *A. phagocytophilum* infections in northwestern California.

Key words: *Anaplasma phagocytophilum*, domestic dog, gray foxes, *Urocyon cinereoargenteus*, *Ixodes pacificus*, *Ixodes texanus*, mesocarnivore, ticks.

INTRODUCTION

Granulocytic anaplasmosis (GA) is an emerging tick-borne zoonotic disease caused by *Anaplasma phagocytophilum* (Walker and Dumler, 1996). There has been a 75% increase in human case reports since 1999 when the Centers for Disease Control and Prevention designated human granulocytic anaplasmosis (HGA) as a nationally notifiable disease (Centers for Disease Control and Prevention, 2007). Clinical manifestations of HGA range from asymptomatic to severe morbidity, sometimes including a hospitalizing febrile episode and, rarely, mor-

tality (Bakken et al., 1996; Walker and Dumler, 1997). Wide-ranging sequelae have been observed in domestic animals, although clinical signs have not been reported in infected wild animals (Pusterla et al., 2002; Foley et al., 2003; Stuenkel et al., 2003; Tate et al., 2005).

Anaplasma phagocytophilum is transmitted by some *Ixodes* species ticks (Richter et al., 1996; Kramer et al., 1999) including *Ixodes ricinus* (the sheep tick) in Europe (Lillini et al., 2006), *Ixodes persulcatus* (the taiga tick) in Russia and Asia (Eremeeva et al., 2006), *Ixodes scapularis* (the black-legged tick) in eastern North America (Pancholi et al., 1995),

and *Ixodes pacificus* (the western black-legged tick) in western North America (Richter et al., 1996). *Ixodes pacificus* parasitizes a wide variety of vertebrates, including reptiles, birds, and mammals, in 55 of the 58 counties of California at elevations ranging from sea level to approximately 2,150 m (Furman and Loomis, 1984; Centers for Disease Control and Prevention, 2008).

The primary wildlife reservoir of *A. phagocytophilum* in the western United States remains uncertain, although dusky-footed woodrats (*Neotoma fuscipes*) have been implicated as a natural reservoir in California (Nicholson et al., 1999; Foley et al., 2002). Several species of carnivores from California have been documented with exposure to *A. phagocytophilum*, including 46% of coyotes (*Canis latrans*; Pusterla et al., 2000), 17% of mountain lions (*Felis concolor*; Foley et al., 1999), and 93% of American black bears (*Ursus americanus*; Brown, unpubl. data). These larger animals rarely support large numbers of *I. pacificus* immatures (Furman and Loomis, 1984; Castro and Wright, 2007) and are infrequently polymerase chain reaction (PCR)-positive (Foley et al., 1999), suggesting that carnivores do not serve as important reservoirs of *A. phagocytophilum*. However, these carnivores may be competent sentinel species for the detection of *A. phagocytophilum* due to their relatively large home ranges, long life spans, and common exposure to *I. pacificus* nymphs and adults (Eisen et al., 2004).

Gray foxes have received little attention for their possible role in the ecology of GA. Gray fox populations can occur at high densities (Trapp and Hallberg, 1975; Fritzell and Haroldson, 1982), overlap spatially with the reported HGA case distribution (Centers for Disease Control and Prevention, 2007), commonly cohabit areas and structures with humans (Hoff et al., 1974; Harrison, 1997; Neale and Sacks, 2001), and may interact with domestic animals (Riley et al., 2004).

Thus, peri-urban foxes could enhance tick exposure to domesticated animals and humans by carrying ticks harboring *A. phagocytophilum* from wildland habitats to the urban interface. Domestic dogs (*Canis familiaris*) in northern California often are exposed to *I. pacificus* and have relatively high antibody prevalences to *A. phagocytophilum* and other zoonotic pathogens (Foley et al., 2007; Henn et al., 2007). However, although many dogs tend to remain near homes, foxes generally are unrestricted in their movements across the wildland-urban interface (Harrison 1997). Our goals were to describe exposures of *A. phagocytophilum* as well as tick infestation in gray foxes from an *A. phagocytophilum*-enzootic area in northern California, and to evaluate gray foxes for their potential as sentinels of *A. phagocytophilum*.

MATERIALS AND METHODS

Study site

Field sampling was conducted on the Hoopa Valley Indian Reservation (HVIR; 40°03'01''N, 123°40'29''W) in Humboldt County, California, USA (Fig. 1). The reservation is an approximate square with 19-km sides and an area greater than 360 km² (Singer and Begg, 1975; Hoopa Valley Indian Reservation, 2007). The study area is bisected by the Trinity River and elevations on the reservation vary between 76 m in the valley floor to 1,170 m above sea level (Singer and Begg, 1975). Most HVIR inhabitants reside in an urban zone near the valley floor. The vegetation within this area includes Douglas fir (*Pseudotsuga menziesii*), Pacific madrone (*Arbutus menziesii*), tanoak (*Lithocarpus densiflorus*), California blackberry (*Rubus ursinus*), California wild grape (*Vitis californica*), manzanita (*Arctostaphylos* sp.), and annual grasses (Singer and Begg, 1975). Vegetation outside of the valley floor includes Douglas fir, big-leaf maple (*Acer macrophyllum*), Pacific madrone, tanoak, California blackberry, manzanita, California wild grape, and annual grasses (Singer and Begg, 1975). The valley floor and the outlying areas are herein distinguished as urban and backcountry zones, respectively.

