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SPATIAL AND TEMPORAL VARIATION IN THE SEROPREVALENCE OF CANINE HEARTWORM ANTIGEN IN THE ISLAND FOX

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ABSTRACT: Island foxes (*Urocyon littoralis*) are endemic to six of the eight California Channel Islands (USA). The island fox is classified as a threatened species by the State of California, and recently three of the six subspecies have experienced abrupt population declines. As part of a continuing effort to determine the cause of the declines, we tested island fox serum samples collected in 1988 (n = 176) and 1997–98 (n = 156) over the entire geographic range of the species for seroprevalence of canine heartworm (*Dirofilaria immitis*) antigen. Using a commercially available enzyme-linked immunosorbent assay (PetChek®, Idexx Laboratories, Westbrook, Maine, USA) we detected heartworm antigen in four of the six populations of island foxes. On San Miguel and Santa Rosa Islands, seroprevalence in adult foxes was >85% (n = 62) in 1988 and increased to 100% (n = 24) in 1997–98. On Santa Cruz Island, seroprevalence in adult foxes decreased from 83% (n = 30) to 58% (n = 26), whereas on San Nicolas Island, seroprevalence increased from 25% (n = 32) to 77% (n = 30) during the same period. All of the pups assayed (n = 33) were seronegative. The seroprevalences of heartworm reported herein for the four populations of island foxes are the highest yet reported for a fox species. However, additional demographic data reported elsewhere suggests that heartworm has not been a major factor in the recent declines of island fox populations.

Key words: *Dirofilaria immitis,* heartworm antigen, island fox, seroprevalence, survey, *Urocyon littoralis.*

INTRODUCTION

Island foxes (*Urocyon littoralis*) are a unique dwarf form endemic to the six largest of the eight Channel Islands located off the coast of southern California (USA; Moore and Collins, 1995). A flagship species for the Channel Islands, island foxes are California's only endemic carnivore. They are currently listed as threatened by the State of California (California Department of Fish and Game, 1987) and were considered a species of special concern by the federal government (USFWS, 1989). Their limited geographic distribution, small population sizes, low genetic variablity and lack of exposure to potentially lethal canine diseases makes this species particularly vulnerable to extinction (Garcelon et al., 1992; Roemer et al., 1994; Goldstein et al., 1999). Recently, three of the six island fox populations have undergone dramatic population declines and are now facing extirpation (Coonan et al., 1998; Roemer, 1999). As part of an ongoing effort to understand the factors responsible for the declines, we serologically assayed for canine heartworm (*Dirofilaria immitis*), a filarial nematode with potentially lethal consequences for canids (Strickland, 1998), across the entire geographic range of the island fox.

MATERIALS AND METHODS

We surveyed island fox sera from the six California Channel Islands where foxes occur including San Miguel (33°02′N, 120°18′W), Santa Rosa (33°57′N, 120°06′W), Santa Cruz (34°0′N, 119°45′W), San Nicolas (33°14′N, 119°30′W), San Clemente (32°55′N, 118°30′W), and Santa Catalina (33°24′N, 118°24′W). The California Channel Islands are characterized by warm, dry summers and mild, wet winters. Annual precipitation is variable, owing to the influence of the El Niño-Southern Oscillation phenomenon that brings occasional periods of heavy winter rains

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TABLE 1. Seroprevalence (%) to canine heartworm detected in adult island foxes, in 1988 and 1997–98, across the entire geographic range of the fox. Sample size is in parentheses. Chi-square analyses represent comparisons between sampling periods. The Chi-square value for San Miguel Island includes Santa Rosa Island because of small sample sizes in 1997–98. All pups sampled in 1988 ($n = 5$) and 1997–98 ($n = 28$) were seronegative.

	1988	1997–98	χ^2	P
Northern Channel Islands				
San Miguel Island	96 (24)	100 (18)	2.5	=0.11
Santa Rosa Island	87 (38)	100 (6)		
Santa Cruz Island	83 (30)	58 (26)	4.5	≤0.03
Southern Channel Islands				
San Nicolas Island	25 (32)	78 (30)	16.5	≤0.0001
San Clemente Island	0 (30)	0 (31)		
Santa Catalina Island	0 (17)	0 (17)		

or, alternately, periods of drought. Mean monthly temperatures on Santa Cruz Island (1961 to 1971) varied between 12 and 21 C and mean annual precipitation (1904 to 1993) was 50 cm (Junak et al., 1995). The southern islands are typically warmer and drier; mean annual precipitation on San Clemente Island is 16 cm (Keegan et al., 1994).

