

## SEROLOGIC SURVEY OF THE ISLAND SPOTTED SKUNK ON SANTA CRUZ ISLAND

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**ABSTRACT.**—Two rare endemic carnivores occur on California's northern Channel Islands: island spotted skunks (*Spilogale gracilis amphiala*) and island foxes (*Urocyon littoralis*). As insular carnivores, both may be particularly vulnerable to novel pathogens. We tested sera from 31 island spotted skunks on Santa Cruz Island, where both skunks and foxes occur sympatrically, to establish their exposure to disease agents. One skunk (3%) tested positive for canine heartworm, and 3 skunks (10%) tested positive for canine parvovirus. None showed evidence of exposure to canine adenovirus, canine distemper virus, canine herpesvirus, *Leptospira* bacteria (serovars *pomona*, *canicola*, and *icterohaemorrhagiae*), or pseudorabies virus. Spotted skunks on Santa Cruz Island apparently have low susceptibility or exposure to these pathogens and are unlikely to be reservoirs of these diseases for island foxes.

**Key words:** *Spilogale gracilis amphiala*, *island spotted skunk*, *Santa Cruz Island*, *canine heartworm*, *canine parvovirus*, *canine adenovirus*, *canine distemper virus*, *canine herpesvirus*, *Leptospira*, *pseudorabies virus*.

Islands typically support few terrestrial mammals because they have poor capacity for overwater dispersal. Insular carnivores are especially rare because their low carrying capacities increase their risk of extinction when land area is limited (Heaney 1984, Purvis et al. 2000). Thus, California's Channel Islands are unusual in supporting 2 endemic carnivores—the island fox (*Urocyon littoralis*) and the island spotted skunk (*Spilogale gracilis amphiala*). Taxa on islands evolve in isolation and frequently lack natural defenses to predators and pathogens, further elevating extinction risk (Van Riper et al. 1986, Bowen and Van Vuren 1997, Roemer et al. 2001), and carnivores appear particularly susceptible to diseases (Young 1994, Macdonald 1996). Consequently, diseases are a primary conservation concern for both island foxes and island spotted skunks.

Within the last 10 years 4 island fox subspecies have declined drastically, resulting in their federal listing as endangered (USFWS 2004) and necessitating captive propagation to avert their extinction (Coonan 2003). Predation by Golden Eagles (*Aquila chrysaetos*) and the recent appearance of canine distemper are assumed to be the primary causes of this decline

(Timm et al. 2000, Roemer et al. 2001, USFWS 2004), consistent with the profile of a naive island species. Currently, the health of island foxes is closely monitored (T. Coonan personal communication).

In contrast, relatively little is known about the population dynamics and health status of island spotted skunks, which inhabit the 2 largest Channel Islands (Santa Cruz and Santa Rosa), where they are sympatric with island foxes. Prior to the decline of the fox, island spotted skunks were very rare (Laughrin 1980, Crooks 1994). Although they are still uncommon and remain a state-listed species of special concern (Williams 1986, Crooks and Van Vuren 2000), their numbers have increased concurrent with the decline of the fox (Crooks and Van Vuren 2000, Roemer et al. 2001), presenting an opportunity to perform health screening essential for the conservation of both foxes and skunks on the Channel Islands. Previously, skunks were so rare that acquiring adequate sample sizes was difficult (Crooks 1994, Crooks and Van Vuren 1995). Thus, baseline hematologic and serum chemistry parameters and data on ectoparasite loads have emerged only recently (Crooks et al. 2003,

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Crooks et al. 2004). Such basic health information is important both because skunks may serve as reservoirs for diseases that threaten island foxes and because, as a vulnerable insular endemic subspecies, they have conservation value in their own right.

The goal of this study was to establish baseline disease status by conducting a serological analysis to assess exposure of the island spotted skunk to pathogens on Santa Cruz Island. We tested for disease agents previously detected in island foxes that are known to cause morbidity or mortality to canids, including canine heartworm, canine adenovirus, canine distemper virus, canine herpesvirus, canine parvovirus, and *Leptospira* bacteria (Garcelon et al. 1992, Crooks et al. 2001, Roemer et al. 2001). In addition, we tested for pseudorabies virus, a disease of swine, because feral pigs were present on the island. All these disease agents appeared capable of infecting mephitids or mustelids (Trainer and Karstad 1963, Keymer and Epps 1969, Karstad et al. 1975, Barker et al. 1983, Snyder et al. 1989, Kimber et al. 2000, Leighton and Kuiken 2001).