Trapping and sampling

Gray foxes were trapped with 81×25×31-cm model 108 Tomahawk traps (Gabriel and

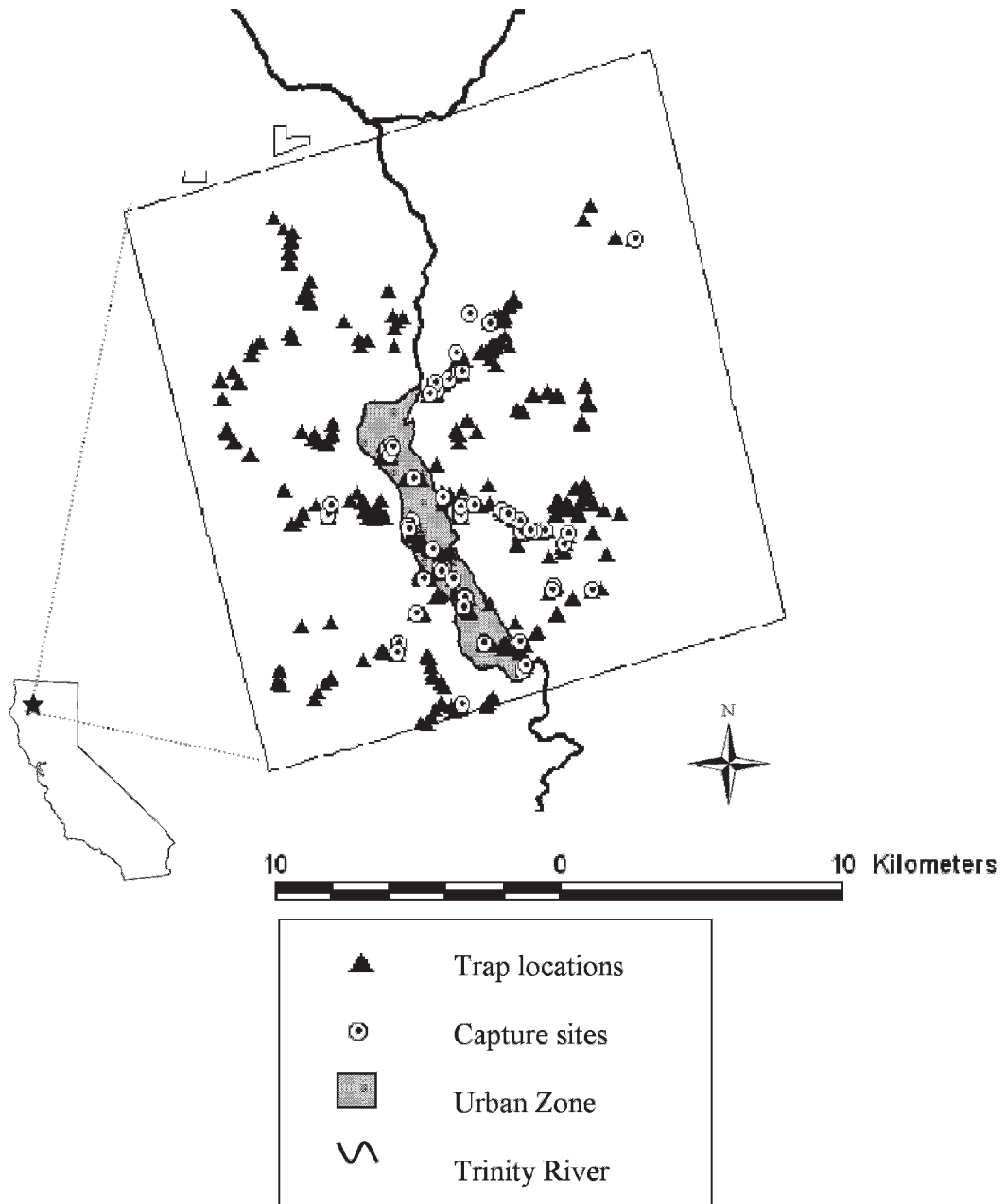


FIGURE 1. Map of trap locations and gray fox (*Urocyon cinereoargenteus*) captures from June 2003 to October 2004 within the Hoopa Valley Indian Reservation, Humboldt County, California, USA. Each trap location is representative of ≥ 2 trap placements.

Wengert, 2005; Tomahawk Live Trap Company, Tomahawk, Wisconsin, USA) with an insulated wooden box attached to the rear of the trap. Wooden boxes were lined with waterproof insulation paneling, which inhibited bacterial and fungal growth (Kemlite

Corporation, Joliet, Illinois, USA). Traps were placed ≥ 30 m from a road edge and parallel to any large downed woody debris or within grass or brush areas with evidence of fresh gray fox feces or tracks. Trap treadle systems were lined with vegetative material and the trap

exterior was covered with a burlap sack and vegetation to conceal and provide insulation for trapped animals.

Fresh chicken was used as bait and traps were checked at ≤ 14 -hr intervals. Captured foxes were weighed and then anesthetized with 20 mg/kg ketamine (Fort Dodge Animal Health, Fort Dodge, Iowa, USA) and 4 mg/kg xylazine (Wildlife Pharmaceuticals, Inc., Fort Collins, Colorado, USA) via intramuscular injection. Blood was collected into sterile Vacutainer tubes containing ethylenediamine-tetraacetic acid (EDTA) and stored at -20 C until analysis. Each fox received a uniquely numbered modified Roto[®] ear tag (Premier 1 Supplies, Washington, Iowa, USA) in each ear for future identification. All observed fleas and ticks were collected and stored in 70% ethanol with 5% glycerol for later identification. Additional samples were collected and archived for future research (Botzler and Armstrong-Buck, 1985) including genetic tissue, fecal samples, digital tooth-wear photos, digital body-condition photos, and morphometric data. A dose of 0.125 mg/kg of yohimbine (Lloyd Laboratories, Shenandoah, Iowa, USA) was administered intravenously after all samples and data were collected and all foxes were released at their capture locations. Foxes recaptured ≤ 4 wk of a previous sampling, and those estimated to be ≤ 3 mo of age, were released without processing.

