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Serologic Evidence of Nonfatal Rabies Exposure in a Free-ranging Oncilla (*Leopardus tigrinus*) in Cotapata National Park, Bolivia

Sharon L. Deem,[1,5,6] **Rolan Davis,**[2] **and Luis F. Pacheco**[3,4] [1] Field Veterinary Program, Wildlife Conservation Society, 2300 Southern Boulevard, New York, New York 10460, USA; [2] College of Veterinary Medicine, Kansas State University, Manhattan, Kansas 66506, USA; [3] Estación Biológica Tunquini, Instituto de Ecología, Casilla 10077, Correo Central, La Paz, Bolivia; [4] Centro de Estudios en Biología Teórica y Aplicada (BIOTA), Casilla 9641, La Paz, Bolivia; [5] Current address: Department of Animal Health, Smithsonian's National Zoological Park, Washington, D.C. 20008, USA; [6] Corresponding author (email: deems@si.edu)

ABSTRACT: A clinically healthy free-ranging oncilla (*Leopardus tigrinus*) was live-trapped in Boliva in 2000. Based on serology, we concluded that this animal was exposed to feline panleukopenia virus, *Toxoplasma gondii,* and rabies virus. The rabies virus-neutralizing antibody titer (>70 IU/ml) in this oncilla was unusual for an asymptomatic animal exposed to street virus and at a level expected in animals exposed to a large amount of virus, clinically affected, or vaccinated. Based on a subsequent 18 mo of radiotracking, we concluded that the oncilla had a nonfatal exposure to rabies virus.

Key words: Bolivia, feline panleukopenia virus, *Leopardus tigrinus,* oncilla, rabies, serology, *Toxoplasma gondii.*

Rabies is a highly fatal, acute viral encephalomyelitis caused by RNA viruses in the genus *Lyssavirus*, family *Rhabdoviridae* (Rupprecht et al., 2001). All warmblooded animals are susceptible to rabies virus infection, although susceptibility varies among species. Felids have intermediate susceptibility; less than foxes (*Vulpes* spp.), coyotes (*Canis latrans*), and wolves (*Canis lupus*), but greater than domestic dogs and primates (Podell, 1994). Additionally, susceptibility is influenced by the quantity of virus introduced, viral strain, age of the animal, site of inoculation, immunocompetence, and prior vaccination (Carey and McLean, 1983). Although clinical disease does not always result from infection with rabies virus, the outcome is often fatal following clinical disease (Murphy, 1985). However, there are reports of humans and animals that have recovered following confirmed clinical rabies (Fekadu, 1991). Here we describe serologic evidence of exposure of a clinically healthy, free-ranging oncilla (*Leopardus tigrinus*) to rabies virus that was subsequently followed via telemetry for a period of 18 mo.

In March 2000, a 1.6-kg, adult female, free-ranging oncilla was captured in Cotapata National Park, Bolivia (16°11′37″S, 67°52′14″W) (Pacheco et al., 2001). The oncilla was captured by using a tomahawk trap (81×24×24 cm) (Tomahawk Live Trap, Tomahawk, Wisconsin, USA) baited with a live chicken. The oncilla was immobilized with tiletamine-zolazepam combination (Telazol®, Fort Dodge Laboratories, Fort Dodge, Iowa, USA), 7.5 mg intramuscularly (IM) delivered by blowdart (Telinject® USA, Saugus, California, USA) and supplemented with 25 mg of IM ketamine (Ketaset®, Fort Dodge Laboratories) by syringe. During immobilization, a physical examination was performed, body measurements were taken, the oncilla was fitted with a radiocollar (Advanced Telemetry Systems, Inc., Isanti, Minnesota, USA) with activity/mortality sensors, and biomaterials were collected. A fecal sample was collected from the trap and preserved in 10% formalin for endoparasite identification. Hair was plucked from the dorsal thoracic region and stored in a paper envelope for genetic analysis. Blood was collected from the jugular vein and immediately placed in a serum separator tube (Corvac, Sherwood Medical, Saint Louis, Missouri, USA). The blood tube was placed in the shade until clot formation and then the serum was separated by centrifugation in a portable 12-volt centrifuge (Mobilespin, Vulcan Technologies, Gransview, Missouri, USA) at 3,000 × G for 15 min and stored in liquid nitrogen.

