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# BIOMETRIC AND DISEASE SURVEILLANCE OF AN INSULAR POPULATION OF FERAL PIGS ON SANTA CRUZ ISLAND, CALIFORNIA

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Abstract—Feral pigs (*Sus scrofa*) can harbor parasites and other infectious agents that may potentiate sylvatic transmission to native wildlife such as the federally protected *Urocyon littoralis santacruzae* (Santa Cruz island fox) and *Spilogale gracilis amphiala* (island spotted skunk). When the wild boar population on Santa Cruz Island (SCI) was scheduled for eradication, an effort was made to gather reference data on a potential reservoir of parasites and pathogens for future epidemiology and management, should health issues arise among native biota. Blood samples (256) were collected and necropsies (77) were performed to document the health, age, disease exposure, and biometric characteristics of the feral pig population. Samples were conveniently collected during five field visits between July 3 and November 22, 2005 from representative sections of the island. Serum samples were tested for antibodies to *Brucella spp*., pseudorabies virus, *Trichinella spiralis*, *Toxoplasma gondii*, and six serovars of *Leptospira spp*. Tests were negative (with 95% confidence of less than 2.2% prevalence), except for 32 (14%) individuals that had very low antibody titers (1:100–200) to one or more serovars of *Leptospira*. The existence of low antibody titers for *Leptospira* suggests exposure to this agent and perhaps some interaction between feral pigs and other land or sea mammals of the island. Necropsy examination of 77 carcasses was unremarkable. Histologically, mild non-specific background lesions were noted in various organs, interstitial pneumonia being the most commonly seen (38%). No parasites were detected at necropsy from stomach and small or large intestines. Fecal samples from 68 animals examined for parasites/parasite parts detected *Eimeria sp*. oocysts (coccidia, 2.9%), *Demodex sp*. (mite, 2%), and *Metastrongylus sp*. (lungworm, 9%). External parasites that were detected included *Pulex irritans* (human flea), *Ixodes pacificus* (western black-legged tick), and *Haematopinus suis* (hog louse). Data suggests exposure of SCI pigs to several Leptospire serovars that are atypical in pigs. There is no evidence that wild pigs or the eradication efforts on SCI posed a conservation threat to the SCI fox or other SCI species.

## INTRODUCTION

The presence of parasites and infectious diseases in Santa Cruz Island (SCI) feral pigs (*Sus scrofa*), which have potential sylvatic transmission to insular wildlife populations of conservation interest such as SCI fox (*Urocyon littoralis santacruzae*) or the island spotted skunk (*Spilogale gracilis amphiala*), has yet to be described. During the decline of the endangered island fox on Santa Catalina Island (SCaI), questions were raised as to whether a particular disease may have been present on the island prior, and particularly if a previously-eradicated non-native species such as the pig could have served as a reservoir for the pathogens. The information presented here on the presence of pathogens on the island and the behavior of these pathogens would be useful in the case of possible future emerging diseases in protected wildlife on SCI. Additionally, human exposure to zoonotic diseases of feral pigs, as well as sylvatic exposure, is potentiated by hunting activities through exposure to carrion (Neiland 1970; Zygmont et al. 1982; Currier 1989; Weigel et al. 1996; Hutton et al. 2006; Lloyd-Smith et al. 2007). The presence of one of these diseases may influence management decisions

during eradication efforts. This project was undertaken to document the health status of a subset of the feral pig population during the process of their eradication. Some background history and potential effects of sylvatic and zoonotic transmission of diseases from of feral pigs are introduced herein.

*History*

The initial introduction of the feral pig to SCI in 1852 was for commercial purposes. Within the year, the enterprise was abandoned and the pigs went feral (More 1857). Hog cholera (Classical Swine Fever Virus) was purposely introduced on the island three times, once prior to 1944 and at two different times in the 1950s, in an attempt to control or eradicate the pigs with no success (Wheeler 1944; Corn 1987a). In 1987, serologic testing for hog cholera was performed on 31 animals from the SCI pig population and did not detect exposure (APHIS 1987).

Pigs have had multiple biological effects on the island ecosystem, such as competition with native species for food resources (Schuyler 1988), reducing the regeneration of coast live oak (*Quercus agrifolia*) and other woody-species (Peart et al. 1994), and rooting impacts on two rare endemic plants (*Arabis hoffmannii* and *Thysanocarpus conchuliferus*) (Klinger 2003) and seven of the nine listed plant species on SCI (EIS 2002).

*Conservation Considerations*

This serosurvey was generalized for diseases with sylvatic and zoonotic potential. The inferences for conservation are dependant upon the potential virulence of such diseases in naïve island natives and whether they are passed in carrion. Diseases passed in carrion vary in importance and include pseudorabies virus (PRV) (Weigel et al. 1996), *T. gondii* (Frenkel et al. 1970), *T. spiralis*, *B. abortus*, and *B. suis* (Neiland 1970; Zygmont et al. 1982).

SCI fox have been observed feeding on feral pig carrion; therefore, PRV could be a potentially serious threat to foxes, skunks, and carnivorous birds during eradication activities if the virus was present in the pig carcasses. PRV produces acute disease and high mortality in most non-porcine secondary hosts (E. Hahn, personal communication 2007). PRV has been shown experimentally to be lethal in blue foxes (*Alopex lagopus*), producing an acute illness progressing from anorexia, depression, and coma with meningioencephalitis (Quiroga et al. 1995).

