## Research Article

# Serological Detection of Viral Infections in Captive Wild Cats from Costa Rica

### Kinndle Blanco,<sup>1</sup> Roberto Peña,<sup>2</sup> Carmen Hernández,<sup>3</sup> Mauricio Jiménez,<sup>4</sup> Luis Nazario Araya,<sup>4</sup> Juan José Romero,<sup>4</sup> and Gaby Dolz<sup>4</sup>

<sup>1</sup>Research Direction, Universidad Nacional, P.O. Box 86-3000, Heredia, Costa Rica

<sup>2</sup> Cataratas de la Paz Zoo, Monterrey, Alajuela, P.O. Box 502-1000, San José, Costa Rica

<sup>3</sup> Las Pumas Rescue Center, P.O. Box 89-5700, Cañas, Guanacaste, Costa Rica

<sup>4</sup> School of Veterinary Medicine, Universidad Nacional, P.O. Box 304-3000, Heredia, Costa Rica

Correspondence should be addressed to Kinndle Blanco, kinnvet@yahoo.es

Received 3 May 2010; Revised 29 August 2010; Accepted 8 February 2011

Academic Editor: G. F. Browning

Copyright © 2011 Kinndle Blanco et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Serum samples from a total of 44 wildcats, 28 margays (*Leopardus wiedii*), 10 ocelots (*Leopardus pardalis*), four jaguaroundis (*Herpailurus yaguaroundi*), one oncilla (*Leopardus tigrina*), and one jaguar (*Panthera onca*) were obtained between January 2001 and August 2002 from the Profelis Centre for rehabilitation of wild felids, located in the northwestern region of Costa Rica. Forty three samples were tested for antibodies against feline immunodeficiency virus (FIV) and p27 antigen of feline leukemia virus (FeLV), 42 samples for antibodies against feline parvovirus (FPV), and 30 for antibodies against feline calicivirus (FCV). None of the samples contained detectable antibodies against FIV or p27 antigen of FeLV, all samples contained antibodies against FPV, and one sample contained antibodies against FCV.

#### **1. Introduction**

Six new world felids are found in Costa Rica: the margay (*Leopardus wiedii*), the ocelot (*Leopardus pardalis*), the jaguaroundi (*Herpailurus yaguarondi*), the oncilla (*Leopardus tigrina*), the jaguar (*Panthera onca*), and the puma (*Puma concolor*), all of which are listed in Appendix I of the Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES 2010). An approach to help the survival of these species in the country has been the release of animals born in captivity or living in captivity into protected zones. Infections by potentially dangerous agents in wild cats are seen occasionally [1, 2], so the aim of the present study was to investigate the presence or absence and the number of captive felids with evidence of prior infection with feline immunodeficiency virus (FIV), feline leukemia virus (FeLV), feline parvovirus (FPV), and feline calicivirus (FCV).

#### 2. Materials and Methods

A total of 44 serum samples were collected between January 2001 and August 2002 from wild cats living in captivity in

the Profelis Centre for rehabilitation and release of wild felids into protected zones, located in north-western Costa Rica (latitude 9°50'27"N, longitude 84°59'54"W). The samples originated from 28 margays, 10 ocelots, 4 jaguaroundis, one oncilla, and one jaguar. The following information was collected from individual wildcats: age (1-4 years, 5-8 years, and >8 years), gender, whether they had been held illegally as pets (pets) or tourist attractions (tourist attraction) (for a few months up to three years) until they were confiscated by the Ministry of Environment, Energy, and Telecommunications (MINAET) and transferred to Profelis, whether they had lived mainly in the forests before they were captured by dealers but were immediately confiscated by MINAET and transferred to Profelis (forest), or whether they were born in a rescue centre (born in captivity), housing (living alone or living with other felids in cages), and their vaccination history (vaccinated once: with an inactivated vaccine against FCV, FPV, and FHV-1 from Fort Dodge, Iowa, U.S.A. approx. 10 years before sample was taken or never vaccinated).

All animals were clinically healthy at the time of sample collection. They were immobilized with 10% ketamine