Table 1 summarizes the serologic tests

TABLE 1. Resutls of serology on a free-ranging oncilla (*Leopardus tigrinus*) from Cotapata National Park, Bolivia.

Pathogen	Serologic test[a] (titer considered positive)	Serologic results
Canine distemper virus	Serum neutralization (1:8)	Negative
Dirofilaria immitis	ELISA[b]	Negative
Feline calicivirus	Serum neutralization (1:8)	Negative
Feline herpesvirus	Serum neutralization (1:32)	Negative
Feline immunodeficiency virus	ELISA (NA)	Negative
Feline coronavirus	KELA (1:4)	Negative
Feline leukemia virus	ELISA (NA)	Negative
Feline panleukopenia virus	HAI (1:8)	1:20
Leptospira interrogans (18 serovars)	Microagglutination (1:100)	Negative
Neospora caninum	IFAT (1:100)	Negative
Rabies virus	RFFIT (>0.5 IU/ml)	>70 IU/ml
Toxoplasma gondii	KELA (1:48)	1:74

[a] ELISA = enzyme-linked immunosorbent assay; NA = not applicable; KELA = kinetics-based enzyme-linked immunosorbent assay; HAI = hemagglutination inhibition; IFAT = indirect fluorescent antibody test; RFFIT = rapid fluorescent focus inhibition test.

[b] Enzyme-linked immunosorbent assay for *D. immitis* antigen.

performed, methods used, and the antibody titer defined as positive by laboratories conducting the tests. Rabies antibody testing was conducted at the Kansas State Veterinary Diagnostic Laboratory, Kansas State University (KSU, Manhattan, Kansas, USA) by using the rapid fluorescent focus inhibition test (RFFIT) (Smith et al., 1996). An additional five oncilla sera collected from cats in Brazilian zoos in 1989 and 1990 as part of a biomedical survey as described in Swanson et al. (2003) also were tested for rabies virus antibodies. Sera from this survey had been stored at −70 C until 2003. Serology for antibodies against feline immunodeficiency virus and feline leukemia virus and the *Dirofilaria immitis* antigen test were conducted at the Oklahoma Animal Disease Diagnostic Laboratory (Oklahoma State University, Stillwater, Oklahoma, USA). All other serologic tests were conducted at the New York State Veterinary Diagnostic Laboratory (Cornell University, Ithaca, New York, USA). The fecal sample was processed by using both sugar and zinc sulfate flotation methods at Cornell University.

At capture, the oncilla was judged nonpregnant and clinically healthy based on physical examination. *Toxocara* sp., *Ancylostoma* sp., *Capillaria aerophila,* and *Capillaria putorii* ova, *Aelurostrongylus abstrusus* larvae, and *Lynxacarus* sp. eggs and adult mites were present in the feces. Antibodies to feline panleukopenia virus (1:20), rabies virus (>70 IU/ml), and *Toxoplasma gondii* (1:74) were detected (Table 1). The RFFIT for rabies virus antibody was repeated by using an initial serum dilution of 1:100 (due to the minimal amount of serum available). The results of this second RFFIT detected antibodies (>50 IU/ml), thus confirming the initial RFFIT results. All five Brazilian oncilla tested had rabies virus antibody titers <0.05 IU/ml.

Feline panleukopenia is a highly contagious parvovirus that causes clinical disease in domestic cats, wild felids, mustelids, procyonids, and viverrids (Scott, 1990). Antibodies to feline panleukopenia virus also have been detected in many free-ranging felids, including bobcats (*Lynx rufus*) (Wassmer et al., 1988), Florida panthers (*Puma concolor coryi*) (Roelke et al., 1993), leopard cats (*Prionailurus bengalensis*) (Ikeda et al., 1999), and lions (*Panthera leo*) (Spencer, 1991). The low

antibody titer in the oncilla may indicate exposure to the virus or a nonspecific cross-reaction.

