Infectious Diseases of Dogs and Cats on Isabela Island, Galapagos

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Background: Vaccination and importation of dogs and cats are prohibited in the Galapagos, resulting in a uniquely isolated population. The purpose of this study was to determine the prevalence of infectious diseases of dogs and cats that impact their health, could spill over to native wildlife, or sentinel diseases of concern to humans.

Hypothesis: The isolation of dogs and cats in the Galapagos protects them from diseases common in mainland populations.

Animals: Ninety-five dogs and 52 cats presented during a neutering campaign.

Methods: A prospective cross-sectional study was performed. Blood was collected for serological and DNA evaluation of a panel of infectious diseases.

Results: Antibodies against parvovirus (100%), parainfluenza virus (100%), adenovirus 1/2 (66–67%), and distemper virus (22%) were present in dogs. Dirofilaria immitis was also common in dogs (34%), with lower prevalences of Wolbachia piapiens (22%), Bartonella sp. (13%), Ehrlichia/Anaplasma spp. (1%), and Mycoplasma haemocanis (1%) observed. Antibodies against panleukopenia virus (67%), Toxoplasma gondii (63%), calicivirus (44%), and herpesvirus 1 (10%) were detected in cats. Feline leukemia virus antigen, feline immunodeficiency virus antibody, or coronavirus antibodies were not detected. Bartonella sp. (44%) infections were common in cats, but only one was infected with M. haemofelis.

Conclusions and Clinical Importance: Despite their relative seclusion from the rest of the world, cats and dogs of Isabela were exposed to many pathogens found in mainland South America. Parasite prophylaxis, neutering, and strict enforcement of animal movement restrictions would control a majority of the diseases. In the absence of vaccination, a reservoir of susceptible animals remains vulnerable to new disease introductions.

Key words: Bartonellosis; Dirofilaria immitis; Distemper virus; Parvovirus; Toxoplasmosis; Zoonoses.

The Galapagos archipelago comprises 13 major islands and more than 100 smaller land masses and rock outcroppings straddling the equator approximately 600 miles off the coast of Ecuador. Although approximately 16,000 people live on four of the larger islands, 97% of the islands are park land protected from human activity. The Galapagos are home to at least 6,000 species of animals and plants, more than half of which are found nowhere else on the Earth. The native mammalian species are the Galapagos sea lion, fur seal, rice rat, and Galapagos red and hoary bats. Transient maritime visits have occurred since the 1500s, and the Galapagos were formally colonized by Ecuador in 1832. Accompanying these human activities was the introduction of nonnative species, either as intentional agricultural introductions or as incidental passengers on boats and planes. Invasive species also arrived naturally on ocean and wind currents. These introduced species are believed to be the greatest threat to the unique biodiversity of the Galapagos via predation, competition, infectious diseases, and habitat destruction.1

The Galapagos National Park Service (SPNG) oversees conservation in the Galapagos National Park and Galapagos Marine Reserve. An inspection and quarantine system (SICGAL) was established in 1998 to limit immigration and prevent the introduction and spread of exotic and invasive species to and between the islands. This includes a prohibition against animal movement and against the use of canine and feline vaccines. Eradication of nonnative feral goats, pigs, donkeys, cattle, poultry, cats, and dogs has periodically involved hunting and poisoning.2,5 In 2004, the SPNG launched a trial program to control dogs and cats by neutering. In May 2004, volunteers from the United States3 and the islands conducted a neutering campaign on Isabela Island supervised by the SPNG, SICGAL, and the municipal Control and Management of Introduced Species committee (CIMEI) for the island. The pilot program was deemed a success and expanded to other islands of the Galapagos in subsequent years.