Blood samples from domestic dogs were collected in March 2004 during an annual spay and neuter clinic conducted by The Humane Society of the United States, Rural Area Veterinarian Services (Salinas, California, USA). For each dog, blood was collected and stored in sterile EDTA tubes. Ectoparasites were not collected from dogs.

Tick identification, serology, and PCR

Adult and nymphal-stage ticks were identified using taxonomic keys (Cooley and Kohls, 1945; Furman and Loomis, 1984). Serology for both foxes and dogs was conducted via indirect immunofluorescence antibody assays (IFA; Dumler et al., 1995) on *A. phagocytophilum* NCH-1 strain substrate slides (VMRD, Pullman, Washington, USA) and samples were considered positive if a strong green fluorescence within morulae was observed at a dilution of $\geq 1:25$. Positive and specific-pathogen-free negative dog controls were included on each slide.

All DNA was extracted from gray fox whole blood using the Dneasy Tissue Kit (Qiagen, Valencia, California, USA), according to manufacturer's instructions. A quantitative real-

time PCR assay targeting the *msp2* protein gene was performed to determine if an animal was bacteremic (Drazenovich et al., 2006). Samples were considered positive if they had a cycle threshold value (C_T) < 40 and characteristic amplification plots.

Data analysis

Seasons were designated as spring (20 March to 20 June), summer (21 June to 21 September), fall (22 September to 20 December), and winter (21 December to 19 March). Data collected during the same season of different years were combined for analyses of seasonal variation. Weather parameters (mean monthly temperature, relative humidity, and precipitation) did not exceed normal values as reported by the Western Regional Climate Center in combined months either year (Western Region Climate Center, Reno, Nevada, USA).

A circular buffer of 129 ha, consistent with the maximum expected gray fox home-range in the western USA, was projected around every capture location (Fuller, 1978; Kodani, 1996; Matthews, 2000; Cypher, 2003). An "urban" fox capture was one whose buffer overlapped the urban zone. A fox whose buffer did not overlap with any part of the urban zone was classified as a "backcountry" fox. Foxes captured in both areas were labeled as "multiple use" foxes.

All statistical analyses were conducted using Number Cruncher Statistical Software (NCSS[®], Kaysville, Utah, USA). Numeric data were screened for normality with a Kolmogorov-Smirnov test before analyses and nonparametric tests were used to analyze nonnormally distributed data.

Fisher's exact tests were used to test for differences in antibody prevalence between the capture areas, sexes, and seasons. An Armitage test was used to analyze for trends in proportions of seropositive foxes through the seasons. A chi-square test was used to test for differences in antibody prevalence between foxes and domestic dogs. Each fox captured ≥ 4 wk from its previous capture was considered an independent data point for PCR analyses because positive PCR results indicate a current infection in the individual (Walker and Dumler, 1996). Fisher's exact tests were used to test for differences in prevalence of PCR-positive foxes between the capture areas, seasons, and sexes.

A generalized linear model analysis of variance was used to test for differences in mean infestation levels of ticks among the seasons, the capture areas, and the sexes. Pair-

wise contrast among seasons was performed with the Tukey-Kramer adjustment for multiple comparisons. A Mann-Whitney *U*-test was used to compare numbers of ticks collected from backcountry and urban foxes.

RESULTS

Traps were set for 1,522 trap-nights with a mean (\pm SE) of 108.7 (\pm 70.7) trap-nights per month between June 2003 and October 2004. Trapping effort was distributed equally between the backcountry and urban regions with 761 trap-nights per region. Fifty-four individual foxes, with 16 recaptures of 10 individuals, were sampled and 23 additional foxes were released without sampling. We captured 33 male and 21 female foxes, 61% and 39%, respectively. Of the 54 individual foxes, 31 were designated urban foxes and 23 were designated backcountry foxes. No recaptured foxes were captured in both zones. The sex ratio of captured foxes did not differ between urban (male:female; 1.2:1) and backcountry (2.3:1) foxes ($\chi^2=1.20$, $df=1$, $P=0.27$). The longest distance a fox moved between captures was 1,237 m, (mean \pm SE = 613 m \pm 139.0 m).

We recovered 488 identifiable ticks from the 70 processed foxes, including 47 larvae, 145 nymphs, and 296 adults; an additional 12 ticks were unidentifiable due to damage during their removal. Identified adult ticks included 191 *I. pacificus* (101 females and 90 males), six *Ixodes texanus* females, 98 *Dermacentor variabilis* (51 females and 47 males), and one *Dermacentor occidentalis* male. We also collected 143 *I. texanus* nymphs and two *I. pacificus* nymphs.

Forty-seven percent (33 of 70) of the gray foxes were infested with *I. pacificus* adults, 3% (2 of 70) with *I. pacificus* nymphs, 8.6% (6 of 70) with *I. texanus* adults, 24% (17 of 70) with *I. texanus* nymphs, 37% (26 of 70) with *D. variabilis* adults, and 1% (one of 70) with *D. occidentalis* adults. There was a significant difference ($Z=2.086$, $P=0.037$) between the number of ticks removed from indi-

vidual foxes in the backcountry (mean \pm SE = 9.8 \pm 2.07) and urban areas (mean \pm SE = 5.1 \pm 1.10).