#### METHODS

Santa Cruz Island, located 40 km south of Santa Barbara, California, experiences a maritime Mediterranean climate with periodic cool winter rains typically beginning in November and hot, dry summers beginning in May. We live-trapped skunks in August 2000 and January 2001 throughout most of the island with single-door box traps baited with commercial cat food and fruit paste (Nick Wyshinski, Berwick, PA). Animals were temporarily marked so that individuals could be identified within sampling periods. Trap location, sex, weight, and other distinguishing characteristics were used to identify individuals between sampling periods. We assumed all captured skunks were young adults or adults based on typical late-spring litter production (Howard and Marsh 1982) and age-specific weight criteria (Crabb 1994, Crooks 1994). All captured skunks appeared healthy.

Captured skunks were manually restrained for processing, because use of anesthesia may have influenced blood parameters. A veterinarian removed 3–4 mL of blood (<1% of body mass) from the jugular vein. A portion was prepared for hematological analysis, and

the remainder was placed in sterile serum-separator tubes, allowed to clot for  $\geq 2$  hours, and then centrifuged at 3000 rpm for up to 10 minutes. On the same day as centrifugation, serum was extracted from clotted samples with Pasteur pipettes and placed in 1.5 mL Eppendorf vials. Serum samples were maintained at approximately 4°–6°C for 1–4 days prior to analysis. Each skunk was released unharmed at the point of capture.

We used a commercially available micro-well enzyme-linked immunosorbent assay (ELISA) to detect heartworm antigen in skunk sera (DiroChek Synbiotics Corporation, San Diego, CA), with testing performed by Home-Vet Hospice (Davis, CA). All other testing was conducted by personnel at the Animal Health Diagnostic Laboratory, Cornell University (Ithaca, NY), who used serum neutralization tests to screen samples for antibodies to canine distemper virus, canine adenovirus 1 (infectious canine hepatitis), and canine herpesvirus and agglutination assays to detect antibodies to canine parvovirus (hemagglutination inhibition), pseudorabies virus (latex agglutination), and *Leptospira* (micro-agglutination). We tested for 3 *Leptospira* serovars: *pomona*, present in sea lions and pigs in California; *canicola*, occurring in dogs; and *icterohaemorrhagiae*, detected in island foxes on Santa Cruz Island (Roth 1970, Garcelon et al. 1992, Gulland et al. 1996). Starting dilutions for distemper, adenovirus, herpesvirus, and parvovirus tests were 1:10 with the exception of 1 distemper and 3 herpesvirus samples started at 1:20 because sera showed toxicity at lower dilutions. Pseudorabies and *Leptospira* dilutions were 1:4 and 1:100, respectively.

#### RESULTS

We collected blood from 31 different skunks. Serologic testing revealed positive results for only 2 pathogens (Table 1). One skunk ( $n = 31$ ) tested positive for canine heartworm antigens. Three skunks ( $n = 30$ ) exhibited antibodies to canine parvovirus at titer levels of 1:10. Limited volumes of serum from some individuals precluded some tests and resulted in variable sample sizes (Table 1).

#### DISCUSSION

Island spotted skunks exhibited a low prevalence of disease exposure relative to island

TABLE 1. Prevalence of positive tests for pathogens among island spotted skunks sampled on Santa Cruz Island during August 2000 and January 2001.

Pathogen	<i>n</i>	Prevalence (%)
Canine heartworm	31	3
Canine parvovirus	30	10
Canine adenovirus	29	0
Canine distemper virus	29	0
Canine herpesvirus	29	0
<i>Leptospira pomona</i>	28	0
<i>Leptospira canicola</i>	28	0
<i>Leptospira icterohaemorrhagiae</i>	28	0
Pseudorabies virus	29	0