Island foxes were trapped using welded-wire box traps (23 × 23 × 66 cm, Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) arranged in rectangular grids or set as line transects. Foxes were sexed and then aged according to tooth eruption and wear patterns on the first upper molar (Wood, 1958) and were assigned to one of five age classes. Foxes were classified as pups (Age Class 0, 1- to 9-mo-old), young adults (Age Class 1, 9-mo to 2-yr-old), adults (Age Class 2, 2- to 3-yr-old), mature adults (Age Class 3, 3- to 5-yr-old) and old adults (Age Class 4, ≥5-yr-old). Five to 10 ml of blood were taken from the femoral vein of unanaesthetized foxes just prior to release.

We assayed 176 fox serum samples (171 adults and 5 pups) from 1988 and 156 samples (128 adults and 28 pups) from 1997–98 for antigen of *D. immitis*. Heartworm seroprevalence was determined using a commercially available, enzyme-linked immunosorbent assay (ELISA) that detects the presence of circulating antigen mainly originating from the reproductive tract of adult, female worms (PetChek®, Idexx Laboratories, Westbrook, Maine, USA).

Antigen-based ELISA methods are highly sensitive, correctly classifying from 75% to 99% of confirmed heartworm infections, are able to detect heartworm antigen as early as 6 mo postinfection, consistently yield positive results with five or more adult worms, and typically do not cross-react with other parasite antigens (Dzimianski and McCall, 1986; Bland et al., 1995). Clinical trials assessing the accuracy of antigen-based ELISAs have been primarily restricted to domestic dogs. For ethical and legal reasons we could not euthanize and necropsy captured animals to assess the accuracy of PetChek for diagnosing heartworm infection in island foxes. However, we did retest 40 serum samples with another commercially available ELISA kit (DiroCHEK®, Synbiotics Corporation, San Diego, California, USA) for comparison with PetChek. We tested for association between variables via chi-square analysis (SYSTAT 7.0, SPSS Inc., Chicago, Illinois, USA) using a significance level of $P \leq 0.05$.

RESULTS

Dirofilaria immitis was not detected in any of the pups, but was detected in adult foxes from the three northern Channel Islands and from one southern Channel Island (Table 1). Overall seroprevalence among adults from the four populations that tested positive was 74%; overall seroprevalence was 72% in 1988 and 78% in 1997–98. There was no association between seroprevalence and sex ($\chi^2 = 0.4$, $P = 0.83$). Seroprevalence in adult foxes differed among islands in 1988 ($\chi^2 = 47.6$, $P < 0.0001$), and was nearly significant in 1997–98 ($\chi^2 = 7.1$, $P = 0.07$). On Santa Rosa and San Miguel Islands, seroprevalence was >85% in both 1988 and in 1997–98 (Table 1). Heartworm seroprevalence in adult foxes decreased on Santa Cruz Island from 1988 to 1997–98, but in-

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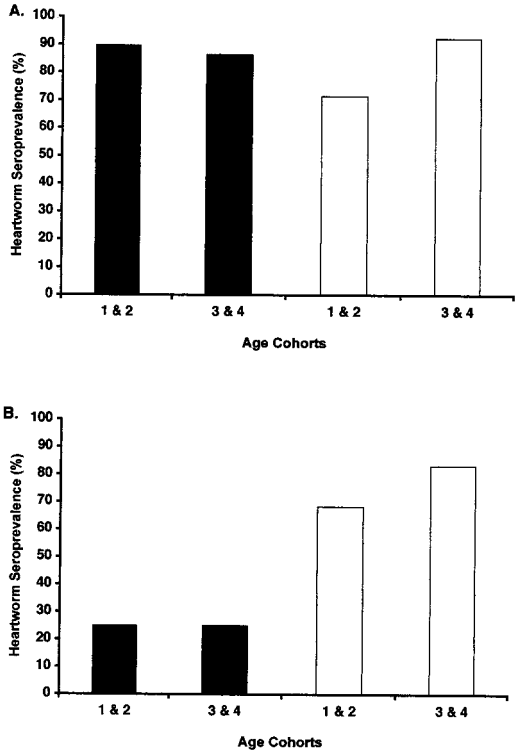