Mean numbers of *I. pacificus* adults on foxes was greater in winter and spring than in fall and summer ($F=28.17$, $df=3$, $P<0.001$, $n=70$) (Table 1) and in backcountry foxes (mean \pm SE = 4.4 \pm 0.54, $n=30$) compared with urban zone captures (mean \pm SE = 1.5 \pm 0.46, $n=40$; $F=17.57$, $df=1$, $P<0.001$, $n=70$). There was no difference in numbers of *I. pacificus* adults collected from male (3.38 \pm 0.47) and female foxes (1.90 \pm 0.53; $F=0.59$, $df=1$, $P=0.44$). *Ixodes texanus* nymphs also varied by season ($F=3.06$, $df=3$, $P=0.035$), with higher mean numbers in spring compared to fall, winter, and summer (Table 1). There was no difference in the number of foxes infested with *I. texanus* nymphs between the areas of capture (urban: 1.30 \pm 1.07, backcountry: 3.00 \pm 1.24; $F=0.86$, $df=1$, $P>0.05$) or sex of the fox (male: 1.38 \pm 0.108, female: 2.87 \pm 1.22; $F=0.68$, $df=1$, $P>0.05$). *Dermacentor variabilis* removed from foxes was significantly more common in spring and summer compared to winter and fall ($F=4.10$, $df=3$, $P=0.010$; Table 1). There was no difference in numbers of *D. variabilis* adults between the areas of capture (urban: 1.45 \pm 0.40, backcountry: 1.33 \pm 0.46; $F=0.15$, $df=1$, $P=0.70$) or host sex (male: 1.13 \pm 0.41, female: 1.74 \pm 0.46; $F=0.61$, $df=1$, $P=0.44$).

Of the 70 fox captures, 36 were seropositive for antibodies to *A. phagocytophilum* (Fig. 2). Foxes seropositive upon initial capture were seropositive at all subsequent recaptures. Antibodies to *A. phagocytophilum* in foxes were most prevalent in the spring (11 of 15, 73%) and least prevalent in the fall (5 of 22, 23%) ($\chi^2=12.54$, $df=3$, $P=0.0057$). There was a decreasing trend in proportions of seropositive foxes from the summer to winter ($Z=-2.87$, $df=3$, $P=0.002$). Backcountry foxes (16 of 23, 70%) had higher seroprevalence to *A. phagocytophilum*

TABLE 1. Mean numbers (\pm SE) of nymphal and adult tick species removed from gray foxes (*Urocyon cinereoargenteus*) during each season from Hoopa Valley Indian Reservation, Humboldt County, California, USA, June 2003 to October 2004.

Tick species	Season							
	Winter (n=9) ^a		Spring (n=15)		Summer (n=24)		Fall (n=22)	
	Nymph	Adult	Nymph	Adult	Nymph	Adult	Nymph	Adult
<i>Ixodes texanus</i>	0.66 \pm 2.27 (n=6) ^b	0.11 \pm 0.11 (n=1)	6.93 \pm 1.76 (n=104)	0.20 \pm 0.11 (n=3)	0.83 \pm 1.39 (n=20)	0.08 \pm 0.05 (n=2)	0.59 \pm 1.44 (n=13)	0
<i>Ixodes pacificus</i>	0	8.44 \pm 0.98 (n=76)	0.06 \pm 0.06 (n=1)	6.13 \pm 0.75 (n=92)	0.04 \pm 0.04 (n=1)	0.71 \pm 0.60 (n=17)	0	0.27 \pm 0.63 (n=6)
<i>Dermacentor occidentalis</i>	0	0	0	0	0	0.04 \pm 0.04 (n=1)	0	0
<i>Dermacentor variabilis</i>	0	0	0	2.20 \pm 0.66 (n=33)	0	2.67 \pm 0.52 (n=64)	0	0.05 \pm 0.55 (n=1)

^a Total foxes sampled during a season are indicated to the right of the season.

^b Total numbers of ticks of a species and stage removed during a season are indicated below tick means and standard errors.

than urban foxes (12 of 31, 39%) ($\chi^2=5.04$, $df=1$, $P=0.024$). Of all captured foxes, 37% (10 of 27) of the seropositive foxes were female and 55% (18 of 33) were male. This difference was not significant ($\chi^2=0.25$, $df=1$, $P=0.62$).

Twenty-eight (31%) of the 90 dogs sampled at Hoopa Valley Indian Reservation were seropositive for antibodies against *A. phagocytophilum*. Although backcountry foxes were more likely (16 of 23, 70%) to be seropositive than domestic dogs ($\chi^2=11.39$, $df=1$, $P<0.01$), there was no difference in the prevalence of antibodies against *A. phagocytophilum* between domestic dogs and urban foxes (12 of 31; 39%) ($\chi^2=0.60$, $df=1$, $P=0.44$).

Six (9%) of the 70 fox samples were PCR-positive for *A. phagocytophilum*, including four (13%) of the 30 urban and two (6%) of the 34 backcountry foxes. Differences between the areas (2 of 36 [5%] backcountry foxes; 4 of 34 [12%] urban foxes; $\chi^2=0.86$, $df=1$, $P=0.35$), seasons (spring, 1 of 15 [7%]; summer, 3 of 24 [13%]; fall, 2 of 34 [6%]; winter, 0 of 9 [0%]; $\chi^2=1.39$, $df=3$, $P=0.70$), or sexes (male, 4 of 33 [12%]; female, 2 of 21 [10%]; $\chi^2=0.88$, $df=1$, $P=0.77$) were not significant. None of the recaptured foxes were PCR-positive at any subsequent recaptures.

DISCUSSION

This is the first documentation of exposure of gray foxes to *A. phagocytophilum*; we also document a new host as well as a geographic extension for *I. texanus*. Our findings increase an understanding of the ecology of *A. phagocytophilum* maintenance in northern California and provide evidence for the potential value of foxes as sentinels for *A. phagocytophilum* infection within this region.