Isabela is the largest and most volcanically active island in the Galapagos archipelago. It has a diverse landscape, rising from beaches of sand and lava rock, mangroves and lagoons near sea level, through dry forests of cactus and scrubland, to the rain forests of the highlands, and finally to barren lava flows surrounding the 6 active volcanoes. The island has the greatest diversity of native animal species and is best known for its giant tortoises, marine iguanas, and Darwin finches. In addition to a human population of 1,500, the island is colonized by many invasive species, including cats and
dogs. Of particular concern is the hunting of endangered iguanas by free-roaming dogs and of birds by cats.6,7 Most of the residents inhabit the coastal fishing village of Puerto Villamil, and a smaller number live on farms in the highlands. In Puerto Villamil, the dogs freely roam in the village and on the beaches where the iguanas live. Most dogs are friendly and associate with a family residence where they return at night. Some cats also appear to associate with a home or business, whereas others are stray or feral. Most dogs and cats live outdoors, and it is unusual for them to be confined in any way. Pets are primarily fed uncooked fish scraps or leftovers from family meals, and parasiticides developed for human and food animal use are provided to almost half of the dogs.2 No animals receive heartworm prophylaxis. A census performed by the CIMEI just before the neutering campaign in 2004 estimated the presence of approximately 320 dogs and 150 cats on the island.

Because dogs and cats may not be transported between the islands and vaccines are prohibited, Isabela has an isolated population of dogs and cats. The neutering program provided a unique opportunity to evaluate the health of the dogs and cats in this environment. To the authors’ knowledge, there are no published reports on infectious diseases in dogs and cats in the Galapagos. The purpose of this study was to determine the prevalence of a broad panel of infectious diseases of dogs and cats that impact their health, that could spill over to native wildlife, and that sentinel the presence of diseases of concern to human beings.

Materials and Methods

Study Participants
A permit for collection and exportation of blood samples from cats and dogs participating in the neutering project was obtained from SICGAL. The study population included 95 dogs (30 females, 65 males) and 52 cats (26 females, 26 males) presented for neutering in May 2004. The animals either were voluntarily presented by their owners (34) or, in the case of feral cats (18), were captured in humane traps. The ages ranged from approximately 6 weeks to adult for cats, and from 6 months to adult for dogs. Of the 52 cats, 15 were juveniles <6 months of age. A majority of animals sampled resided in the seaside village of Puerto Villamil. Only 3 dogs and 3 cats were sampled from the farms of the highlands. Based on the CIMEI census of 320 dogs and 150 cats, the samples represented 30% of the estimated dog population and 35% of the estimated cat population.

Sample Collection
Blood was collected by jugular venipuncture into EDTA tubes at the time of neutering. Blood films were prepared immediately, air dried, and stained with Wright-Giemsa stain upon return to the United States. After centrifugation on site, the plasma and blood cells were separated into plastic cryovials and stored at approximately 4°C until being returned to the United States 1–2 weeks after collection. The samples were then frozen at −20°C pending analysis.

Sample Analysis
All assays were performed and interpreted following the standard operating procedures for each laboratory or the manufacturer’s instructions. Plasma from canine blood samples was tested for specific antibodies by ELISA (Ehrlichia canis,8 Borrelia burgdorferi,8 Leishmania donovani), Western blot (Trypanosoma cruzi), microscopic agglutination (Leptospira interrogans serovars canicola, grippotyphosa, hardjo, icterohaemorrhagiae, pomona), IFA (Babesia canis, Babesia gibsoni Asian genotype, Babesia conradii), hemagglutination inhibition (canine parvovirus [CPV]), and virus neutralization (canine distemper virus [CDV], canine adenovirus 1 and 2 [CAV1/2], canine parainfluenza virus [CPV], and canine enteric coronavirus [CE Cv]). The plasma was also tested for Dirofilaria immitis antigen by ELISA.8 DNA was extracted from blood cells for PCR assays (Bartonella spp., Ehrlichia/Anaplasma/Neorickettsia/Wolbachia spp., and Hemoplasma spp.). The Ehrlichia/Anaplasma/Neorickettsia/Wolbachia spp. PCR assay gives a shared amplion for all genera and so amplified products were sequenced to confirm pathogen species identification.