FIGURE 1. Seroprevalence (%) to canine heartworm antigen in young adult island foxes (Age Classes 1 and 2) and old adult island foxes (Age Classes 3 and 4) from the northern Channel Islands (A) and San Nicolas Island (B) in 1988 (black bars) and 1997–98 (white bars). There was no difference in seroprevalence owing to age class during either sampling period on the northern Channel Islands (1988: $\chi^2 = 0.2$, $P = 0.63$, 1997–98: $\chi^2 = 2.34$, $P = 0.1$), or on San Nicolas Island (1988: $\chi^2 = 0.0$, $P = 1.0$, 1997–98: $\chi^2 = 0.9$, $P = 0.35$).

creased on San Nicolas Island during this same period (Table 1).

There was no association between age class of adult foxes and seroprevalence in 1988 or in 1997–98 on the three northern islands with heartworm or on San Nicolas Island (Fig. 1). Seroprevalence in young adult foxes (Age Classes 1 and 2) was similar to seroprevalence in older foxes (Age Classes 3 and 4).

The proportion of older adults (Age Class 3 and 4) in the fox populations with heartworm declined between 1988 and 1997–98 ($\chi^2 = 13.0$, $P \leq 0.0003$) but the proportion of older adults did not change in the two populations without heartworm ($\chi^2 = 0.9$, $P = 0.35$). Fox populations with heartworm had 72 (58%) older individuals in 1988 but only 25 (32%) in 1997–98. Fox populations without heartworm had 11 (23%) older individuals in 1988 and 15 (32%) in 1997–98.

The results of the two ELISA methods were similar. Of the 40 samples assayed with both methods, 20 were seropositive with PetChek and 17 (85%) of these were seropositive with DiroCHEK; the remaining 20 samples were seronegative with both ELISA methods.

DISCUSSION

In this study we recorded a much higher prevalence of heartworm in island foxes than previously reported for other fox species. Although heartworm infection varies by species and area for wild canids, heartworm prevalence in gray foxes (*U. cinereoargenteus*), the closest relative to island foxes (Goldstein et al., 1999), has been low. Reported heartworm prevalence in gray foxes ranges from 0% to 24% (Crowell et al., 1978; Simmons et al., 1980; Lavoipierre et al., 1986; Wixsom et al., 1991). Reported prevalence for red fox (*Vulpes vulpes*) ranges from 4% to 28% (Stuht and Youatt, 1972; King and Bohning, 1984). Recent prevalence of heartworm in coyotes (*Canis latrans*) is higher (up to 90%), and coyotes are now considered a significant reservoir for heartworm in some areas (Sacks, 1998).

The high seroprevalence of heartworm recorded in island foxes is surprising compared with the low prevalences observed in the gray fox and in other mainland canids. The values we report may be relatively high due to the greater sensitivity of the antigen-based ELISA methods. Earlier studies of heartworm prevalence in canids looked for adult worms in the heart, lungs and associated vessels, or searched for microfilariae in tissues and blood. Because low nematode intensities may be missed in gross examination and occult infections will be missed when searching for micro-

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filariae, these methods may be less sensitive than the antigen-based serologic methods (Brunner et al., 1988).

Early serologic techniques used to diagnose heartworm infection were based on the detection of antibodies produced by the infected host. These methods proved to be of limited value because the same antibodies were produced in response to other parasitic infections as well. Subsequently, more accurate antigen-based ELISAs (e.g., Petchek and DiroCHEK) were developed that reacted with specific antigens released by adult female *D. immitis,* thereby reducing cross-reactivity (Dzimianski and McCall, 1986). Antigen-based ELISA methods have been shown to distinguish infections of *D. immitis* from other parasitic infections including *D. repens, Dipetalonema reconditum,* and some genera of intestinal parasites including *Ancylostoma, Toxocara, Trichuris,* and *Uncinaria* (Dzimianski and McCall, 1986; Brunner et al., 1988; J. W. McCall, unpubl. data). Despite their lack of cross-reactivity with the above mentioned genera, it is possible that both ELISA methods are cross-reacting with antigen from another untested parasite.