Ixodes pacificus is a common tick in California that feeds on a large number of host species, including mid- to large-sized carnivores (Furman and Loomis, 1984;

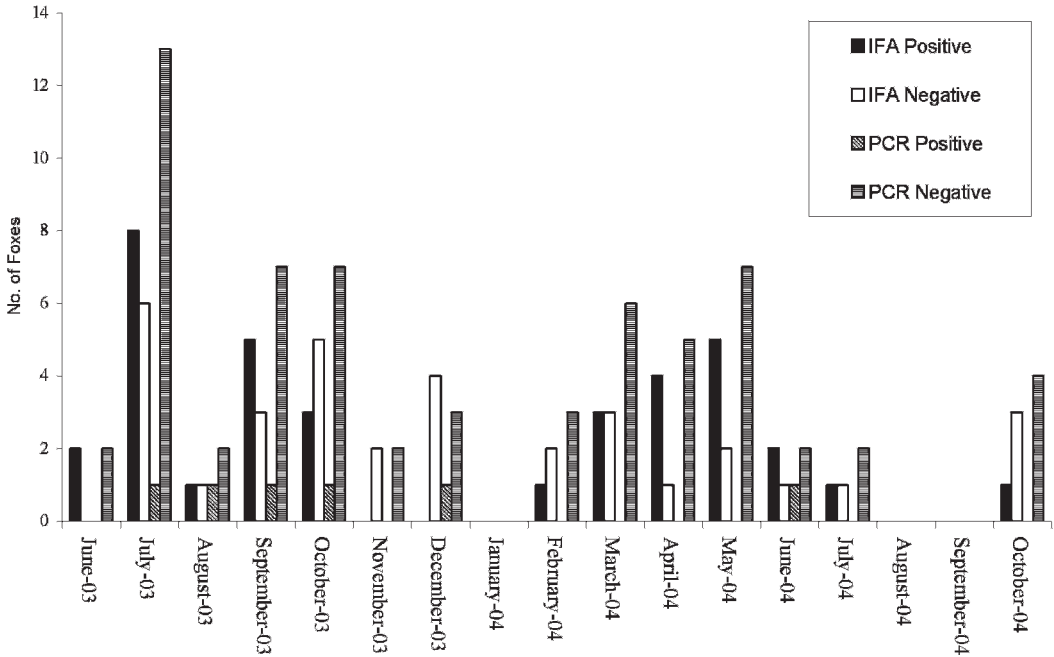


FIGURE 2. Number of foxes with antibodies reactive with *Anaplasma phagocytophilum* based on an immunofluorescent antibody test and polymerase chain reaction detections of *A. phagocytophilum* in gray foxes (*Urocyon cinereoargenteus*) trapped on the Hoopa Valley Indian Reservation, Humboldt County, California, USA, during June 2003 to October 2004.

Castro and Wright, 2007). In northern California coast foothills, *Ixodes pacificus* adults generally have a peak emergence during fall and winter months with 90% decline of the adult cohort by late June (Padgett and Lane, 2001). *Ixodes pacificus* adults were removed from foxes year-round during this study and this pattern could be influenced by several abiotic factors. Survival and the seasonal extension of questing *Ixodes* ticks are positively correlated with relative humidity (Loye and Lane, 1988; Perret et al., 2004). The HVIR is located only 18–25 km from the Pacific Ocean, has a heavily forested landscape, and is subject to frequent coastal fog during the summer months. In nearby northern California landscapes, inundation of summer fog can drop a total of 42.5 cm and 36.3 cm of precipitation during the summer in sites 15 km and 46 km inland, respectively (Azevedo, 1974; Dawson, 1998). Humidity levels during the summer months on the HVIR

can stem from other sources, such as moisture from the Trinity River corridor as well as from local perennial streams, creeks, and springs throughout the study area. This increase in humidity could potentially prolong questing and survival time of *I. pacificus*, thus increasing exposure to tick-borne pathogens for wildlife, domesticated animals, and humans.

Based on our results, foxes from the low-elevation urban zone were less likely to be infested with *I. pacificus* than those captured in the backcountry. The backcountry zone of the HVIR is dominated by brush and trees (Singer and Begg, 1975). In contrast, the urban zone is dominated by grasses and forbs, with few trees and associated leaf litter (Singer and Begg, 1975) providing less suitable microhabitat for immature tick survival (Eisen and Lane, 2000). Density of *I. pacificus* adults has been positively correlated with high brush density, presence of both uphill and

downhill slopes, and trail systems (Li et al., 2000); these are common characteristics of the backcountry of the HVIR. Removal of only two *I. pacificus* nymphs was not surprising given that they feed primarily on small vertebrate hosts such as rodents and lizards (Furman and Loomis, 1984; Eisen and Eisen, 1999; Casher et al., 2002; Castro and Wright 2007); accordingly, immature *I. pacificus* were found in high numbers on rodents in forest stands of different types in the backcountry zone at the HVIR (Whitaker, 2003). Thus, the two immature *I. pacificus* likely represent incidental findings.

Ixodes texanus occurs on gray foxes in several states within the USA, but has not been previously documented on gray foxes in California or in the HVIR. Our detection is a geographic range extension for this tick species within the state of California (Darsie and Anastos, 1957; Furman and Loomis, 1984). *Ixodes texanus* is commonly known as a “mustelid tick” or “raccoon tick” because of its close association with these taxa (Darsie and Anastos, 1957). In this study, adult female *I. texanus*, but no males, were infrequently collected and this is consistent with earlier reports (Cooley and Kohls, 1945; Ouellette et al., 1997). *Ixodes texanus* nymphs in West Coast states have not been reported previously (Furman and Loomis, 1984). Nymphal *I. texanus* are found year-round on various hosts in the eastern USA with a peak emergence between April and June (Furman and Loomis, 1984; Ouellette et al., 1997; Kollars and Oliver, 2003). *Ixodes texanus* has been suggested to be a potential vector for several tick-borne pathogens, including *A. phagocytophilum* and *Rickettsia rickettsii* (Sonenshine et al., 2002; Dugan et al., 2005).