Without the presence of cats (felidae) on the island to shed infective oocysts, a sylvatic transmission cycle of *T. gondii* would depend directly upon carnivorism of encysted meat or nerve tissue (Frenkel et al. 1970). The SCI fox has apparently been living with exposure to *T. gondii* without significant impact on the population. A serologic survey (Garcelon et al. 1992) showed a low antibody prevalence of *T. gondii* in SCI fox, and recent work has shown persistence of the low prevalence in SCI fox of *T. gondii* (Clifford et al. 2006). Although not an apparent conservation threat to the SCI fox, *T. gondii* can infect all warm-blooded animals, including pigs and foxes (Jungersen et al. 2002), and infection in the fox can be asymptomatic or can cause central nervous system or other pathology. *Toxoplasma* was found concurrently with distemper virus in the single fox carcass recovered during the 1999 decline of the SCaI fox (Timm et al. 2009). Recently *T. gondii* and *Sarcocystis neurona* were recognized to cause encephalitis in marine mammals (Dubey et al. 2003). Without adjacent felidae to perpetuate *T. gondii* in the SCI ecosystem, carcasses of marine mammals that beach on SCI should be considered as a potential source of exposure. Marine mammals documented to have *T. gondii* infection include California sea lions (*Zalophus californianus*) (Dubey et al. 2003), harbor seals (*Phoca vitulina*) (Lambourn et al. 2001), and southern sea otters (*Enhydra lutris nereis*) (Kreuder et al. 2003). It has also been recovered from a dolphin fetus (*Tursiops aduncus*) (Jardine and Dubey 2002) and has been isolated from carnivorous birds such as raptors (Lindsay et al. 1993).

Although not a conservation threat, *T. spiralis* can cause myositis, infect both sylvatic and domesticated animals, and be maintained by carnivores such as foxes, skunks, and swine (Murrell et al. 1987). Improper hunting practices contribute to transmission and spread of this infection among wildlife (Pozio 2000). Anaerobic metabolism of the larvae in nurse cells allows their survival in extremely decomposed carcasses. Spread of *Trichinella spiralis* via ingestion of infected musculature is low in swine and high in foxes (Smith 1985).

Although *B. abortus* and *B. suis* have been documented to cause infections in canines (Neiland 1970; Shin and Carmichael 1999), and *B. canis* is known to cause abortion in canids, the effects of *B. canis* and *B. suis* on canids have not been reported to be a direct cause of death. (Neiland 1970). The abortive effects of these strains on canids are in question (Woodroffe and Ginsberg 1997); therefore, these strains of *Brucella* are not considered to be a major conservation threat to the island fox.

Leptospirosis is an acute febrile zoonosis infecting a broad range of mammalian hosts and is re-emerging globally (Lloyd-Smith et al. 2007). It is generally accepted that the major mode of transmission of leptospirosis is by exposure to contaminated water or animal tissues. The various serovars of *L. interrogans* differ widely in their host interactions (Lloyd-Smith et al. 2007). The epizootic infections typical of *Leptospira* tend to be periodic (Gulland et al. 1996). These cycles may be suggestive of either host susceptibility, climactic change, and increase in population density of reservoir hosts and/or "accidental" hosts (Gulland et al. 1996). A central tenant of the epidemiology of leptospirosis is the distinction between maintenance hosts and accidental hosts for a given serovar, or equivalently, between host-adapted and non-host-adapted serovars (Lloyd-Smith et al. 2007). Classically, maintenance hosts develop a chronic, largely asymptomatic infection in their kidneys, and may shed leptospires in their urine for months or years, while accidental hosts experience acute infections with symptoms ranging from malaise to multi-organ failure and death (Lloyd-Smith et al. 2007). There is also a recognized co-adaptation on the part of *Leptospira* strains. Typically there are host-adapted and non-host-adapted strains of leptospires. Host-adapted strains cause mild disease, abortion, a high percentage of seropositives in the host population, and are shed in the urine for long periods (Heath and Johnson 1994; Gulland et al. 1996). Non-adapted strains cause sporadic severe disease in the host, low prevalence of seropositive hosts, and are usually only shed for short periods by host individuals (Gulland et al. 1996). In addition, the behavior of *Leptospira* is often geographically specific, so that within a geographic region, certain serovars are prevalent and become adapted to a particular maintenance host (Bolin and Cassells 1992). Species and epidemiological investigations involve differentiating between maintenance-host populations and accidental-host populations for the serovar in a particular ecosystem (Hathaway 1981). Characteristics of infection with a particular serovar are often considerably different in different hosts; consequently, leptospirosis is more likely to pose a conservation threat when it cross-infects to a new species host, because it is often more virulent in the accidental host. For instance, domestic dogs are considered maintenance hosts for *L.i.* serovar Canicola, and incidental hosts for the other serovars (Moore et al. 2006). A common example of this phenomenon of increased virulence in an incidental host is the (recent) resurgence of acute and chronic leptospirosis in domestic canines, most often caused by *L.i.* serovars of Grippotyphosa or Pomona (Brown et al. 2003). Some of the recent thinking on *Leptospira* epidemiology hypothesizes that an accidental host may sometimes become a maintenance host through a process of an epidemic, when the survivors of the infection sometimes co-adapt and "non-host-adapted" serovars may become "host-adapted" (Gulland et al. 1996; Lloyd-Smith et al. 2007). Therefore, it may be important to know about the existence of reservoirs of leptospires, what the typical maintenance hosts and the actual maintenance hosts may be, and how the organisms are behaving in an ecosystem where managers are seeking to protect and conserve threatened or endangered species.