Plasma from feline blood samples was tested for specific antibodies by ELISA (Bartonella spp., D. immitis,8 feline immunodeficiency virus [FIV],8 feline coronavirus [FCoV],8 feline panleukopenia virus [FPV],8 feline calcivirus [FCV],8 feline herpesvirus 1 [FHV],8 and Toxoplasma gondii) and for specific antigens by ELISA (D. immitis,8 feline leukemia virus [FeLV]8). DNA was extracted from blood cells for PCR assays (Bartonella spp., Hemoplasma spp., Ehrlichia/Anaplasma/Neorickettsia/Wolbachia spp.). Blood smears stained with Wright-Giemsa reagent were evaluated by microscopy for hemoparasites.

Statistical Analysis
Prevalence was defined as the proportion of tested animals with positive test results. 2x2 statistics were used to compare prevalence between males and females, and juveniles and adults.1 Odds ratios (ORs), 95% confidence intervals (CI), and P-values were calculated for statistically different prevalences by univariate logistic regression.1 Values of P < .05 were considered to be significant. Binomial proportion 95% CI were calculated for infection prevalences.

Results
Dogs were commonly seropositive for antibodies against CPV, CAV-1/2, and CPIV (Table 1). Only 22% were seropositive for CDV exposure and none was seropositive for CECoV. All dogs were negative for antibodies against E. canis, B. burgdorferi, T. cruzi, Babesia spp., and 5 serovars of L. interrogans. Antibodies to L. donovani were detected in 4 dogs. D. immitis antigen was detected in 34% of the dogs. Most (66%) of the D. immitis-positive dogs were also PCR-positive for the filarial endosymbiont Wolbachia pipientis, whereas none of the D. immitis-negative dogs carried W. pipientis. Anaplasma platys (1 dog) was the only Ehrlichia/Anaplasma/Neorickettsia group organism identified by PCR and genetic sequencing. Mycoplasma haemocanis DNA was amplified from 1 dog. A total of 13 dogs were PCR positive for 1 of 3 Bartonella spp., but none of the infected dogs carried more than a single species. Three of the D. immitis-positive dogs were coinfected with Bartonella spp. (2 with B. clarridgeae, 1 with B. elizabethae), and 3 other D. immitis-positive dogs were coinfected with L. donovani. When the prevalence of infections was compared between male and female dogs, the only significant difference was a higher rate in males of CAV-1 (77% in males versus 47% in females; OR = 3.8, 95% CI = 1.4–10.7; P = .007) and CAV-2 (75% in males versus 47% in
females; OR = 3.5; 95% CI = 1.9–9.7; P = .01). Of the 64 dogs with CAV-1 antibodies, all but 1 also had CAV-2 antibodies. The CAV-1 antibody titer exceeded (48/64 dogs, 75%) or equaled (15/64 dogs, 23%) the CAV-2 antibody titer in all but 1 dog.

Of the cats tested, none was positive for FeLV antigen, FIV antibodies, or FCoV antibodies, but antibodies against FCV and FPV were common (Table 2). A majority of the cats had antibodies to T. gondii (63%) and Bartonella spp. (75%). One cat had D. immitis antibodies, but all cats were negative for D. immitis antigen. Mycoplasma haemofelis DNA was amplified from the blood of 1 cat, and all cats were negative for Ehrlichia/Anaplasma/Neorickettsia/Wolbachia group DNA. Bartonella henselae or B. claridgeae DNA was amplified from the blood of 23 cats; 8 cats were coinfected with both species. No blood parasites were evident on stained blood films. When the prevalence of infections was compared between male and female cats, the only significant difference was a higher rate of B. henselae DNA in females (59% in females versus 19% in males; OR = 4.2; 95% CI = 1.1–17.8; P = .04). On comparing infection prevalence in juvenile (<6 months) and adult cats, adults had a higher rate of T. gondii (84% in adults versus 13% in juveniles; OR = 33.6; 95% CI = 5.0–293.6; P < .0001) and FCV (60% in adults versus 7% in juveniles; OR = 20.5; 95% CI = 2.3–463.9; P = .001).