Exposure to *A. phagocytophilum* has not been reported in gray foxes; however, observed prevalence in foxes (50%) was similar to the prevalence (46%) reported from coyotes sampled in California (Pusterla et al., 2000). Also, 93% of black bears sampled within HVIR had antibodies to *A.*

phagocytophilum (Brown, unpubl. data); bears were sampled in both urban and backcountry areas in the HVIR and their large home ranges make it impossible to determine the geographic location at which they were infected (Brown et al., 2004).

The home range buffer used (129 ha) in this study was probably an overestimate of true home range size. Fuller (1978) found female gray foxes in the Sacramento Valley to have a mean home range of 122 ha, Kodani (1996) found female gray foxes in southern California to have a 110-ha home range and males a 71-ha home range, and Matthews (2000) reported home ranges of 54 ha and 58 ha, for males and females, respectively, in southern California. Though distance between recaptures ranged widely, the lack of crossover between urban and backcountry zone captures and differences in *A. phagocytophilum* prevalence between the two areas warrants further investigation.

The seroprevalence among urban foxes (39%) was similar to the overall seroprevalence among domestic dogs (31%). Based on personal observation, care of domestic dogs varies within the HVIR; some owners leash and confine their pets within their property boundaries, whereas others allow their pets to roam freely. During the study, one fox carcass was found near a group of domestic dogs; it had superficial and deep puncture wounds on the skull and along the dorsal midline section; predation by domestic dog was suspected to be the cause of death. At the time of this study there were no tribal laws that restricted roaming of domestic dogs within HVIR. Up to 50% of domestic dogs were seropositive to *A. phagocytophilum* in rural communities adjacent to the study area, whereas dogs from coastal communities had significantly lower prevalences (Foley et al., 2007).

In conclusion, we propose that gray foxes may be valuable sentinel species in determining *A. phagocytophilum* exposure risks for domestic animals and humans.

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LITERATURE CITED

- AZEVEDO, J., AND D. L. MORGAN. 1974. Fog precipitation in coastal California forests. *Ecology* 55: 1135–1141.
- BAKKEN, J. S., J. KRUEH, C. WILSON-NORDSKOG, R. L. TILDEN, K. ASANOVICH, AND J. S. DUMLER. 1996. Clinical and laboratory characteristics of human granulocytic ehrlichiosis. *Journal of the American Medical Association* 275: 199–205.
- BOTZLER, R. G., AND S. B. ARMSTRONG-BUCK. 1985. Ethical considerations in research on wildlife diseases. *Journal of Wildlife Diseases* 21: 341–345.
- BROWN, R. N., M. BELLTAWN, M. W. GABRIEL, J. L. SAJECKI, J. M. HIGLEY, AND J. E. FOLEY. 2004. Evidence of infections by *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l. in American black bears, woodrats, and domestic dogs from inland forest habitats of northern California. *In* Wildlife Disease Association Annual Conference, Arcata, California, 28 August–3 September, 222 pp.
- CASHER, L., R. LANE, R. BARRETT, AND L. EISEN. 2002. Relative importance of lizards and mammals as hosts for ixodid ticks in northern California. *Experimental and Applied Acarology* 26: 127–143.
- CASTRO, M. B., AND S. A. WRIGHT. 2007. Vertebrate hosts of *Ixodes pacificus* (Acari: Ixodidae) in California. *Journal of Vector Ecology* 32: 140–149.
- CENTERS FOR DISEASE CONTROL AND PREVENTION. 2007. Summary of notifiable diseases—United States, 2005. *Morbidity and Mortality Weekly Report* 54: 2–92.
- . 2008. *Established and reported distribution of Ixodes scapularis and Ixodes pacificus by county*. www.cdc.gov/ncidod/dvrd/ehrlichia/Natural_Hx/Tickmap.htm. Accessed May 2008.
- COOLEY, R. A., AND G. M. KOHLIS. 1945. The genus *Ixodes* in North America. *National Institute of Health Bulletin* 184: 1–246.
- CYPHER, B. L. 2003. Foxes. *In* Wild mammals of North America: biology, management and conservation, G. A. Feldhamer, B. C. Thompson, and J. A. Chapman (eds.). Johns Hopkins University Press, Baltimore, Maryland, pp. 511–546.
- DARSIE, R. F., AND G. ANASTOS. 1957. Geographical distribution and host of *Ixodes texanus* Banks (Acarina, Ixodidae). *Annals of the Entomological Society of America* 50: 295–301.
- DAWSON, T. E. 1998. Fog in the California redwood forest: Ecosystem inputs and use by plants. *Oecologia* 117: 476–485.
- DRAZENOVICH, N., J. FOLEY, AND R. N. BROWN. 2006. Use of real-time quantitative PCR targeting the msp2 protein gene to identify cryptic *Anaplasma phagocytophilum* infections in wildlife and domestic animals. *Vector-Borne and Zoonotic Diseases* 6: 83–90.
- DUGAN, V. G., J. K. GAYDOS, D. E. STALLKNECHT, S. E. LITTLE, A. D. BEALL, D. G. MEAD, C. C. HURD, AND W. R. DAVISON. 2005. Detection of *Ehrlichia* spp. in raccoons (*Procyon lotor*) from Georgia. *Vector-Borne Zoonotic Disease* 5: 162–171.
- DUMLER, J. S., K. M. ASANOVICH, J. S. BAKKEN, P. RICHTER, R. KIMSEY, AND J. E. MADIGAN. 1995. Serologic cross-reactions among *Ehrlichia equi*, *Ehrlichia phagocytophila*, and human granulocytic *Ehrlichia*. *Journal of Clinical Microbiology* 33: 1098–1103.
- EISEN, L. T., AND R. J. EISEN. 1999. Abundance of ticks (Acari: Ixodidae) infesting the western fence lizard, *Sceloporus occidentalis*, in relation to environmental factors. *Experimental and Applied Acarology* 23: 731–740.
- , AND R. S. LANE. 2000. Spatial and temporal variation in the density of *Ixodes pacificus* (Acari: Ixodidae) nymphs. *Environmental Entomology* 29: 272–280.
- , R. J. EISEN, AND R. S. LANE. 2004. The roles of birds, lizards, and rodents as hosts for the western black-legged tick *Ixodes pacificus*. *Journal of Vector Ecology* 29: 295–308.
- EREMEEVA, M. E., A. OLIVEIRA, J. B. ROBINSON, N. RIBAKOVA, N. K. TOKAREVICH, AND G. A. DASCH. 2006. Prevalence of bacterial agents in *Ixodes persulcatus* ticks from the Vologda Province of Russia. *Annals of the New York Academy of Sciences* 1078: 291–298.
- FOLEY, J. E., P. FOLEY, M. JECKER, P. K. SWIFT, AND J. E. MADIGAN. 1999. Granulocytic ehrlichiosis and tick infestation in mountain lions in California. *Journal of Wildlife Diseases* 35: 703–709.