In the relatively insular environment of SCI, the possibilities of hosts are limited. There are only four indigenous land mammals on the island: deer mouse (*Peromyscus maniculatus*), western harvest mouse (*Reithrodontomys megalotis*), spotted skunk, and SCI fox (L. Laughrin, personal communication 1991). Only very few cattle remain localized on the island today, but in the past, cattle, sheep, and pigs were wide-ranging. The sheep (Schuyler 1988) and nearly all the cattle were removed by the early 1990s. The pigs are now considered eradicated. Seals and sea lions, primarily *Phoca vitulina* (harbor seal) and *Zalophus californianus* (California sea lion), with occasional *Mirounga angustirostris* (Northern elephant seal) and vagrants "haul out" on SCI beaches. Carcasses of other marine mammals, *Enhydra lutris* (California sea otter), *Eschrichtius robustus* (California gray whale), and others may

occasionally wash up on SCI beaches. Eight species of bats (Brown 1980) should also be considered as a source of exposure of SCI natives to potential pathogens.

Because of the behavior of *Leptospira* organisms, there are many literature citations of maintenance and incidental hosts that parallel SCI species. The pig has been considered a primary maintenance host for *L.i.* Pomona (Hathaway 1981). In North America striped skunks (*Mephitis mephitis*) are recognized as maintenance hosts for this serovar as well (McKeever et al. 1958; Kingscote 1986). Leptospirosis due to *L.i.* Pomona, has been reported in a small sample of red foxes in Canada causing severe hemorrhagic disease. In this study, the fox appeared to be functioning as an amplifier (accidental) host but not as a maintenance host (Kingscote 1986). *L.i.* Pomona infection is affecting the survival of California sea lions and is thought to have important consequences on population dynamics of the species (Gulland et al. 1996). Although the pig is not considered a primary host for *L.i.* serovar Icterohaemorrhagiae, it was the most prevalent of eight serovars tested in wild swine in Texas (Corn et al. 1986). The rat is considered the primary host for *L.i.* Icterohaemorrhagiae (Ward et al. 2004). Dogs are considered maintenance hosts for serovar Canicola (Moore et al. 2006). Skunks and rats are thought to serve as incidental hosts for *L.i.* Canicola (Roth et al. 1961; Ward et al. 2004) and it has been reported in bats (Matthias et al. 2005) and in other canids. Raccoons are considered to be a primary maintenance host to *L.i.* Grippotyphosa. Skunks have also been cited as showing evidence of exposure to this serovar (Richardson and Gauthier 2003). This was also the most common serovar seen in foxes in Germany (Müller and Winkler 1994). Although cattle are maintenance hosts for *L. borgptersenii* serovar Hardjo, pig, horse, and sheep are also known to carry the infection (Hathaway 1981). *L.b.* Hardjo has also been detected in bats in the Amazon (Matthias et al. 2005). The domestic pig is considered the primary host for *L.i.* serovar Bratislava (S. Hietala, personal communication 2007).

*Pulex irritans* and *Ixodes pacificus* are thought to be "host-sharing" with the SCI fox and spotted skunk (Crooks et al. 2001; Crooks et al. 2004). *P. irritans* is a possible vector for wild canid disease such as plague (McGee et al. 2006). *I. pacificus* has been known to infest deer mice, western harvest mice, wild turkey (*Meleagris gallopavo*; Lane et al. 2006), and the island fox) (Castro and Wright 2007) and is an important vector of *Borrelia burgdorferi*, the causative agent of Lyme disease. Lyme disease has been reported to cause multisystemic lesions in foxes in Hokkaido, Japan (Isogai et al. 1994). Since Lyme disease does not tend to be epizootic, it represents a low threat to conservation efforts.

*Zoonoses*

Feral swine carry several diseases that pose a zoonotic threat to humans. Human exposure to carcasses after a hunt can potentiate infections such as brucellosis (from *B. abortus* and *B. suis*) (Currier 1989) leptospirosis (Lloyd-Smith et al. 2007), toxoplasmosis, trichinosis, and sarcoptic mange (Hutton et al. 2006). Lyme disease may also be transmitted to humans via *I. pacificus* (Burgdorfer et al. 1985).

## MATERIALS AND METHODS

To maximize sample yield, five field sample periods were performed in conjunction with early trapping and aerial hunting operations between July 3 and November 22, 2005. Seventy-seven field necropsies were performed, and 256 blood samples were conveniently collected during the eradication process. As part of the eradication strategy, the island was arbitrarily fenced into five zones based primarily upon fencing convenience (Fig. 1). True random sampling was not possible due to logistical constraints, but all pigs dispatched during the sample periods were bled for serology. Since porcine blood is prone to rapid hemolysis, rendering the samples unfit for some serologic analyses, we made efforts to draw blood from carcasses in the field within five minutes of dispatch. Global Positioning System (GPS) locations of the pigs were recorded by the hunters and plotted in a random subset, 241/256 (94%), of pigs sampled (Fig. 1). This subset of sample locations represented a variety of habitats, including grassland (52%), coastal sage brush (17%), shrubland (13%), chaparral (6%), oak woodland (4%), fennel (4%), human land use (3%), and riparian (1%). Barren and forest habitats were not represented. Sage and chaparral habitats were particularly