Cats are the only definitive hosts of *T. gondii*, which occurs wherever felids are present (Dubey, 1994). Finding a moderate antibody titer for *T. gondii* in the oncilla is similar to other studies of captive and free-ranging felid species (Roelke et al., 1993; Cheadle et al., 1999; Silva et al., 2001).

The rabies virus-neutralizing antibody titer in this oncilla was unusual for an asymptomatic animal exposed to street virus and at a level expected in animals exposed to a large amount of virus, clinically affected, or vaccinated (Niezgoda et al., 1997, 2002). The World Health Organization (WHO) standard for an antibody level indicative of adequate vaccination in humans is 0.5 IU/ml (WHO, 1992). Sera from naïve animals and humans are routinely <0.5 IU/ml in the KSU laboratory (Davis, unpubl. data). Five explanations are possible for the high rabies titer in the oncilla: the oncilla was previously vaccinated, the oncilla had a true inapparent infection, the oncilla had an infection and had recovered from clinical disease, the titer was due to nonspecific virus-neutralizing substances, and this was a false-positive result from laboratory error.

The possibility that this free-ranging oncilla was an escaped captive animal that had previously been vaccinated is highly improbable in that most domestic animals in the region do not receive rabies vaccines. Additionally, one of us (L.F.P.) has lived and worked in the region for 5.5 yr and has never heard of an oncilla being kept as a captive pet in the region. There are well-documented cases indicating chronic rabies virus infection, although the epidemiologic significance of a chronic carrier state is unknown (Jackson, 2002). Documented cases also exist of animals that have recovered from rabies, with and without neurologic sequelae (Bell, 1975; Niezgoda et al., 1997). Therefore, it is possible that the oncilla in this report either had an inapparent infection or had recovered from clinical rabies. Nonspecific virus-neutralizing substances have been found in several species (Bell, 1975), but nonspecific inhibition has not been found at a level above 1:25 (0.25 IU/ml) in any species tested to date (J. Smith, pers. comm.). Additionally, we tested five oncilla with the RFFIT and all of these animals had titers <0.05 IU/ml. Therefore, it is highly unlikely that the titer (>70 IU/ml) in the Bolivian oncilla was due to nonspecific substances. Although one cannot entirely rule it out, it is also unlikely that a titer of this level is related to laboratory error.

Current knowledge of rabies ecology includes that viral exposure may or may not lead to a productive viral infection, which may or may not lead to a detectable immune response; natural immunity to rabies varies depending in part on the species and virus variant; and some animals are carriers that remain clinically normal and yet can shed the virus and infect and kill other species, including humans (Carey and McLean, 1983). Serum neutralizing antibodies have been detected in other wild carnivores, such as African wild dogs (*Lycaon pictus*) (Gascoyne et al., 1993), Indian mongooses (*Herpestes auropunctatus*) (Everard and Everard, 1985), raccoons (*Procyon lotor*) (McLean, 1972), striped skunks (*Mephitis mephitis*) (Carey and McLean, 1983), and red foxes (*Vulpes vulpes*) (Baradel et al., 1988), in rabies-endemic areas.

Rabies is an endemic disease within the domestic dog population of much of South America, including many parts of Bolivia (Widdowson et al., 2002). Additionally, occasional cases of wildlife rabies in terrestrial species have been reported from throughout many portions of Latin America (Rupprecht et al., 2001). Our 18-mo period of telemetry on the oncilla after the blood collection supports a nonfatal exposure to rabies virus. Based on our findings, we cannot state whether this oncilla was a chronic carrier or simply exposed to the

rabies virus. Additionally, we cannot conclusively state how the oncilla in this report was exposed to rabies virus. However, domestic dogs and cats are commonly seen in Cotapata National Park (Deem, unpubl. data) and many Aymara Indian villages, within and surrounding the park, have domestic animals. Additionally, vampire bats (*Desmodus rotundus*), common in the region, are another potential source of exposure for this oncilla (Constantine, 1988).

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