Discussion

The results of this survey indicate that some canine and feline pathogens with a cosmopolitan distribution have been introduced into the Galapagos and are now endemic in the local dog and cat populations on Isabela Island. The dogs and cats were exposed to several common viruses, including CPV, FPV, CDV, CAV, CPiV, FCV, and FHV. None of the tested animals was exposed to enteric coronaviruses or infected with FeLV or FIV. Two thirds of the dogs were seropositive for CAV. The CAV-1 antibody titer exceeded or equaled the CAV-2 antibody titer in all but 1 dog, suggesting that CAV-1 was the predominant serotype and that the detection of CAV-2 reactivity may have been because of nonspecific cross-reactivity, which is common for CAV. None of the viruses endemic in the dogs and cats on Isabela infects human beings, reptiles, or birds. Marine mammals are susceptible to some strains of CDV and FCV, although host range and transmission modes are poorly understood.

CDV and FCV are highly contagious and are spread by virus shed in airborne droplets, in fecal material, or by direct contact with infected animals or fomites. The dogs and cats in Puerto Villamil frequently visit the beaches, and so there is potential for indirect contact with the beach-dwelling Galapagos sea lions. However, during a distemper outbreak in dogs in 2001, serological tests of sea lions in the area showed no evidence of exposure to CDV.

Paroviral exposure in particular appeared to be ubiquitous within the dog and cat populations. Several puppies and adult dogs were observed with hemorrhagic gastroenteritis during the May 2004 sample collection period, but on-site testing of feces for parovirus antigen

### Table 1. Results of testing for infectious diseases in 95 dogs from Isabela Island, Galapagos.

| Infectious Agent | Assay     | No. of Positive | Prevalence (%) | 95% CI |
|------------------|-----------|-----------------|----------------|--------|
| Bartonella henselae | PCR       | 8               | 8              | 3.14   |
| Bartonella clarridgeae | PCR   | 2              | 2              | 6.34   |
| Bartonella elizabethae | PCR   | 4              | 4              | 0.8    |
| Ehrlichia/Anaplasma/Neorickettsia spp. | PCR | 1              | 1              | 1–1.3  |
| Mycoplasma haemocanis | PCR   | 1              | 1              | 1–1.3  |
| Mycoplasma haemofelis | PCR   | 1              | 1              | 1–1.3  |
| Toxoplasma gondii | PCR  | 1              | 1              | 1–1.3  |
| T. gondii | IgG antibody | 62              | 62             | 48, 75 |

CI, confidence interval; NA, not applicable.

### Table 2. Results of testing for infectious diseases in 52 cats from Isabela Island, Galapagos.

| Infectious Agent | Assay     | No. of Positive | Prevalence (%) | 95% CI |
|------------------|-----------|-----------------|----------------|--------|
| Bartonella henselae | PCR   | 18             | 35             | 22, 48 |
| Bartonella clarridgeae | PCR   | 13             | 25             | 13, 37 |
| Bartonella elizabethae | PCR   | 0              | 0              | 0–6.2  |
| Bartonella spp. | Antibody  | 39            | 75             | 63, 87 |
| Canine adenovirus-1 | Antibody | 64           | 67             | 58, 77 |
| Canine adenovirus-2 | Antibody | 63           | 66             | 57, 76 |
| Canine parainfluenza virus | Antibody | 95         | 100            | 96, 100 |
| Canine parvovirus | Antibody  | 95            | 100            | 96, 100 |
| Canine enteric coronavirus | Antibody  | 0            | 0              | NA     |

CI, confidence interval; NA, not applicable.
or collection of paired acute and convalescent serum samples for seroconversion was not possible. Because most of the dogs and cats presented for neutering appeared healthy and vaccination is prohibited, the high parvovirus antibody prevalence suggests that these animals were either subclinically infected or had recovered from infection at the time of testing. Parvoviruses are not known to affect the native species found in the Galapagos, but these viruses undergo rapid evolution and have occasionally increased their host range to new species, as evidenced by the jump from cats to dogs in the late 1970s.13,14

