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Ocelots on Barro Colorado Island Are Infected with Feline Immunodeficiency Virus but Not Other Common Feline and Canine Viruses

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ABSTRACT: Transmission of pathogens from domestic animals to wildlife populations (spillover) has precipitated local wildlife extinctions in multiple geographic locations. Identifying such events before they cause population declines requires differentiating spillover from endemic disease, a challenge complicated by a lack of baseline data from wildlife populations that are isolated from domestic animals. We tested sera collected from 12 ocelots (*Leopardus pardalis*) native to Barro Colorado Island, Panama, which is free of domestic animals, for antibodies to feline herpes virus, feline calicivirus, feline corona virus, feline panleukopenia virus, canine distemper virus, and feline immunodeficiency virus (FIV), typically a species-specific infection. Samples also were tested for feline leukemia virus antigens. Positive tests results were only observed for FIV; 50% of the ocelots were positive. We hypothesize that isolation of this population has prevented introduction of pathogens typically attributed to contact with domestic animals. The high density of ocelots on Barro Colorado Island may contribute to a high prevalence of FIV infection, as would be expected with increased contact rates among conspecifics in a geographically restricted population.

Key words: Barro Colorado Island, FIV, *Leopardus*, ocelot, serology.

The spillover of pathogens from domestic animal species has been a source of numerous outbreaks in wildlife populations with disastrous consequences (Daszak et al., 2000). Evaluating the risk of spillover in wildlife populations is complicated by a lack of baseline data from populations isolated from domestic animals (Munson and Karesh, 2002). Most surveys are performed in populations in proximity to human settlements and domestic animals because the threat of adventitial disease is presumed to be greater in such locations. However, without appropriate baseline data, it is difficult to determine whether the presence of a pathogen represents an introduction from domestic animals, signifying a potential threat to the wildlife population, or whether the pathogen has been historically present in the wild population.

Exposure to multiple pathogens typically considered to reside in domestic animal reservoirs have been documented in ocelots (*Leopardus pardalis*), including feline herpes virus (FHV), feline calicivirus (FCV), feline corona virus (FCoV), feline panleukopenia virus (FPV), feline leukemia virus (FeLV), and canine distemper virus (CDV; Schmitt et al., 2003; de Carvalho Ruthner Batista et al., 2005; Filoni et al., 2006; Fiorello et al., 2007). Fiorello et al. (2007) reported that from a sample of 10 ocelots sampled in Kyaa-Iya del Gran Chaco National Park (Bolivia) and the adjacent area, seven and 10 had antibodies to CDV and FCV, respectively. Although this national park is approximately 40 km from human settlement, villagers frequently hunt in areas adjacent to the national park with dogs (*Canis familiaris*). This makes it difficult to determine whether the exposure of these ocelots to CDV and FCV represents the presence of an endemic wildlife disease, or whether it occurs as a result of direct or indirect contact with domestic animals in areas adjacent to the national park.

Unlike the aforementioned diseases, wild felid infection with feline immunodeficiency viruses (FIV; family *Retrovir-*

idae, genus *Lentivirus*) is not suggestive of cross-species transmission. Domestic cat (*Felis catus*) FIV has been identified in a wild felid only once (Nishimura et al., 1999), and transmission of different strains of FIV among captive or free-ranging nondomestic felids has been documented on few occasions (Carpenter et al., 1996; Troyer et al., 2005; Franklin et al., 2007a). Rather, species-specific FIV strains have been identified in almost all cases of nondomestic cat infections where FIV genotype analyses were performed; this relationship has been demonstrated with a virus that was isolated from an ocelot (Troyer et al., 2005).