Variable	Categories	Felid species				
		Margay	Ocelot	Yaguaroundi	Oncilla	Jaguar
Number of positive wild felids/number of wild felids tested	FPV	28/28	10/10	4/4	1/1	1/1
	FCV	0/28	1/10	0/4	0/1	ND
	FIV	0/28	0/10	0/4	0/1	0/1
	FeLV	0/28	0/10	0/4	0/1	0/1
Age	1–4 years	11/28	3/10	3/4	1/1	1/1
	5–8 years	6/28	3/10	1/4	0/1	0/1
	>8 years	13/28	4/10	0/4	0/1	0/1
Sex	Female	13/28	6/11	0/4	0/1	1/1
	Male	15/28	4/11	4/4	1/1	0/1
Modus vivendi	Illegal pets/tourist attractions	16/28	9/10	2/4	0/1	1/1
	Forest	0/28	1/10	1/4	1/1	0/1
	Born in captivity	5/28	0/10	0/4	0/1	0/1
	ND*	7/28	0/10	1/4	0/1	0/1
Cage	Alone	2/28	0/10	2/4	1/1	1/1
	With other felids	21/28	4/10	2/4	0/1	0/1
	ND	5/28	6/10	0/4	0/1	0/1
Vaccination history	Vaccinated once	16/28	3/10	2/4	1/1	0/1
	Not vaccinated	6/28	0/10	1/4	0/1	1/1
	ND*	6/28	7/10	1/4	0/1	0/1

TABLE 1: Description of wild felids tested for antibodies against FPV, FCV, FIV, and p27 antigen of FeLV.

\* ND: not determined.

hydrochloride (Ketaset, Fort Dodge, Iowa, U.S.A.) and 2% xylazine (Rompun, Bayer). Blood was collected from the medial saphenous or the jugular vein. The serum was separated and stored at  $-20^{\circ}$ C until arrival at the School of Veterinary Medicine. Due to the small sample sizes, our priority was first to test for antibodies against FIV and p27 antigen of FeLV, then antibody titres against FPV and finally antibodies against FCV. Since serum from the oncilla was very limited, only analysis for FPV and FCV was carried out.

A commercial kit (Cite-Combo, IDEXX Co., Portland, U.S.A.) was used to detect antibodies against FIV and p27 antigen of FeLV, according to the manufacturer's instructions. To detect antibodies to FPV, an haemagglutination inhibition assay was performed as described previously [3], using feline parvovirus from the Eclipse 3 vaccine (Fort Dodge, Iowa, U.S.A.) as antigen, after formalin and heat inactivation. Virus and erythrocyte controls were assayed on each plate [4]. Antibodies against FCV were detected in a serum neutralization (SN) assay as described elsewhere [5] using 100–200 TCID<sub>50</sub> of FCV-CR1, a field strain from Costa Rica isolated from a cat with upper respiratory tract disease [6]. Virus controls (FCV-CR1), cell controls (CRFK, Crandell feline kidney cells), and positive and negative control sera were included on each plate.

#### 3. Results

The descriptions of the wild felids tested are given in Table 1. In the present study, neither antibodies against FIV (0/43) nor p27 antigen of FeLV (0/43) were detected; however, all

wild felids analysed had detectable serum antibodies against FPV (42/42).

The distribution of the FPV antibody titres among the wild cats were as follows: 160 (4), 320 (11), 640 (7), 1,280 (8), 2,560 (11), and 5,120 (1). A total of 22 (52.4%) of the 42 FPV positive wild cats were vaccinated 10 years ago with an inactivated vaccine and had titres ranging from 160 to 2,560, whereas six (14.3%) animals born in the Profelis Centre, and which had never been vaccinated, had the same titre ranges (160 to 2,560). For the remaining 14 wild cats, the vaccination history could not be determined.

Only one male ocelot, 5-to-8 years old, had a low antibody titre (4) against FCV. This animal came to the Profelis Centre in 1999, after being confiscated by MINAET in a banana plantation located in the Caribbean region, where it had been kept illegally as pet for approximately 2 years. At the Profelis Centre, the ocelot was kept alone in a cage, but close to other wild felids.

#### 4. Discussion

No antibodies against FIV and no p27 antigen of FeLV were detected in agreement with a serological study performed in Peten, Guatemala [7]. A previous study by Blanco et al. [4] detected a low prevalence of FeLV (16.6%) and FIV (8.8%) in domestic cats in the greater metropolitan area of Costa Rica, which may represent a risk for wild felid populations [1]. The high proportion of animals exposed to FPV is in accordance with the high prevalence (92%) reported in domestic cats in the metropolitan area of Costa

Rica [4]. This suggested natural exposure of wild cats to the stable and resistant FPV [8], rather than through vaccination, which was performed at least 10 years previously with an inactivated vaccine. It is noteworthy that only 17.8% of urban domestic cats from Costa Rica that were positive for FPV had been vaccinated against the agent [4]. One ocelot contained detectable serum antibodies against FCV, in accordance with results reported by Blanco [9], who detected a relatively low prevalence (30.1%) of serum antibodies against FCV in domestic cats of Costa Rica, and contrasts with other studies that have reported greater prevalences of FCV exposure among domestic cats [10, 11]. The results obtained in Costa Rica may be due to the antigen source used in the SN assay. A great variety of antigenic variants of FCV have been reported in the literature, and it has been shown that different FCV isolates are not neutralized by serum antibodies induced by other FCV strains [12], questioning the reliability of FCV serological tests. Thus far, antibodies against FCV have only been detected in ocelots from the Amazon rain forest [13].