Distemper outbreaks have occasionally occurred in the Galapagos. One outbreak in 2001 originated on the island of Santa Cruz and killed more than 300 dogs.2,12 A month later, dogs began to die of distemper on Isabela. Approximately 300 dogs died in the Isabela outbreak, leaving only 5 or 6 dogs remaining in Puerto Villamil and approximately 30 dogs on the highland farms or as strays. On both islands, the source of the infection was believed to be illegally introduced dogs. Authorities responded to the outbreak by quarantining healthy dogs indoors, culling sick dogs, and burning carcasses. Humans traveling from the mainland and between islands were treated with disinfectant in an attempt to prevent reintroductions of the virus. A door-to-door census conducted on Isabela 15 months after the 2001 distemper outbreak revealed that the dog population had rebounded to 197.2,12 At the time of the neutering campaign in May 2004, a small portion of the dogs had CDV antibodies, but the antibody titers were low compared with those usually observed in vaccinated dogs or dogs recovering from infection. The presence of antibodies may be because of decreased specificity of the assay when using plasma (versus serum), because of cross-reactivity with a related virus, or because of previous exposure to CDV. The small number of antibody-positive dogs, coupled with low antibody titers and prohibition of vaccination, suggests that most of the canine population on Isabela Island is susceptible to distemper and a new epidemic could occur. The rapid recovery of the canine population so soon after its decimation by distemper illustrates the difficulty of controlling invasive species in the absence of ongoing programs. A similar phenomenon occurred after an eradication program on Isabela in 1981 in which the dog population was reduced from an estimated 300–500 members to < 100 by means of poisoning.5 After the precipitous decline of the population because of the culling, it recovered to its original baseline by the 2001 distemper outbreak, after which it again recovered by the time of the neutering campaign in 2004.

Dogs and cats that live outdoors in tropical climates are at increased risk for diseases transmitted by biting insects. Although many species may be incidentally infected with *D. immitis*, canids are considered to be the true natural reservoir. One third of the dogs in the current study were infected with *D. immitis*, indicating that this parasite is endemic in the dog population of Isabela and that the local mosquito population is capable of transmitting infective larvae. Of the heartworm-infected dogs, two thirds also had evidence of exposure to *W. pipiens*, an endosymbiont found in a variety of human and animal filarial parasites and now believed to play an important role in the pathology of filarial diseases.15,16 Although it is believed that 100% of heartworms carry *W. pipiens*, it was not detected in all heartworm-infected dogs. This may be because of the use of serum rather than whole blood in the PCR assay, because microfilaria, a rich source of *W. pipiens*, are not present in serum. Detection of *W. pipiens* in the circulation is most common during heartworm larval molting and at the time of heartworm death. In addition to the dogs, 1 cat had evidence of exposure to *D. immitis*. A study in the early 1980s made reference to finding *D. immitis* in dogs on Isabela, but the prevalence of infection was not reported.5 This same study reported that 77% of dogs on Floreana Island had *D. immitis* microfilaria and 18% of cats had serum antibodies against *D. immitis*. To the authors’ knowledge, there are no other reports on the prevalence of *D. immitis* infection in dogs and cats in the Galapagos or mainland Ecuador. Only a few of the 13 South American countries have reported heartworm infections in dogs, including Peru, Colombia, and Brazil, and the prevalence ranges from 2% in Brazil to 5% in Colombia.17 Feline heartworm infection is seldom reported in South America but has been documented in Brazil.17

Infection with *Borrelia* spp., *Babesia* spp., and the *Ehrlichia/Anaplasma* spp. group of tick-transmitted diseases is endemic in South American dogs. *Ehrlichia canis*, *B. canis*, and *B. burgdorferi* infections have been identified in dogs in Argentina, Brazil, Chile, and Venezuela,18–22 whereas infections with *A. platys* and *Anaplasma phagocytophilum* have been reported in dogs in Venezuela.21,23 In Brazil, the seroprevalence for *E. canis* in dogs is 23–30%,18,20 and 36–67% for *B. canis*.20,24 Of the dogs and cats in the Galapagos tested for exposure to these tick-borne diseases, only 1 dog was found to be infected with *A. platys*. There are no published reports on the presence of ixodid ticks infected with *Borrelia* or *Ehrlichia/Anaplasma/Babesia* organisms in the Galapagos, and it is unknown whether this dog was initially exposed to infected ticks on Isabela Island or outside of the Galapagos.