foxes. Although foxes on Santa Cruz Island have tested positive for canine heartworm at rates of  $\geq 58\%$  (assessed with the same or similar ELISA tests; Roemer et al. 2000, Crooks et al. 2001), only 3% of skunks tested positive. Heartworm is a filarial nematode (*Dirofilaria immitis*) that primarily affects canids, especially domestic dogs (*Canis familiaris*). It is vectored by mosquitoes and causes a serious, progressive, and potentially fatal condition by obstructing blood flow in the heart and adjacent blood vessels (Georgi and Georgi 1990). Reports of heartworm affecting mustelids are isolated (Williams and Dade 1976, Snyder et al. 1989, McCall 1998) and no reports exist for mephitids. The Dirochek test, intended for domestic dogs, yielded sensitivities (true positive classification rate) of 71%–89% and specificities (true negative classification rate) of 94%–100% when used with sera from domestic dogs and cats (*Felis catus*) in a range of conditions (Atwell 1988, Courtney and Zeng 1995, Snyder et al. 2000, Courtney and Zeng 2001). If accuracy of the Dirochek test were similar when applied to sera from spotted skunks, we would expect false positives at rates of up to 6%, while false negatives could occur at rates of up to 29%. The absence of heartworm microfilaria in the blood of 9 heartworm-positive foxes from Santa Cruz Island (Knott test; K.R. Crooks, C.A. Scott, and D.H. Van Vuren unpublished data) and the absence of adult heartworms in necropsied foxes from throughout the Channel Islands (Coonan et al. 2005) suggest that foxes may have exhibited false positive reactions to the heartworm test; the same could be true for skunks. False positives might be caused by cross-reactions from anti-

genically related parasites (Roemer et al. 2000, Crooks et al. 2001, Sacks et al. 2002).

We found a low prevalence (10%) of antibodies to canine parvovirus in island spotted skunks. In foxes on Santa Cruz Island, canine parvovirus antibodies were reportedly prevalent in 1988 (59%; Garcelon et al. 1992) and in the early 2000s (47%, 2000–2003; Clifford et al. 2006), but were not detected in samples taken in the mid-1990s (1994–1997; Roemer et al. 2001). The virus, transmitted via the fecal-oral route, is long lived in the environment (Barker and Parrish 2001). Infections are frequently mild, although the disease can cause mortality, generally through gastroenteritis (Barker and Parrish 2001). The low prevalence of antibodies in spotted skunks and the low titer levels in positive samples (1:10) suggests a false positive result, perhaps arising from a nonspecific reaction (E. Dubovi, Cornell University, personal communication; Gardner et al. 1996). Skunks may be relatively resistant to parvovirus. Wild striped skunks (*Mephitis mephitis*) in Ontario showed a low prevalence (1.3%,  $n = 157$ ) of canine parvovirus antibodies, and experimentally exposed striped skunks did not sustain the virus and produced few or no canine parvovirus antibodies (Barker et al. 1983).

Although we did not detect canine adenovirus antibodies among island spotted skunks, they were found in 58% of island foxes sampled in the mid-1990s (Roemer et al. 2001) and 5% of foxes sampled from 2001 to 2003 (Clifford et al. 2006). Adenovirus is highly contagious; it is shed in urine, feces, and nasal secretions and is stable in the environment (Woods 2001). A variety of carnivores, including canids, mustelids, and mephitids (Karstad et al. 1975, Woods 2001), are susceptible to the disease, which can be fatal. Indeed, an antibody prevalence of 63% was detected in a serologic survey of striped skunks in the eastern United States (Alexander et al. 1972). Thus, our results suggest that spotted skunks on Santa Cruz Island have a low exposure or susceptibility to the virus.

Antibodies for canine distemper virus were not detected in Santa Cruz Island foxes in 1988 or in the mid-1990s (Garcelon et al. 1992, Roemer et al. 2001), but at least 14% of foxes sampled during 2001–2003 exhibited antibodies to the virus (Clifford et al. 2006). Canine distemper is a highly contagious virus,

but transmission generally requires close contact with oral and nasal fluids because the virus is short lived in the environment (Deem et al. 2000, Williams 2001). All carnivores appear susceptible to infection, and mortality is near 100% in domestic ferrets (*Mustela putorius furo*) and black-footed ferrets (*Mustela nigripes*; Deem et al. 2000). Striped skunks may be more resistant (Diters and Nielsen 1978, Williams 2001); the prevalence of antibodies in striped skunks in Maryland was 8% (Jamison et al. 1973). Our failure to detect canine distemper antibodies indicates a low exposure or susceptibility of spotted skunks to this virus on Santa Cruz Island.