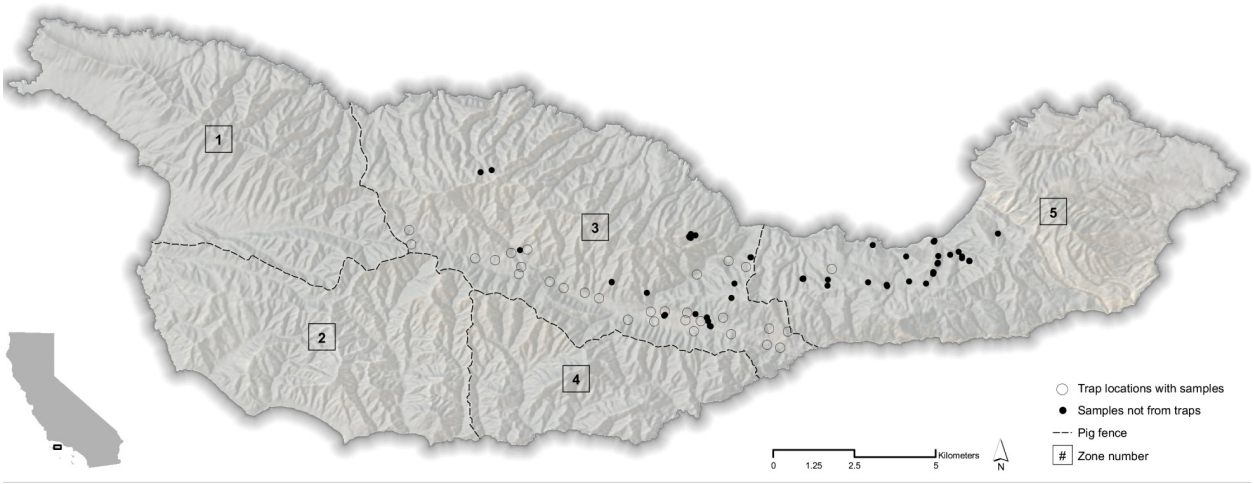


Figure 1. Santa Cruz Island showing numbered hunting zones, and locations of where pig carcasses for sampling were collected.

underrepresented in the study, and grasslands and scrublands were highly overrepresented, likely due to ease of targeting and trap setting in those areas. Sampling was convenience-based, since field visits were coordinated with times of early trappings in each region and most traps were set at sites with road access and where a higher density of pigs was expected. Pigs from non-trap locations (10% of total) were also sampled during each field period. The disparity between the age and sex of individuals sampled for serology, compared with a larger island-wide population subset, was 49% juveniles (84/172), and 51% adults (88/172), versus 68% juveniles (519/761) and 32% adults (242/761) ($\chi^2_1$=22.15, p<0.001); 40% sows (84/211) and 60% boars (127/211), versus 36% sows (1288/3531) and 64% boars (2243/3531) ($\chi^2_1$=0.81, p=0.37).

Age was estimated by visual examination of tooth eruption patterns and individuals were assigned to one of five age classes according to the classification protocol of Matschke (1967): I (<1 week to 6 weeks), II (7–19 weeks), III (20–51 weeks), IV (12–21 months), and V (>21 months).

Field necropsy examination was performed by either a pathologist or a practicing veterinarian (HK, KB) on 77 swine. Tissues of palatine tonsil, mandibular lymph node, lung, spleen, liver, brain, heart, thymus, diaphragm, small intestine, spiral colon, caecum, and kidney were collected in 10% buffered formalin (pH 7.2) for histopathologic evaluation. Paired tissues of palatine tonsil, mandibular lymph node, lung, spleen, liver, and brain were collected in separate sterile bags and stored frozen. Additional tissues from animals exhibiting gross lesions were collected when indicated. The small intestine, spiral colon, and caecum were examined for parasites and 5 g of contents from each were pooled and preserved in 10% formalin. A random subset of 54 of these pigs was examined for the presence of ectoparasites with an emphasis on a variety of organisms rather than quantitative numbers. Parasites were collected, preserved in 10% formalin, and shipped to the University of Tennessee for evaluation (SP).

Formalin fixed tissues were shipped to California Animal Health and Food Safety Laboratory (CAHFS), San Bernardino Branch, for histologic evaluation. Samples were processed by standard techniques, and tissues were sectioned at 4μm thick and stained with hematoxylin and eosin and examined microscopically (HK). In an individual with diseased tissues, lesions were described and then catalogued by organ affected, i.e., hepatitis would count as one "lesion," nephritis as a separate lesion, and pneumonia as a third. Causative agents such as parasites were only cited if visualized, or if inferred by the pattern of pathologic findings. Due to the incidence of pneumonia detected, a random subset of five individuals with interstitial pneumonia was screened for porcine circovirus using immunoperoxidase (IPX) histochemistry technique (HK).

Blood was drawn from the heart or chest cavity with focus on obtaining samples within five minutes of dispatch. We evaluated 240 samples for *Brucella* and *PRV* and 223 for *Leptospira* antibodies at CAHFS. Antibody specific to PRV was evaluated using a commercially available latex agglutination assay developed for domestic swine[1] (Schoenbaum et al. 1990). Buffered acidified plate agglutination (BAPA) was used to screen for antibody to *Brucella spp*. using *B. abortus* antigen supplied by the United States Department of Agriculture (USDA) National Veterinary Service Laboratory (NVSL) according to the procedure described in Uniform Methods and Requirements (UM&R). The screening assay, though designed to detect *B. abortus* exposure, is known to be antigenically cross-reactive with *Brucella suis* (Nielsen et al. 1999); therefore negative tests for *B. abortus* likely rules out *B. suis* as well (S. Hietala, personal communication 2007). A standard microagglutination test (microAT) was used to screen for six *Leptospira* serovars, including *L. interrogans* serovar Pomona, *L.i.* serovar Bratislava, *L.b.* serovar Hardjo, *L.i.* serovar Grippotyphosa, *L.i.* serovar Icterohemorragiae, and *L.i.* serovar Canicola. The microagglutination assay was performed using serial twofold dilutions of serum starting at a 1:100 dilution (Cole et al. 1973). Reference antigen and serovar-specific control antisera were supplied by the USDA NVSL. Positive serologic reactions were interpreted as evidence of exposure to disease rather than confirmation of active infection in the wild pig sampled.