We tested 12 ocelots from an estimated total population of 30 animals (Ziegler, 2002) from Barro Colorado Island (BCI), Panama, for antibodies to FHV, FCV, FCoV, FPV, CDV, and FIV. Samples also were tested for FeLV antigen. Our objective was to acquire baseline antibody prevalence data from a population of ocelots isolated from domestic animals. Barro Colorado Island (1,600 ha; 9°9′N, 79°51′W) is a hilltop that was isolated from the mainland in 1914 when the Chagres River was dammed to create Lake Gatun as part of the Panama Canal. The minimum distance between the island and the mainland is 200 m, although small islands break up this interval in some places. There are no domestic animals permitted on BCI, and poaching of native fauna is limited or nonexistent because the island is heavily guarded (Wright et al., 2000). The area of BCI was not well developed before the creation of the canal, and it is unlikely that there were any domestic animals at this site before 1913. Movement of ocelots between the island and the mainland has not been studied in detail, although a low level of ocelot emigration from BCI has been documented in that one radiocollared BCI ocelot was tracked to the mainland. Other predators may likely limit cross-water movement of ocelots as evidenced by the killing of another radiocollared BCI ocelot by a crocodile. Additional felid species residing on BCI include margay (*Leopardus wiedii*) and jaguarundi (*Herpailurus yagouaroundi*). Puma (*Puma concolor*) are detected regularly, and jaguars (*Panthera onca*) rarely; neither of these species reside permanently on the island (Moreno et al., 2006).

Ocelots were trapped with metal and wooden box-traps from January 2001 to May 2004 and sedated with either a tiletamine and zolezapam premixture (Telazol®, Fort Dodge Animal Health, Fort Dodge, Iowa, USA) or ketamine hydrochloride and xylazine. Whole blood samples were collected in untreated serum tubes. Samples were spun at 25 × G for 10 min, and the serum was separated from the coagulated cells. Samples were stored at −70 C or −20 C until serology was performed with serum for five common feline and canine viruses at the Colorado State University Diagnostic Laboratory (see Table 1 for a list of pathogens and assays used).

A triple chemiluminescent immunoblot with antigen preparations from three distinct strains of feline lentivirus (domestic cat FIV; puma lentivirus, PLV; and African lion lentivirus) was used to test for antibodies to FIV (Franklin et al., 2007a). Use of this multi-antigen-based immunoblot has been shown to enhance sensitivity without loss of specificity in the detection of wild felid FIV (Franklin et al., 2007b). A commercial enzyme-linked immunosorbent assay (ELISA) (FIV/FeLV Combo SNAP™ test; Idexx Inc., Westbrook, Maine, USA) was used as a comparison to FIV immunoblot and for detection of FeLV antigen. Deoxyribonucleic acid was extracted using a standard phenol chloroform protocol (Sambrook and Russell, 2001) using the coagulated blood cell volume (~2 ml). Nested PCR was performed using degenerate primers designed from the conserved reverse transcriptase region of FIV *pol*, using GenBank sequences of FIV (accession nos. M25381 and U11820), PLV (acces-

TABLE 1. Results of serologic testing of 12 Barro Colorado Island ocelots.

Pathogen	Method	*n*	+	Equivocal	−
Feline calicivirus (FCV)	Serum Neutralization (SN)	12	0	NA[a]	12
Canine distemper virus (CDV)	SN	12	0	NA[a]	12
Feline herpes virus (FHV)	SN	12	0	NA[a]	12
Feline corona virus (FCoV)	Hemagglutination Inhibition (HI)	12	0	NA[a]	12
Feline panleukopenia (FPV)	Immunofluorescent Antibody (IFA)	12	0	NA[a]	12
Feline leukemia virus (FeLV)	Antigen ELISA[b]	12	0	0	12
Feline lentivirus	Immunoblot	12	6	1	5
Feline lentivirus	Antigen ELISA[b]	12	5	2	5

[a] NA = not applicable.

[b] FIV/FeLV Combo SNAP test (Idexx).

sion no. U03982), and FIV-Oma (*Otocolobus manul*; accession no. U56928) (Troyer et al., 2005). Positive and negative controls, including FIV-positive and -negative laboratory domestic cats and FIV-positive pumas, bobcats, and African lions (*Panthera leo*) were run concurrently. The immunoblot, ELISA, DNA extraction techniques, and PCR protocols and the relative sensitivity and specificity of each are discussed in detail in Franklin et al. (2007b).

Except for FIV, all test results were negative (Table 1). These negative results are inconsistent with previous studies in which exposure to all six viruses other than FIV is reported (Schmitt et al., 2003; de Carvalho Ruthner Batista et al., 2005; Filoni et al., 2006; Fiorello et al., 2007). We do not believe that this disparity between studies can be attributed to small sample size, because with one exception our sample size was larger than these previous studies. In addition, very high antibody prevalence rates for some of these pathogens have been reported, including a 70% and 100% antibody prevalence rate for CDV and FCV, respectively, in ocelots from Bolivia (Fiorello et al., 2007). Moreover, the diagnostic tests used in our study and these previous studies also were similar.