A low prevalence of canine herpesvirus antibodies was found in Santa Cruz Island foxes in 1988 (10%; Garcelon et al. 1992), and in the early 2000s (4%; Clifford et al. 2006), but we failed to detect antibodies to the virus in skunks. Canine herpesvirus is spread via bodily secretions and is unstable in the environment (Appel 1987). The disease can be fatal in young animals including domestic dogs (Appel 1987) and coyote pups (Evermann et al. 1984). Our result suggests that the exposure or susceptibility of skunks to the virus is low. Similarly, a survey of 25 striped skunks in Maryland found no antibodies to canine herpesvirus (Jamison et al. 1973).

We found no evidence of *Leptospira* exposure in spotted skunks for serovars *pomona*, *canicola*, and *icterohaemorrhagiae*. Santa Cruz Island foxes exhibited a low prevalence ( $\leq 14\%$ ) of antibodies to serovar *icterohaemorrhagiae* but did not show exposure to *canicola* and were not tested for *pomona* exposure in both 1988 and the mid-1990s (Garcelon et al. 1992, Roemer et al. 2001). *Leptospira* sp. is a spirochete bacterium represented by about 200 serovars. Infected individuals transmit the bacteria in their urine to surface water and soil, where bacteria can persist for several weeks (Leighton and Kuiken 2001). Typically, host species are very susceptible to infection by a given serovar and act as carriers, while non-hosts may be less susceptible but develop leptospirosis disease, with severity ranging from mild to fatal (Leighton and Kuiken 2001). Domestic animals and sea lions are commonly infected (Leighton and Kuiken 2001) and represent potential reservoirs of the disease for Santa Cruz Island. Sea lions frequent the island's shores; a wide variety of domestic animals were present historically; and the pets of

boaters continue to access the island. The bacteria apparently can infect any mammal (Leighton and Kuiken 2001), including eastern spotted skunks (*S. putorius*; McKeever et al. 1958), and antibodies are frequently detected in striped skunks, especially serovars *icterohaemorrhagiae* and *pomona*, with *canicola* also present (Roth 1970 and references therein). Our results suggest that island spotted skunks have low susceptibility to serovar *icterohaemorrhagiae*, which is known to be present, although skunks could potentially harbor other serovars.

We did not detect antibodies to pseudorabies, a herpesvirus found primarily in swine and widespread in feral pigs (Stallknecht and Howerth 2001). The exposure of island foxes to pseudorabies has not been tested. Carnivores, including striped skunks, have contracted pseudorabies, almost always when in close physical association with pigs or when infected experimentally (Trainer and Karstad 1963, Stallknecht and Howerth 2001). Spotted skunks on Santa Cruz Island live in proximity to feral pigs, potentially encountering and ingesting pig carcasses, the primary mode of transmission of pseudorabies to nonsuids. A limited serosurvey showed no antibodies to pseudorabies among feral pigs on Santa Cruz Island in 2000–2001 (E. Aschehoug personal communication). Thus, the absence of exposure evidence likely indicates that skunks are not exposed to the virus. Because the disease is usually fatal in carnivores (Stallknecht and Howerth 2001), we may not have found evidence of exposure to pseudorabies if occasional transmissions result in mortalities.

In conclusion, spotted skunks on Santa Cruz Island appeared to have very low exposure or susceptibility to several exotic pathogens, most of which occur in island foxes. They exhibited a low prevalence of canine heartworm antigens and canine parvovirus antibodies and showed no evidence of canine adenovirus, canine distemper virus, canine herpesvirus, *Leptospira* bacteria (serovars *pomona*, *canicola*, and *icterohaemorrhagiae*), or pseudorabies exposure. We cannot exclude the possibility that some of our findings were false negatives arising from transposition of tests developed for domestic animals onto wild animals (Gardner et al. 1996). Nonetheless, serological surveys of wild animals using tests developed for domestic animals remains a standard means of

assessing exposure to pathogens. Negative results can also arise despite the presence of the pathogen in the population if infected hosts fail to produce antibodies or when antibody levels decline over time (Scott 1988). The low prevalence of pathogen exposure, however, suggests that spotted skunks likely are not serving as reservoirs of these disease agents for island foxes. As island carnivores, spotted skunks remain at risk from novel pathogens for which we did not test and also from pathogens for which we did test if population, community, or ecosystem changes alter the susceptibility of skunks to infection and disease (Scott 1988).

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