- , V. KRAMER, AND D. WEBER. 2002. Experimental infection of dusky-footed wood rats (*Neotoma fuscipes*) with *Ehrlichia phagocytophila* sensu lato. *Journal of Wildlife Diseases* 38: 194–198.
- , C. M. LEUTENEGGER, J. STEPHEN DUMLER, N. C. PEDERSEN, AND J. E. MADIGAN. 2003. Evidence for modulated immune response to *Anaplasma phagocytophila* sensu lato in cats with FIV-induced immunosuppression. *Comparative Immunology, Microbiology and Infectious Diseases* 26: 103–113.
- , R. N. BROWN, M. W. GABRIEL, J. HENN, N. DRAZENOVICH, R. KASTEN, S. L. GREEN, AND B. B. CHOMEL. 2007. Spatial analysis of the exposure of dogs in rural north-coastal California to vector-borne pathogens. *The Veterinary Record* 161: 653–657.
- FRITZELL, E. K., AND K. J. HAROLDSON. 1982. (*Urocyon cinereoargenteus*) mammalian species. *The American Society of Mammalogy* 189: 1–8.
- FULLER, T. K. 1978. Variable home-range sizes of female gray foxes. *Journal of Mammalogy* 59: 446–449.
- FURMAN, D. P., AND E. C. LOOMIS. 1984. The ticks of California (Acari: Ixodida). *Bulletin of the California Insect Survey* 25: 1–239.
- GABRIEL, M. W., AND G. M. WENGERT. 2005. Assessment of the modification of stress-causing environmental factors in an improved nest box design for mesocarnivores. In *Annual Meeting of the Western Section of The Wildlife Society*, Sacramento, California, 19–21 January, 43 pp.
- HARRISON, R. L. 1997. A comparison of gray fox ecology between residential and undeveloped rural landscapes. *Journal of Wildlife Management* 61: 112–122.
- HENN, J. B., M. W. GABRIEL, R. W. KASTEN, R. N. BROWN, J. H. THEIS, J. E. FOLEY, AND B. B. CHOMEL. 2007. Gray foxes (*Urocyon cinereoargenteus*) as a potential reservoir of a *Bartonella clarridgeiae*-like bacterium and domestic dogs as sentinels for zoonotic arthropod-borne pathogens in northern California. *Journal of Clinical Microbiology* 45: 2411–2418.
- HOFF, G. L., W. J. BIGLER, S. J. PROCTOR, AND L. P. STALLINGS. 1974. Epizootic of canine distemper virus infection among urban raccoons and gray foxes. *Journal of Wildlife Diseases* 10: 423–428.
- HOOPA VALLEY INDIAN RESERVATION. 2007. *Climate statistics*. www.hoopa-nsn.gov/government/statistics.htm. Accessed May 2007.
- KODANI, C. H. 1996. Home range and diet of California gray fox (*Urocyon cinereoargenteus californicus*) in the Cleveland National Forest, Orange County, California. M.A. Thesis, California State University, Fullerton, California, 49 pp.
- KOLLARS, M. T., AND J. H. OLIVER. 2003. Host associations and seasonal occurrence of *Haemaphysalis leporispalustris*, *Ixodes brunneus*, *I. cookie*, *I. dentatus*, and *I. texanus* (Acari: Ixodidae) in southern Missouri. *Journal of Medical Entomology* 40: 103–107.
- KRAMER, V. L., M. P. RANDOLPH, L. T. HUI, W. E. IRWIN, A. G. GUTIERREZ, AND D. J. VUGIA. 1999. Detection of the agents of human ehrlichioses in ixodid ticks from California. *American Journal of Tropical Medicine and Hygiene* 60: 62–65.
- LI, X., C. A. PEAVEY, AND R. S. LANE. 2000. Density and spatial distribution of *Ixodes pacificus* (Acari: Ixodidae) in two recreational areas in north coastal California. *American Journal of Tropical Medicine and Hygiene* 62: 415–422.
- LILLINI, E., G. MACRI, G. PROIETTI, AND M. SCARPULLA. 2006. New findings on anaplasmosis caused by infection with *Anaplasma phagocytophilum*. *Annals of the New York Academy of Sciences* 1081: 360–370.
- LOYE, J. E., AND R. S. LANE. 1988. Questing behavior of *Ixodes pacificus* (Acari: Ixodidae) in relation to meteorological and seasonal factors. *Journal of Medical Entomology* 25: 391–398.
- MATTHEWS, R. E. 2000. Activity and home range of the gray fox in the chaparral of southern California. M.S. Thesis, California State University, Fullerton, California, 46 pp.
- NEALE, J. C. C., AND B. N. SACKS. 2001. Food habits and space use of gray foxes in relation to sympatric coyotes and bobcats. *Canadian Journal of Zoology* 79: 1794–1800.
- NICHOLSON, W. L., M. B. CASTRO, V. L. KRAMER, J. W. SUMNER, AND J. E. CHILDS. 1999. Dusky-footed wood rats (*Neotoma fuscipes*) as reservoirs of granulocytic ehrlichiae (*Rickettsiales: Ehrlichiae*) in northern California. *Journal of Clinical Microbiology* 37: 3323–3327.
- OUELLETTE, J., C. S. APPERSON, P. HOWARD, T. L. EVANS, AND J. F. LEVINE. 1997. Tick-raccoon associations and the potential for Lyme disease spirochete transmission in the coastal plain of North Carolina. *Journal of Wildlife Diseases* 33: 28–39.
- PADGETT, K. A., AND R. S. LANE. 2001. Life cycle of *Ixodes pacificus* (Acari: Ixodidae): Timing of developmental processes under field and laboratory conditions. *Journal of Medical Entomology* 38: 684–693.
- PANCHOLI, P., C. P. KOLBERT, P. D. MITCHELL, K. D. REED, JR., J. S. DUMLER, J. S. BAKKEN, S. R. TELFORD, III, AND D. H. PERSING. 1995. *Ixodes dammini* as a potential vector of human granulocytic ehrlichiosis. *Journal of Infectious Disease* 172: 1007–1012.
- PERRET, J. L., O. RAIS, AND L. GERN. 2004. Influence of climate on the proportion of *Ixodes ricinus* nymphs and adults questing in a tick population. *Journal of Medical Entomology* 41: 361–365.
- PUSTERLA, N., J. S. CHAE, R. B. KIMSEY, J. B. PUSTERLA, E. DEROCK, J. S. DUMLER, AND J. E.