We analyzed 256 serum samples for *Toxoplasma gondii* antibodies by the modified agglutination test (MAT) using formalin fixed tachyzoites as antigen (bioMerieux Laboratories, Lyon, France) at the University of Tennessee, College of Veterinary Medicine (UTCVM). Titers of 1:32 or greater were considered positive. Serum samples were analyzed for *Trichinella spiralis* antibodies (SafePath Laboratories ELISA), using microwells coated with *T. spiralis* excretory-secretory antigen. Samples with absorbance of 0.3 optical density units or higher were considered positive (SP). Serum was not analyzed for exposure to the introduced CSF (hog cholera) or any other "federally reportable" disease because we could not gain permission from U.S. Department of Agriculture Animal and Plant Health Inspection Service to test for these diseases.

Preserved external parasites and fecal samples were shipped to the UTCVM for evaluation. External parasites were examined microscopically for identification. A random subset of 69 fecal samples was collected from the 77 necropsies. Five grams of fecal material was taken from each of the small intestine, large intestine, and caecum and then combined into single specimens. Fecal samples were concentrated using centrifugal sugar flotation (Sheather's sugar solution specific gravity 1.275) and formalin-ethyl acetate sedimentation, and then examined microscopically (SP).

Comparisons between sexes and juveniles and adults were evaluated using Chi-Square for a 2X2 contingency table with a Yates correction for continuity. We also report 95% confidence intervals (95% CI) for the true prevalence of all pathologies within the population based on sample prevalence.

## RESULTS

The sampling fraction of the total wild swine population of SCI tested for PRV and *Brucella* was 5% (240/5048); for *Toxoplasma* and *Trichinella* it was 5% (256/5048); and for the six serovars of *Leptospira* it was 4% (223/5048). Eighty-five percent of all sera and 90% of the carcasses were from Zone III; whereas 15% of the sera and 10% of the carcasses were from Zone V (Fig. 1). The sex ratio of the 211 sampled swine was 127/84 (boars/sows). Of these, the age class V adults had the largest sex disparity (48/19). This sex ratio is slightly more skewed than the larger island-wide sample of age class V pigs (105/53) (S. Morrison, personal communication 2007).

No antibody titers were detected for *Brucella*, PRV, *Toxoplasma* or *Trichinella*. While it is not possible to calculate 95% confidence intervals for these null results, the upper limit of the 95% confidence interval (assuming a single positive result) suggests that these diseases have a true prevalence of <2.2% in this population.

The overall apparent seroprevalence for *Leptospira* (all serovars) was 14.3% (32/223) (95% CI: 10.6%–18.8%), with 31% (10/32) showing titers

1. Viral Antigens, Meridian Life Sciences, Memphis, TN (http://www.meridianlifescience.com).

Table 1. Apparent seroprevalence of *Leptospira spp*. in feral pigs (*Sus scrofa*) sampled in 2005 from Santa Cruz Island, California.

| *Leptospira* serovar | N | ≥1:100 | Seroprevalence (%) | 95% CI | Std. error |
|---|---|---|---|---|---|
| *L.i.* Canicola | 223 | 3 | 1.3 | 0.4–3.4 | 8.1 |
| *L.i.* Grippo | 223 | 16 | 7.2 | 4.6–10.7 | 6.4 |
| *L.i.* Hargjo | 223 | 0 | 0 | ND* | - |
| *L.i.* Ictero | 223 | 15 | 6.7 | 4.2–10.2 | 6.5 |
| *L.i.* Pomona | 223 | 2 | 0.9 | 0.2–2.8 | 9.4 |
| *L.i.* Bratislava | 223 | 12 | 5.4 | 3.1–8.6 | 6.6 |
| Total # tested** | 223 | 32 | 14.4 | 10.6–18.8 | 6.8 |

≥1:100 dilution titer denotes exposure to one or more *Leptospira* serovars.

*ND – No antibody detected at 1:100.

** Some individuals were positive to one or more serovars

(microAT) 1:100 to one or more serovars of *Leptospira* (Table 1). These low-level titers were considered to be indicative of past exposure rather than evidence of active infections. The seroprevalence of exposure to *Leptospira spp.* relative to sex was 14% (18/127) (95% CI: 9.3%–20.3%) boars and 16% (13/84) (95% CI: 9.4%–23.5%) sows, with one positive sample from an animal of undetermined sex. With respect to age class, the seroprevalence of exposure to *Leptospira* was 17% (14/84) (95% CI: 10.4%–24.8%) juveniles (age class I–III) and 16% (14/88) (95% CI: 9.9%–23.7%) adults (age class IV and V). Samples were positive from four pigs of undetermined age. There was no significant difference in prevalence of *Leptospira* between boars and sows, or between juveniles and adults ($\chi^2_1$<0.01, p>0.99 for both comparisons).

Gross examination was performed on 77 carcasses consisting of 65% (50/77) boars, 32% (25/77) sows, and 3% (2/77) undetermined sex. No gross lesions indicative of population-level disease were noted in any of the animals examined. Sixty-two percent (48/77) (95% CI: 52.4%–71.6%) of the pigs had mild, non-specific microscopic lesions in one or more organs (Table 2). Interstitial pneumonia characterized by mild multifocal lymphocytic infiltrates was seen in 38% (29/77) of the necropsied animals. The distribution of combined lesions among the different groups was 76% (19/25) in sows and 58% (29/50) in boars. The 77 pigs showed various non-specific incidental lesions in different organs. Among the 63 aged individuals, there was no significant difference ($\chi^2_1$=2.19, p = 0.14) in numbers of juveniles (I–III) (83%, 15/18) (95% CI: 62.3%–95.3%) with pathology as compared to adults (IV–V) (60%, 27/45) (95% CI: 46.7%–72.3%). There was, however, a marginally significant number of pigs with lesions in age class V (19/34=56%) (95% CI: 9.9%–23.7%) compared

