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Authors: Napolitano, Constanza, Sacristán, Irene, Beltrán-Saavedra, L. Fabián, Limachi-Quiñajo, Rolando, and Poulin, Elie

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Molecular and Serologic Survey of Pathogens in an Endangered Andean Cat (*Leopardus jacobita*) of the High Andes of Bolivia

Constanza Napolitano,[1,2,5] Irene Sacristán,[3] L. Fabián Beltrán-Saavedra,[4] Rolando Limachi-Quiñajo,[4] and Elie Poulin[1] [1]Instituto de Ecología y Biodiversidad (IEB), Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Ñuñoa, Santiago, Chile; [2]Andean Cat Alliance (AGA), Jenner 152, B La Quinta, Segunda Seccion, Villa Carlos Paz, Cordoba 5152, Argentina; [3]Programa de Doctorado en Medicina de la Conservación, Facultad de Ciencias de la Vida, Universidad Andres Bello, República 440, Santiago, Chile; [4]Zoológico Municipal Vesty Pakos, Av. La Florida s/n, Mallasa, La Paz, Bolivia; [5]Corresponding author (email: cnapolit@uchile.cl)

ABSTRACT: The Andean cat (*Leopardus jacobita*) is one of the most threatened and least known wild felids in the world. Using molecular and serologic tests, we screened a free-ranging Andean cat for 17 pathogens of conservation concern. Results suggested no evidence of infection or exposure. Whether pathogens are a threat for Andean cat populations remains currently unknown.

The Andean cat (*Leopardus jacobita*) is one of the most threatened and least known wild felid species in the world (Villalba et al. 2016). Restricted to the high Andes of Argentina, Bolivia, Chile, and Peru, at mainly 3,000 m elevation, Andean cat populations occur in patchy rocky areas where its main prey, the viscacha (*Lagidium viscacia*) resides (Napolitano et al. 2008). Listed as Endangered by the International Union for Conservation of Nature red list (Villalba et al. 2016) and legally protected in its host countries and by CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora; appendix I), main threats for Andean cats are habitat loss and degradation, particularly by mining activities, unregulated water extraction, and inadequate livestock, agricultural, and tourism practices. Only six Andean cat individuals have ever been captured in the wild (Villalba et al. 2016), and no Andean cats have ever been in zoos or rehabilitation centers. No studies to date have assessed pathogens affecting Andean cat populations.

In March 2016, a 4.1-kg, subadult, male, free-ranging Andean cat was found under unclear circumstances in Patacamaya, a village in La Paz Department, in the central Andes of Bolivia (17°14′17″S, 67°54′52″W; 3,800 m elevation; population 22,110; Fig. 1). The Bolivian Local Environmental Authority rescued the cat and gave temporary custody to the Vesty Pakos Zoo, a legal wildlife center in La Paz City, for medical evaluation in preparation for its release back into its natural habitat. Physical examination, blood biochemistries, and hematology were normal according to domestic cat (*Felis catus*) reference ranges; body condition score was 3 (on a scale of 1 [too thin] to 9 [obese]). Five *Amblyomma* spp. (Acari: Ixodidae) ticks were found on the animal. Blood, serum, oropharyngeal swab and fecal samples were obtained and stored at –20 C for 1 mo.

Total genomic DNA was extracted from blood, swab, and fecal samples using the commercial DNeasy Blood & Tissue and QIAamp DNA Stool Mini kits (Qiagen, Hilden, Germany), respectively, following manufacturer's instructions. We conducted PCR assays and serologic tests to survey 17 pathogens (including those transmitted by ticks) relevant to felids for their high pathogenicity and possible effect in wild populations or because of their importance in public health (Tables 1, 2). Negative (ultrapure water) and positive controls were used in all PCR reactions. Positive controls for each specific pathogen consisted of viremic, clinically ill, or positive domestic cats or Andean cat congeneric species (guigna, *Leopardus guigna*) diagnosed by PCR and confirmed by nucleotide sequencing. As internal controls, we amplified major histocompatibility complex class II genes. At least two independent PCR amplifications were performed for each DNA extraction.

Our results suggested no evidence of infection or exposure (Tables 1, 2). However, proper test validation of serologic assays should be conducted for each specific species (Gilbert et al. 2013). In our study, serologic tests have not been validated for Andean cats because of

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FIGURE 1. The location of the village of Patacamaya, La Paz Department, Bolivia, where a live Andean cat (*Leopardus jacobita*) was recovered and surveyed for 17 viral, bacterial, mycoplasmal, and parasitic pathogens using PCR and serologic testing.

a lack of additional samples and positive individuals. Therefore, serologic results cannot be reliably interpreted.

Studies of pathogen infection in South American wild felids are limited compared with North America or Europe, generally including only serology and a few, mostly captive, individuals. The presence of feline immunodeficiency virus in South America has been reviewed by Teixeira et al. (2012). The feline immunodeficiency virus and feline leukemia viruses have been identified serologically in pumas (*Puma concolor*; Filoni et al. 2006) and in guignas by PCR (Mora et al. 2015). Antibodies against feline panleukopenia virus have been detected in Geoffroy's cats (*Leopardus geoffroyi*; Fiorello et al. 2007), pumas, ocelots (*Leopardus pardalis*), and oncillas (*Leopardus tigrinus*; Filoni et al. 2006). *Mycoplasma haemofelis*, *Candidatus* Mycoplasma turicensis, and *Candidatus* Mycoplasma haemominutum were detected by PCR testing of oncilla, Geoffroy's cat, margay (*Leopardus wiedii*), and ocelot (Willi et al. 2007). *Hepatozoon* spp. were detected by PCR in jaguars (*Panthera onca*; Furtado et al. 2017). Other pathogens, including canine distemper virus, feline herpesvirus, feline calicivirus, feline coronavirus, parvovirus, rabies virus, *Toxoplasma gondii*, *Leptospira interrogans*, *Cytauxzoon* spp., *Bartonella* spp.