- MADIGAN. 2002. Transmission of *Anaplasma phagocytophila* (human granulocytic ehrlichiosis agent) in horses using experimentally infected ticks (*Ixodes scapularis*). *Journal of Veterinary Medicine Series B* 49: 484–488.
- , C. C. CHANG, B. B. CHOMEL, J. S. CHAE, J. E. FOLEY, E. DEROCK, V. L. KRAMER, H. LUTZ, AND J. E. MADIGAN. 2000. Serologic and molecular evidence of *Ehrlichia* spp. in coyotes in California. *Journal of Wildlife Diseases* 36: 494–499.
- RICHTER, P. J., JR., R. B. KIMSEY, J. E. MADIGAN, J. E. BARLOUGH, J. S. DUMLER, AND D. L. BROOKS. 1996. *Ixodes pacificus* (Acari: Ixodidae) as a vector of *Ehrlichia equi* (Rickettsiales: Ehrlichieae). *Journal of Medical Entomology* 33: 1–5.
- RILEY, S. P. D., J. FOLEY, AND B. CHOMEL. 2004. Exposure to feline and canine pathogens in bobcats and gray foxes in urban and rural zones of a national park in California. *Journal of Wildlife Diseases* 40: 11–22.
- SINGER, B. C., AND E. L. BEGG. 1975. Soil survey Hoopa Valley, California. Agriculture Extension Service, University of California, Davis, California, 51 pp.
- SONENSHINE, D. E., R. S. LANE, AND W. L. NICHOLSON (eds.). Ticks (Ixodida). Academic Press, New York, New York, 597 pp.
- STUEN, S., K. BERGSTROM, M. PETROVEC, I. VAN DE POL, AND L. M. SCHOOLS. 2003. Differences in clinical manifestations and hematological and serological responses after experimental infection with genetic variants of *Anaplasma phagocytophilum* in sheep. *Clinical Diagnostic Laboratory Immunology* 10: 692–695.
- TATE, C. M., D. G. MEAD, M. P. LUTTRELL, E. W. HOWERTH, V. G. DUGAN, U. G. MUNDERLOH, AND W. R. DAVIDSON. 2005. Experimental infection of white-tailed deer with *Anaplasma phagocytophilum*, etiologic agent of human granulocytic anaplasmosis. *Journal of Clinical Microbiology* 43: 3595–3601.
- TRAPP, G. R., AND D. L. HALLBERG. 1975. Ecology of the gray fox (*Urocyon cinereoargenteus*): A review. In *The wild canids: Their systematics, behavioral ecology and evolution*, M. W. Fox (ed.). Van Nostrand-Reinhold, New York, New York, pp. 164–178.
- WALKER, D. H., AND J. S. DUMLER. 1996. Emergence of the ehrlichioses as human health problems. *Emerging Infectious Diseases* 2: 18–29.
- , AND ———. 1997. Human monocytic and granulocytic ehrlichioses. Discovery and diagnosis of emerging tick-borne infections and the critical role of the pathologist. *Archives of Pathology and Laboratory Medicine* 121: 785–791.
- WHITAKER, D. A. 2003. Relation of thin and release timber management practices to abundance of woodrats, chipmunks, mice, and ticks within the Hoopa Valley Indian Reservation. M.S. Thesis, Humboldt State University, Arcata, California, 42 pp.

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