Table 2. Histopathological findings for Santa Cruz Island feral pigs (*Sus scrofa*) in 2005 from Santa Cruz Island, California.

| Histopathology findings (N=77) | Number of findings | Incidence |
|---|---|---|
| Interstitial pneumonia | 29 | 38% |
| Pulmonary hemorrhage | 2 | 3% |
| Interstitial nephritis | 1 | 1% |
| Hepatitis | 1 | 1% |
| Myositis | 4 | 5% |
| Sarcocyst | 7 | 9% |
| Eosinophilic enteritis* | 4 | 5% |
| Tonsilitis | 18 | 23% |
| Cervical lymphadenopathy | 5 | 7% |
| Chronic abscess | 3 | 1% |
| Total # pigs with lesion | 48† | 62% |

* Possibly due to parasitic migration.

† Individuals may have ≥1 pathological findings.

to juveniles ($\chi^2_1$=2.80, p=0.094). Thirty-eight percent (29/77) (95% CI: 28.4%–47.6%) of the animals had no remarkable lesions in any of the tissues examined. Although pathologic changes were seen in the liver of one pig and interstitial nephritis of the kidney of another, none of the pigs with positive titers for *Leptospira* showed pathology in the commonly affected organs. Overall, there were no remarkable lesions noted in the swine population to suggest an active population-disease process.

Fecal sample concentration yielded: eggs of *Metastrongylus spp.* (lungworm) in 9% (6/69) (95% CI: 3.9%–16.4%); *Demodex sp.* ("mange" mite) in one fecal sample (95% CI 0.1%–7.7%); *Eimeria sp.* oocysts 3% (2/69) (95% CI: 1.2%–10.9%) and grain mites or their eggs in 17% (12/69) (95% CI: 10.4%–26.6%) of the samples. A few of the samples contained unidentified coccidian oocysts that were not compatible with *Eimeria* or *Isospora* species typical of swine. Twenty percent (14/69) (95% CI: 12.7%–29.9%) contained grain mites or parasite eggs that were considered to be non-specific to swine. External parasites identified on pigs examined included *Haematopinus suis* (hog louse) 80% (43/54) (95% CI: 68.5%–88.1%); *Pulex irritans* 13% (7/54) (95% CI: 6.2%–23.0%); and *Ixodes pacificus* 6% (3/54) (95% CI: 1.5%–13.7%). *H. suis* was detected commonly during all visits, *P. irritans* was seen once in July and the remainder in October and November, and *I. pacificus* was found in October and November only.

## DISCUSSION

In spite of the background pathology present and evidence of exposure to several serovars of *Leptospira*, data suggest that the subset of SCI wild pigs examined was from a generally healthy population with no detectable ongoing population-disease processes. However, since several serovars of *Leptospira* were unexpectedly found in an atypical host, it raises questions as to the potential of cross-infection to an island native as a naïve accidental host.

*Methodology and Data Biases*

The serum sampling had a convenience-based bias, as the majority of traps were set primarily in open areas along roads and in the long central valley that courses longitudinally through the island. Trap-wary pigs would be less likely to be represented. It may be assumed that the subset of pigs along roads would be more likely to interact, due to more convenient travel access, as opposed to some smaller subpopulations that may have been more isolated by geographic barriers. This would likely bias the data to reflect a higher incidence of disease than the total population prevalence.

Contrary to the age ratio observed in the island-wide survey, significantly fewer juveniles than adults were represented in this study (p 0.001). The reason for this discrepancy was undetermined. Nevertheless, adults, having lived longer than juveniles, could have greater opportunities for exposure to diseases endemic to the island. The selection of older individuals, with the expected increased probability of disease exposure over time, and those with external lesions or in poor condition, could potentially bias the necropsy results toward a higher incidence of pathology. However, the data show a marginally greater number of pigs with lesions in juveniles than in adults (age class V). This may be due to some acquired-immunity effect in adults, which may protect surviving individuals (Lankester 2001, 239; Fallon et al. 2003). Although a 30% rate of interstitial pneumonia in the population may appear high, it was comparable to pig farms where a moderate prevalence is considered to be in the range of 30%–70% (Pointon et al. 1984; Radostits et al. 2000). In our opinion, the gross and histologic pathology findings in this pig population represented mild, incidental, non-specific background lesions without evidence of an active population-disease process.

Because of the hemolytic nature of porcine blood there was hemoglobin contamination in some serum samples. Some of the laboratory analyses, particularly the *Leptospira* titers, were more sensitive to hemoglobin contamination. The respective laboratories discarded contaminated samples as invalid for each method; hence, the discrepancy in sample size between the different serologic tests. The serologic assays used in this study were developed for use in domestic species and are not data to reference diagnostic sensitivity and specificity for feral pigs. With most serological testing performed on wildlife species there is a general assumption that individual assays will