None of the pups sampled tested positive for heartworm. The absence of seropositive pups was expected because pups would have been exposed to mosquitoes for only a short time (3 to 5 mo), and because the test used is insensitive to the early stages of infection (Dzimianski and McCall, 1986). It takes from 85 to 120 days following infection for larval worms to migrate to the heart and dogs are not antigen-positive until at least 6 mo after infection; thus it is unlikely that pups would harbor adult worms. Further, the sensitivity of serologic detection of occult infections is influenced by the number of adult worms present (Brunner et al., 1988); if pups had been infected their worm burden would likely have been low.

Other canid populations have shown increasing seroprevalence to heartworm with age (Sacks, 1998), but we did not detect this association in our sample of adult foxes. There were spatial and temporal differences in seroprevalence, however, seroprevalence differed among islands in 1988, and significantly increased on San Nicolas Island between the two sampling periods. On Santa Cruz Island, seroprevalence decreased in adult foxes, but this difference was marginally significant and may actually be smaller than the change (25%) indicated by our samples.

Heartworm was apparently introduced to at least four of the six island fox populations prior to 1988. Before 1970, there were only 12 reported cases of *D. immitis* in domestic dogs from northern California (McGreevey et al., 1970), but by 1980, *D. immitis* was widespread in dogs from mountainous parts of northern California and had appeared in local coyote populations (Weinman and Garcia, 1980). Heartworm has since spread to the foothills of northern California (Sacks, 1998), and autochthonous infections in domestic dogs have been reported as far south as Los Angeles County (Theis et al., 1999). The most probable agents of heartworm dispersal to the Channel Islands are domestic dogs that have been used as working dogs on island ranches and those that have been brought to the islands by recreational boaters and island residents (Roemer et al., 1994). Furthermore, at least two genera of mosquitoes (*Aedes* and *Culex*) known to transmit *D. immitis* (Parker, 1986) are present on the California Channel Islands (Spadoni et al., 1973).

MANAGEMENT IMPLICATIONS

The high seroprevalence of heartworm on San Miguel, Santa Rosa, Santa Cruz and San Nicolas Islands, and the decrease in the proportion of older foxes on these islands from 1988 to 1997–98 suggest that heartworm may have contributed to mortality of older foxes. Although heartworm can have a debilitating effect on the health of wild canids (Weinman and Garcia, 1980; Carlson and Nielsen, 1983; Gortazar et al., 1994), we believe that heartworm has

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probably not been a major factor in the recent population declines of island foxes. First, heartworm was present in the four island fox populations in 1988 or before, and the fox population on San Nicolas Island has not declined between the sampling periods (Roemer, 1999). Thus the population declines are geographically limited to the northern Channel Islands and not completely correlated with the geographic distribution of heartworm seroprevalence in island foxes. Second, during 1993 and 1994, fox density was high (5.5 to 15.9 foxes/km^2) on both San Miguel and Santa Cruz Islands despite a high seroprevalence to heartworm (Coonan et al., 1998; Roemer, 1999). Third, during the years prior to the population declines, survival of pup, yearling and adult island foxes was high (0.75 to 1.0). Annual survival of all age classes began to decline in 1994 and continued to decline through 1998 averaging <0.4 (Roemer, 1999). This suggests that the same agent(s) simultaneously affected the survival of all age classes. Heartworm would not be expected to cause mortality in young foxes as adult worm burdens should be low or non-existent in pups. Finally, recent necropsy results have found no evidence of high nematode intensities or of chronic heartworm disease (L. Munson, pers. comm.). Currently, the only factor that has been linked to the decline in island foxes on the northern Channel Islands is an increase in predation by golden eagles (*Aquila chrysaetos*) (Roemer, 1999).

The observed high seroprevalence of heartworm in island foxes contrasts markedly with the low prevalence recorded for most wild canid populations and raises the question as to whether commercially available, antigen-based ELISA kits cross-react with other parasite antigens and are adequate methods of heartworm diagnosis in wild canids. Determining the actual effect of high heartworm seroprevalence in island fox populations will require additional information on gross pathology and quantification of actual heartworm-induced mortality.

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