TABLE 1. Molecular screening of a free-ranging Andean cat (*Leopardus jacobita*) recovered from Patacamaya, in La Paz Department, Bolivia, and surveyed for 17 viral, bacterial, mycoplasmal, and parasitic pathogens using PCR and serologic testing.

	Molecular screening				
Pathogen	Sample analyzed	Genomic region[a]	PCR conditions	PCR product (bp)	Result
Carnivore protoparvovirus 1	Feces	VP2	Decaro et al. (2010)	83	Negative
Feline leukemia virus	Blood	U3 LTR	Mora et al. (2015)	211	Negative
Feline immunodeficiency virus	Blood	Gag	Mora et al. (2015)	291	Negative
Piroplasmids[b]	Blood	18S rRNA	Baneth et al. (2013)	1,400	Negative
Cytauxzoon felis	Blood	18S rRNA	Millán et al. (2007)	1,726	Negative
Mycoplasma haemofelis	Blood	16S rRNA	Tanahara et al. (2010)	273	Negative
Candidatus Mycoplasma haemominutum	Blood	16S rRNA	Tanahara et al. (2010)	202	Negative
Candidatus Mycoplasma turicensis	Blood	16S rRNA	Tanahara et al. (2010)	138	Negative
Toxoplasma gondii	Feces	Repeated fragment	Homan et al. (2000)	529	Negative
Feline calicivirus	Oropharyngeal swab	Capsid protein	Sykes et al. (2001)	663	Negative
Leptospira spp.	Blood	16S RNA	Lester and Lefebvre (2003)	571	Negative
Ehrlichia spp.	Blood	16S rDNA	Breitschwerdt et al. (2002)	382	Negative

[a] r (with RNA or DNA) = ribosomal.

[b] *Hepatozoon felis*, *Babesia* spp., *Theileria* spp., and *Leishmania* spp.

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TABLE 2. Serologic screening of a free-ranging Andean cat (*Leopardus jacobita*) recovered from Patacamaya in La Paz Department, Bolivia, and surveyed for 17 viral, bacterial, mycoplasmal, and parasitic pathogens using PCR and serologic testing.

Pathogen	Sample	Kit or method	Result
Carnivore protoparvovirus 1	Serum, feces	ImmunoComb Canine VacciCheck Antibody Test Kit (IgG)[a], Anigen Rapid CPV Ag Test Kit[b]	Negative
Feline leukemia virus	Serum	Immunofluorescent Antibody Test[c]	Negative
Feline immunodeficiency virus	Serum	Immunofluorescent Antibody Kit IgG[d]	Negative
Toxoplasma gondii	Serum	OnSite Toxo IgG/IgM Rapid Test[e]	Negative
Canine distemper virus	Serum	Serum neutralization	Negative
Feline coronavirus	Serum	ImmunoComb FCoV Antibody Test Kit (IgG)[a]	Negative

[a] Biogal, Kibbutz Galed, Israel. IgG = immunoglobulin G; FCoV = feline coronavirus.

[b] BioNote, Hwaseong-si, Gyeonggi-do, Republic of Korea. CPV Ag = canine parvovirus-coronavirus antigen.

[c] Veterinary Medical Research & Development, Pullman, Washington, USA.

[d] Fuller Laboratories, Fullerton, California, USA.

[e] CTK Biotech, San Diego, California, USA. IgM = immunoglobulin M.

and *Ehrlichia* spp., have also been described in South American wild felids (Deem et al. 2004; Filoni et al. 2012; Uhart et al. 2012).

Human landscape perturbation is one of the main causes of emerging diseases in wildlife, facilitating interspecific casual encounters and pathogen transmission. The high Andes area is home to approximately 70% of the human population of Bolivia, having a long history of human occupation, land use, and habitat alteration (Villalba et al. 2016). Domestic dogs (*Canis lupus familiaris*) and domestic cats are commonly seen in many Aymara Indian villages (Deem et al. 2004), and contact between wild carnivores and domestic hunting dogs has been recorded (Fiorello et al. 2007). Wild carnivores share a high percentage of parasites and viruses with closely related domestic carnivores (Uhart et al. 2012). Therefore, sympatric domestic carnivores with high densities may act as reservoirs, being the most probable origin for potential pathogen transmission into Andean cat populations. However, Andean cats are habitat and diet specialists. They inhabit remote areas, have low densities, and are solitary, pairing only when mating; thus, both contact with domestic animals and pathogen transmission among conspecifics may be low. Also, in this case, the subadult Andean cat may not have been exposed to pathogens at its early age. These factors may explain why this individual was negative to all screened pathogens. As a comparison, in a contrasting study system in central and southern Chile, the congeneric small wild cat, the guigna, inhabiting low-elevation human-dominated landscapes, was also screened for six of the aforementioned pathogens; 49% (47/96) were positive to at least one of the six pathogens tested for (C.N. unpubl. data).

The dynamics of multihost pathogens in the high Andes carnivore community and the role of domestic carnivores in disease ecology in that area have been largely unstudied. Despite the difficulties of Andean cat sample collection, future investigations should consider including more individuals from different populations, screening more pathogens of conservation concern, and sampling sympatric domestic carnivores. Whether pathogens are a threat for Andean cat populations remains currently unknown. To our knowledge, this study provides the first information on Andean cat pathogens, molecular tools for researchers and managers, and insights to facilitate much-needed health research efforts in the Altiplano ecosystem.

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