Some vector-borne pathogens that infect dogs and cats also infect humans. Thus, infected dogs and cats can serve as reservoirs for maintenance of the pathogen in the vector population and as sentinels for the threat of infection of humans. *Leishmania* spp. are obligate intracellular protozoan parasites transmitted by infected phlebotomine sandflies and other biting insects. Infected dogs play a role in transmission of leishmaniasis to humans by serving as sources of infection for the biting insects.25 In South America, canine leishmaniasis is endemic with increasing prevalence. The seroprevalence for leishmaniasis in dogs ranges from 21 to 67% in Brazil and from 8 to 45% in Peru.29 Most infected dogs are asymptomatic. Canine leishmaniasis is important because infection precedes the occurrence of human cases, and the risk for human infections has been correlated with the prevalence in dogs.29 In an effort to decrease the
potential for human infections, seropositive dogs identified by periodic serosurveys have been culled to decrease the reservoir for infection of biting insects. The elimination of infected dogs is supported by the World Health Organization. Four of the dogs on Isabela Island were seropositive for L. infantum antibodies, suggesting the presence of infected biting insects in the Galapagos and the threat for human infection.

Vector-borne pathogens with zoonotic potential were also observed in both dogs and cats in the Galapagos. Three different species of Bartonella were identified in dogs, whereas cats carried 2 species. These agents are often transmitted by fleas, which are common in the Galapagos. The prevalence rates of B. henselae and B. clarridgeiae infections are similar to those of high flea risk areas in the United States. Among the Bartonella spp. observed, only cats infected with B. henselae are considered to have significant zoonotic risk to immune-competent humans (cat scratch disease).

Nonvector-borne infections in dogs and cats with zoonotic potential include toxoplasmosis and leptospirosis. A majority of cats had evidence of exposure to T. gondii. This infection is acquired by cats from eating infected prey, from being fed undercooked contaminated meat, transplacentally, or by ingestion of sporulated oocysts. T. gondii has an extremely broad host range and can be spread to humans and animals by ingestion of contaminated cat feces, undercooked meat, or infected cats. Toxoplasmosis poses a significant threat to the health of unborn children if the mother is first exposed during pregnancy. Toxoplasmosis has also been associated with disease outbreaks in a number of wildlife species, recently among otters in the coastal waters of California. The prevalence rate for T. gondii in the Galapagos cats is similar to that in cats in other countries. Canine leptospirosis is endemic in South America, where the seroprevalence ranges from 11 to 41% in dogs. Infected dogs serve as reservoirs for infection of humans and wild species such as sea lions. None of the dogs on Isabela Island was seropositive for L. interrogans serovars canicula,icterohemorrhagiae, and grippotyphosa, which are the most commonly reported serovars in South American dogs.

In conclusion, despite their seclusion from the rest of the world, the cats and dogs of Isabela are exposed to many of the same pathogens found in South America and worldwide. This is most likely owing to persistence of endemic infections that were present before the strict control of interisland animal movements. Infections may also be introduced by the illegal smuggling of infected animals or by the adventitious contamination of people and supplies arriving by air or boat. Some of the infections posed a risk to humans or to native wildlife, but most were specific to dogs and cats. Because of the relatively small number of samples used in this study, particularly from cats, failure to identify specific pathogens should not be interpreted as evidence against their presence. Routine use of parasite prophylaxis, neutering, and strict enforcement of animal movement restrictions would control many of the diseases. However, in the absence of a vaccination program, a reservoir of susceptible animals remains vulnerable to new disease introductions.

Footnotes

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