These findings support the hypothesis that these pathogens are not endemic to BCI. The absence of these pathogens may be the result of isolation from domestic animals. Alternatively, the BCI ocelot population, and that of other sympatric felids, may be too small to ensure persistence of these viruses. Sampling of additional ocelots on BCI, and sampling of ocelots and sympatric domestic animals on the mainland, is needed to further investigate these possibilities. Unlike the other viruses we investigated, the prevalence of FIV was higher in our sample than reported previously for ocelots from other studies. Six of the 12 BCI ocelots tested were positive (50%) compared with 0% for 38 captive ocelots from Brazil (Filoni et al., 2003), 0% for 10 wild ocelots in Bolivia (Fiorello et al., 2007), and 6% for 90 free-ranging, wild-born, and captive ocelots (Troyer et al., 2005). These previous studies used a virtually identical immunoblot protocol or the same commercial ELISA test used in this study.

We were unable to amplify genomic FIV sequences from any of the seropositive animals using degenerate PCR primers for FIV. These negative results are consistent with reported difficulties in attempts to amplify and sequence virus from ocelots (Troyer et al., 2005) and other nondomestic cat species. PCR amplification of FIV sequences from nondomestic cats is not sensitive because sequence variation requires use of degenerate primers that do not bind viral sequence efficiently (Troyer et al., 2005; Brennan et al., 2006; Franklin et al., 2007b). Furthermore, proviral load may

be low, thus decreasing the probability of adequate binding between sufficient numbers of primer and target sequences (Brennan et al., 2006). Successful amplification of proviral sequences from puma, bobcat, and domestic cat samples run in parallel to the DNA samples from BCI ocelots suggest that there were not inherent problems with our PCR assay and that antibody-positive ocelots were not infected with a FIV strain associated with other hosts, most notably domestic cats. Because cross-species transmission of FIV strains is rare, we suspect that the virus in our study animals is most likely an ocelot-specific virus and that our assay lacked the sensitivity to detect it due to the aforementioned reasons.

The high density of ocelots on BCI may promote more frequent contact among individuals and enhance FIV transmission, explaining the high antibody prevalence observed in this study. The BCI population has been estimated at 30 ocelots based on camera-trapping surveys (Ziegler, 2002), and because BCI is only 1,600 ha, this represents a higher density ($\sim$2 individuals/km^2) than reported from other locations where densities range from 0.077 to approximately 0.8 ocelots/km^2 (Emmons, 1988; Trolle and Kery, 2003; Maffei et al., 2005; Trolle and Kery, 2005; Di Bitetti et al., 2006). Density-related transmission is supported by the finding that African lion densities are positively correlated with FIV prevalence (Winterbach et al., 2006). Interestingly, only one of five (20%) male BCI ocelots was positive for FIV infection, whereas five or six of the seven females (71–86%) were FIV immunoblot positive ($P>0.05$). No FIV gender bias has been previously reported, but this possibility should be further evaluated because it may provide insight into the mode of FIV transmission within this population.

The implications of FIV infection in the BCI ocelot population are unknown. Although studies have not detected clinical affects of FIV in naturally occurring infections in wild pumas (Biek et al., 2003, 2006a, b), other reports of captive, or in one study free-ranging animals, have detected end-stage or subclinical immunologic dyscrasias (Poli et al., 1995; Bull et al., 2003; Brennan et al., 2006; Roelke et al., 2006). The PCR amplification and sequencing are needed to characterize the virus infecting the BCI ocelots as is further evaluation of its pathogenicity. Development of a primer set from the ocelot sequence reported by Troyer et al. (2005) would be a possible strategy to increase sensitivity of this assay.

In summary, we hypothesize that isolation from domestic animals has protected this population from pathogens that are normally present in domestic animal reservoirs, whereas a high density of ocelots on BCI may have increased transmission of a species-specific FIV. Further study of BCI ocelots and of ocelots and domestic animals on the adjacent mainland is warranted to test these hypotheses.

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