These results suggest that the eventual release of wildlife kept in captivity into protected areas could represent a risk for free-ranging members of the species. If efforts continue to repopulate natural parks and reserves with animals born in captivity or confiscated, appropriate legislation should be applied. Such legislation has been established by the Congress of the Republic of Costa Rica [14], but it is rarely enforced. This legislation stipulates that any candidate for reintroduction should be subjected to a complete health assessment to establish that it is free of relevant infectious agents.

Meanwhile, additional studies to determine the prevalence of other animal pathogens are needed.

#### Acknowledgments

The authors wish to thank Rocío Cortés and Jorge Prendas for their technical assistance.

#### References

- D. L. Murray, C. A. Kapke, J. F. Evermann, and T. K. Fuller, "Infectious disease and the conservation of free-ranging large carnivores," *Animal Conservation*, vol. 2, no. 4, pp. 241–254, 1999.
- [2] P. Daszak, A. A. Cunningham, and A. D. Hyatt, "Emerging infectious diseases of wildlife—threats to biodiversity and human health," *Science*, vol. 287, no. 5452, pp. 443–449, 2000.
- [3] L. E. Carmichael, J. C. Joubert, and R. V. Pollock, "Hemagglutination by canine parvovirus: serologic studies and diagnostic applications," *American Journal of Veterinary Research*, vol. 41, no. 5, pp. 784–791, 1980.
- [4] K. Blanco, J. Prendas, R. Cortes, C. Jimenez, and G. Dolz, "Seroprevalence of viral infections in domestic cats in Costa Rice," *Journal of Veterinary Medical Science*, vol. 71, no. 5, pp. 661–663, 2009.
- [5] S. Dawson, F. McArdle, M. Bennett et al., "Typing of feline calicivirus isolates from different clinical groups by virus neutralisation tests," *Veterinary Record*, vol. 133, no. 1, pp. 13– 17, 1993.

- [6] G. Dolz, M. A. Velandos, C. Jiménez, and R. Cortés, "Isolation of Feline Herpesvirus associated to respiratory disease in cats from Costa Rica," in *Proceedings of the 8th National Congress of Veterinary Medicine*, pp. 22–25, San José, Costa Rica, November 1993.
- [7] A. L. A. Lickey, M. Kennedy, S. Patton, and E. C. Ramsay, "Serologic survey of domestic felids in the Petén region of Guatemala," *Journal of Zoo and Wildlife Medicine*, vol. 36, no. 1, pp. 121–123, 2005.
- [8] U. Truyen, D. Addie, S. Belák et al., "Feline panleukopenia ABCD guidelines on prevention and management," *Journal of Feline Medicine and Surgery*, vol. 11, no. 7, pp. 538–546, 2009.
- [9] K. M. Blanco, Prevalence of antibodies against feline panleukopenia virus, feline herpesvirus serotype 1, feline calicivirus and feline immunodeficiency virus and antigens of feline leukemia virus in domestic cats of the metropolitan area of Costa Rica, D.V.M. thesis, School of Veterinary Medicine, Universidad Nacional, Costa Rica, 2002.
- [10] S. H. Binns, S. Dawson, A. J. Speakman et al., "A study of feline upper respiratory tract disease with reference to prevalence and risk factors for infection with feline calicivirus and feline herpesvirus," *Journal of Feline Medicine and Surgery*, vol. 2, no. 3, pp. 123–133, 2000.
- [11] K. Nakamura, Y. Ikeda, T. Miyazawa et al., "Comparison of prevalence of feline herpesvirus type 1, calicivirus and parvovirus infections in domestic and leopard cats in Vietnam," *Journal of Veterinary Medical Science*, vol. 61, no. 12, pp. 1313– 1315, 1999.
- [12] F. Fenner, E. Gibbs, P. Bachman, D. White, and F. Murphy, "Caliciviridae," in *Veterinary Virology*, pp. 448–449, Academic Press, New York, NY, USA, 2nd edition, 1987.
- [13] C. Filoni, J. L. Catão-Dias, G. Bay et al., "First evidence of feline herpesvirus, calicivirus, parvovirus, and Ehrlichia exposure in Brazilian free-ranging felids," *Journal of Wildlife Diseases*, vol. 42, no. 2, pp. 470–477, 2006.
- [14] Congress of the Republic of Costa Rica (Asamblea de la República de Costa Rica). 1992. Ley de Conservación de la Vida Silvestre, Ley Número 7317 (Conservation Law of Wildlife, Law Number 7317). Gaceta. 235: 18.