detect antibodies with similar performance as was documented for domestic species (Hietala and Gardner 1999). These are considered screening assays with high detection sensitivity, but include the possibility of false positive results due to antigenic cross-reactivity with other organisms sharing similar surface antigens. The BAPA test for *Brucella* is the standard used for herd screening in domestic swine since it has up to 100% sensitivity (Barton 1996; Olsen et al. 1996), although a wide range of specificity values are reported. Since all pigs tested negative for *Brucella*, the specificity of this test is inconsequential. The pseudorabies assay specificity is approximately 95%, also as measured for domestic swine. In the microAT test for *Leptospira*, as in other agglutination assays, serologic cross-reaction is known to occur, and extremely elevated titers are more likely to show cross-reaction to related surface antigens. The tested serotypes do have some shared surface antigens, so cross-reactivity is expected between *L.i.* Bratislava and *L.i.* Pomona, as well as a lower level cross-reactivity between *L.i.* Icterohemmorhagiae and *L.i.* Pomona. For example, a high antibody titer of *L.i.* Bratislava of >1:3200 can cause a low reaction (such as 1:100) in *L.i.* Pomona, or a >1:3200. *L.i.* Pomona can pull up an *L.i.* Bratislava to 1:100. The highest serologic response generally indicates the serovar to which it is exposed, and the higher the titer in one serovar the more likely it is to cross-react and artificially raise the titer in the other serovar. The titers for *Leptospira* measured in this study did not exceed 1:200. This decreases the chances of cross-reaction (S. Hietala, personal communication 2007). There are no published evaluations of *Leptospira* serology performance for swine. In assessments of MAT for other species, however, the sensitivity was low (30%) in early acute-phase cases and increased to 76% in convalescence, while specificity was less than or equal to 97% (Cumberland et al. 1999). Therefore, while the prevalence of *Leptospira* may be underestimated by this technique, the probability of false positives is low.

In those necropsies done by a practicing veterinarian, rather than the pathologist, subtle lesions may have been overlooked, thereby underestimating prevalence of pathologic changes. The lack of population disease seen on gross and microscopic examination is noteworthy in light of the fact that the subset was a purposely biased selection of an older, and therefore more likely exposed, population sample.

*Implications*

Our objective was to document whether there were diseases in SCI pigs that would have zoonotic implications or be of conservation interest to native fauna on or around SCI, including marine mammals and the SCI fox.

*Sarcocystis* found in heart and skeletal muscles of pigs are incidental findings and are of no clinical relevance. The grain mites and the unidentified coccidian oocysts, that were non-specific to swine and were detected in fecal samples, were presumably ingested from an exogenous source. Since grain mites and their eggs were fairly abundant, it is likely they were consumed with the corn that was used for baiting the pig traps. The unidentified coccidian oocysts were likely passed from ingested prey. The *Pulex irritans* and *I. pacificus* found on the pigs may also have been "host-sharing" with the SCI fox and spotted skunk (Crooks et al. 2001; Crooks et al. 2004). The lone *Demodex sp.* mite recovered in a fecal sample is the first documented on SCI. This finding should be considered in the differential diagnosis of chronic skin lesions in the captive island foxes, should they reoccur.

There was no significant difference between juveniles and adults in the prevalence of detectable antibody titers for any of the diseases. For PRV, this is in contrast to prevalence findings in studies of pigs on SCaI (Timm et al. 2005), in Georgia (Pirtle et al. 1989), and in Florida (Van der Leek et al. 1993), where the probability of exposure appeared to increase with age. It had been hypothesized this disparity was due to maternal-sibling grouping of young pigs, thereby reducing exposure. However, recent work by Romero et al. (2001) has shown that transmission of the PRV strain indigenous to feral pigs is preferentially by venereal route, suggesting that exposure is primarily in adults.

Transmission of *Leptospira* is primarily from urine and contaminated water sources (Gummow et al. 1999). After weaning, exposure would be similar in all sex and age groups using a water source, regardless of the size of their home range. There was no significant difference in titers between sexes for

any of the diseases tested, which agrees with the findings of the above authors for PRV.

The most important finding of this study relative to SCI fox conservation was the lack of evidence of exposure of SCI pigs to PRV during the feral pig eradication. This is in contrast with the PRV seroprevalence of 25% in the SCaI pig population (Timm et al. 2005). PRV infection is acute and has a high mortality in experimentally infected foxes (Quiroga et al. 1995) and in most non-porcine secondary hosts in the wild (Thawley and Wright 1982; Weigel et al. 1996; E. Hahn, personal communication 2007). Although there is no evidence implicating PRV in the fox population decline on SCaI, finding PRV antibodies in the SCaI pig population and having a concurrent decline in fox or other wildlife populations during the pig eradication (Timm et al. 2000), where infected pig carcasses are available for scavenging, raises interesting questions regarding potential exposure of wildlife. Further data, such as testing of fox tissues for evidence of exposure to PRV, would be needed to address these questions. Since these coincident circumstances did not exist on SCI, decline of the SCI fox population due to PRV exposure is very unlikely.

The lack of evidence of *Brucella* infection supports the results of previous testing of 61 individuals in the SCI swine population (Corn 1987b) that were also negative (Corn 1987c). This is significant to the hunters participating in the eradication efforts to have low risk of zoonotic transmission of brucellosis.

Although there are no cats on SCI, the finding by Garcelon et al.(1992) of low-level seroprevalence for *Toxoplasma gondii* in SCI foxes may not necessarily be false positive laboratory results, as hypothesized, since the findings were repeated in the recent study (Clifford et al. 2006). However, data suggest that it is unlikely that transmission to foxes by eating wild pig carrion was a contributing factor. *T. gondii* infection could be sustained in the population by SCI foxes scavenging carcasses of marine mammals or eating fresh meat of birds. It is interesting to note that the single fox carcass recovered during the rapid decline of SCaI foxes had evidence of infection of *T. gondii* and the importance of the co-infection is unknown (L. Munson, personal communication 2007).

Unlike SCaI, where *Trichinella* were found in muscle of pigs (Timm et al. 2005), no evidence of *Trichinella* was found in the SCI pig population either by serology, gross examination of muscle tissue, or by microscopic examination of diaphragm tissue for nurse cells. It is doubtful the SCI pig population was a reservoir for infection on the island. There have been suggestions, however, of potential low level cannibalism in foxes in Italy (Rémonti et al. 2005) which could perpetuate transmission of *Trichinella* infection within a fox population without an intermediate host. Regardless of this potential, *Trichinella* infection within a fox population would not represent a conservation threat.

The spectrum of titers for different serovars of *Leptospira* is interesting in light of the variety and history of mammalian inhabitants on SCI over the last 20 years. The current four indigenous land mammals, the past four ranching mammals (including dogs), and marine mammals which "haul-out" or wash up as carcasses on the beaches, as well as the eight recorded bat species (Brown 1980) need to be considered in the epidemiology of *Leptospira.* The expected prevalence of *Leptospira* in a wild swine population is estimated at 5% (E. Bush, personal communication 2008). This data suggests that SCI wild swine were exposed to a number of serovars of *Leptospira*.

*L. i.* Grippotyphosa represents the highest titers of any serovar tested (7%), although there have been no reports of raccoons on SCI. This serovar has been shown experimentally to pass to wild carnivores when fed infected rodents (Reilly 1970), however, recent work has revealed no evidence of antibodies to this serovar in the SCI fox (Clifford et al. 2006).

Although they are the primary host of *L.i.* Icterohaemorrhagiae, there have been no confirmed sightings of rats on SCI. Incidental hosts include mice, skunk, fox, and bat (Clark et al. 1961; Ward et al. 2004; Matthias et al. 2005). Our 6% prevalence rate is not unexpected in light of the fact that Garcelon et al. (1992) reported a prevalence of 14% in the SCI foxes. SCI was the only one of six Channel Islands with foxes to have any evidence of *Leptospira* exposure for the two serovars tested in that study. No antibody titer to *L.i.* Icterohaemorrhagiae was detected in the SCI fox survey in 2001–2003 (Clifford et al. 2006) or SCI spotted skunks in 2000–2001 (Bakker et al. 2006). It

is interesting that by the 2001–2003 survey of the SCI fox, there was no evidence of this serovar in the sample tested.

*Leptospira i.* Bratislava displayed a 5% antibody response in the study population and is reportedly indigenous in feral pigs (Corn et al. 1986). Serologic studies in the United States and Canada indicate that Bratislava has become the serovar that most frequently affects swine (Bolin and Cassells 1992). It is noteworthy that 7% (2/28) of the wild SCI fox population showed evidence of exposure to this serovar (Clifford et al. 2006), but the captive-born SCI fox showed no evidence of exposure. This finding suggests that there was exposure of fox in the wild from the pigs or other mammals. The SCI spotted skunk has not been tested for this serovar. This serovar has been reported in rodents and dogs and it is considered they could serve as carriers; however, Moore et al. (2006) cite dogs as an incidental host for this serovar.

*Leptospira i.* Canicola only produced a positive antibody response in 1% of the study population. The presence of *L.i.* Canicola exposure is interesting since the dog is considered to be the primary reservoir host (Ward et al. 2004; S. Hietala, personal communication 2007). Over the past 20 years, with the exception of pig-hunting dogs, dogs have been typically limited to the main ranch area and/or incidental illegal entry to the shore for short periods from oceancraft. No antibodies to *L.i.* Canicola were reported in the 29 SCI foxes tested by Garcelon et al. (1992), nor by Clifford in 2001–2003, nor in 28 SCI spotted skunks tested in 2000–2001 (Bakker et al. 2006).

*Leptospira i.* Pomona titer levels were also relatively low (1%) in the study population. *L.i.* Pomona has been shown to have a markedly pathogenic effect on California sea lions (*Zalophus californianus*) and appears to be responsible for periodic die-offs along the mainland. There has been suggestion that a source of contamination may be small mammals or pigs living on the Channel Islands (Gulland et al. 1996). No evidence of exposure to this serovar was found during testing of 28 SCI spotted skunks in 2000–2001 (Bakker et al. 2006) and 28 SCI foxes in 2001–2003 (Clifford et al. 2006). These data from SCI pigs suggest that pigs were not a primary source of *L.i.* Pomona to sea lions.

Although data indicate exposure of the SCI pigs to several serovars of *Leptospira*, there has been no report of pathology attributed to Leptospirosis from the necropsies done on the SCI fox population to date (L. Munson, personal communication 2008). The overall conservation threat to the SCI fox from Leptospirosis appears to be low at this time. Because of the behavior of *Leptospira* organisms, the ecology of *Leptospira* in island natives bears monitoring of a variety of serovars, particularly in a threatened or endangered population.

## CONCLUSIONS AND RECOMMENDATIONS

Although there were parasites and background pathology present and there was evidence of exposure to several serovars of *Leptospira* in this sample of SCI wild pigs, this was generally a healthy sub-population with no detectable ongoing population-disease processes. There is no evidence that the wild pigs or the eradication efforts on SCI posed a zoonotic threat to hunters nor a conservation threat to the SCI fox or other island species. These studies underscore the importance of pathogen screening on a species prior to eradication to preserve data and to aid preventative management of sylvatic and zoonotic transmission during the efforts.

These studies and findings supplement other works showing the complexity of interactions between different serovar of *Leptospira* with primary and alternative hosts. It is not likely that Leptospirosis will become a major conservation threat on SCI; however any serovar now documented on the island could potentially become virulent if the un-adapted serovar infected a new host species. With an endangered insular native mammal population on SCI, more effort devoted to land and sea mammal serologic surveillance may be desired to address potential reservoirs, particularly for *Leptospira* and *T. gondii,* in order to anticipate potential sylvatic interactions in the island ecosystem.

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