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EXPOSURE OF FREE-RANGING MANED WOLVES (*CHRYSOCYON BRACHYURUS*) TO INFECTIOUS AND PARASITIC DISEASE AGENTS IN THE NOËL KEMPFF MERCADO NATIONAL PARK, BOLIVIA

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Abstract: Maned wolves (*Chrysocyon brachyurus*) are neotropic mammals, listed as a CITES Appendix II species, with a distribution south of the Amazon forest from Bolivia, through northern Argentina and Paraguay and into eastern Brazil and northern Uruguay. Primary threats to the survival of free-ranging maned wolves include habitat loss, road kills, and shooting by farmers. An additional threat to the conservation of maned wolves is the risk of morbidity and mortality due to infectious and parasitic diseases. Captive maned wolves are susceptible to, and die from, common infectious diseases of domestic dogs (*Canis familiaris*) including canine distemper virus (CDV), canine parvovirus (CPV), rabies virus, and canine adenovirus (CAV). Results from this study show that free-ranging maned wolves in a remote area of Bolivia have been exposed to multiple infectious and parasitic agents of domestic carnivores, including CAV, CDV, CPV, canine coronavirus, rabies virus, *Leptospira interrogans* spp., *Toxoplasma gondii*, and *Dirofilaria immitis*, and may be at increased risk for disease due to these agents.

Key words: Bolivia, *Chrysocyon brachyurus*, infectious diseases, maned wolf, parasitic diseases.

INTRODUCTION

Maned wolves (*Chrysocyon brachyurus*) are neotropic mammals with a distribution south of the Amazon forest from Bolivia, through northern Argentina and Paraguay and into eastern Brazil and northern Uruguay. The maned wolf is listed as a CITES Appendix II species (http://www.cites.org), the United States Fish and Wildlife Service considers it ''endangered'' (http://endangered.fws.gov), and the IUCN lists the species as ''vulnerable'' (http://www.redlist.org). There is no global population estimate, but maned wolves are absent from much of their former geographic range. The primary threat to the survival of the maned wolf is considered to be habitat loss.[31,33] Road kills are the major source of mortality near small parks in Brazil, and farmers also shoot maned wolves that they believe hunt chickens.[32]

The risk of morbidity and mortality due to infectious diseases is a significant concern in the conservation of maned wolves and wildlife in general.[10] Domestic dogs (*Canis familiaris*) are known or suspected reservoirs for agents of infectious diseases, which have devastated populations of wild carnivores in many areas of the world.[1,18,26,33] As human populations expand and come into closer proximity with free-ranging maned wolves, there is increased risk of disease transmission from domestic carnivores to maned wolves.

Although studies of exposure to disease agents of free-ranging maned wolves are lacking, captive maned wolves are known to be susceptible to important infectious agents of domestic dogs including canine distemper virus (CDV), canine parvovirus (CPV), rabies virus, and canine adenovirus (CAV). Morbidity and mortality have been reported for CDV,[7,36] CPV,[2,13,16,27] rabies virus,[35] and CAV.[3] Maned wolves may be susceptible to all the known infectious agents of domestic dogs.

Studies on the parasites of free-ranging or recently captive maned wolves have documented the presence of the giant kidney worm, *Dioctophyme renale*, which is known to destroy the right kidney in infected maned wolves.[6] Parasites of the urinary tract, in addition to *D. renale*,[5] gastrointestinal parasites,[12] and ectoparasites[30] have also been documented in free-ranging maned wolves. In captivity, maned wolves are also known to be susceptible to *Dirofilaria immitis*, the causative agent of heartworm disease, *Echinococcus granulosus*, and gastrointestinal parasites.[28]

The objective of this study was to determine the prevalence of exposure to select infectious and parasitic diseases of maned wolves immobilized as part of an ecologic study in the Noël Kempff Mercado National Park, Bolivia (NKMNP).

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MATERIALS AND METHODS

Study period and site description

From February 2000 to October 2003, four maned wolves were immobilized in the NKMNP as part of a radiotelemetry study. NKMNP includes the Serranía de Huanchaca escarpment and the adjacent lowlands between the Río Itenez (Guaporé) and the Río Paraguá and lies between 13°31′–15°05′S and 60°14′–61°49′W. The park is at the interface of Amazonian forest with grassland ecosystems and includes a number of distinct ecosystems including broadleaf semievergreen forest, dry forest, inundated forest, dry savanna (Cerrado), and inundated savanna.[24]

Sample and data collection and analyses

All maned wolves were immobilized using 100 mg tiletamine plus zolazepam (Telazol®, Fort Dodge Laboratories, Fort Dodge, Iowa 50501, USA; 3.5–4.5 mg/kg, i.m.) delivered through Telinject® (Telinject USA Inc., Agua Dulce, California 91390, USA) plastic darts, using a Telinject® pistol. When necessary, anesthesia supplementation was provided with ketamine (Ketaset®, Fort Dodge; 25–50 mg increments, i.v.). Blood was collected by venipuncture of the jugular vein or the lateral saphenous vein. Blood was immediately placed in serum separator tubes (Corvac Sherwood Medical, St. Louis, Missouri 63103, USA) for all the maned wolves. For two of the maned wolves (CYB 1 and CYB 2), the sample tubes were placed in a cool place until clot formation and then sera were separated by centrifugation (Mobilespin, Vulcan Technologies, Grandview, Missouri 64040, USA) at 3,000 *g* for 15 min and stored in liquid nitrogen. Blood of the other two maned wolves (CYB 3 and CYB 4) was allowed to clot at ambient temperature, and the serum was then decanted and kept cool for 48 hr before storage in a −20°C freezer.

Fecal samples were collected manually from the rectum and preserved in 10% formalin. Ectoparasites were collected and stored in 70% isopropanol from all four maned wolves. Urine was collected from CYB 1 and CYB 2 by cystocentesis using a 22 g, 1.5 inch needle and 12 cc syringe. Urine samples were divided into aliquots for freezing and formalin fixation. The remaining urine was immediately centrifuged at 3,000 *g* for 5 min. Urine sediment was immediately examined by direct microscopic examination in the field.

Samples were transported to the United States of America for laboratory testing. Blood and urine samples were transported on dry or wet ice. Fecal and urine samples were transported in 10% buffered formalin. Ticks were transported in 70% isopropanol. Formalin and alcohol were removed before air travel and refilled on arrival in the United States. All appropriate export and import permits accompanied the samples during transport.

Serologic testing for antibodies to CAV, *Brucella canis*, canine coronavirus, CDV, canine herpesvirus (CHV), CPV, *D. immitis*, *Toxoplasma gondii*, and leptospirosis antibody testing was conducted at the New York State Veterinary Diagnostic Laboratory (Cornell University, Ithaca, New York 14853, USA). The 18 *Leptospira interrogans* serovars tested included *L. ballum*, *L. wolffi*, *L. autumnalis*, *L. tarassovi*, *L. pomona*, *L. hardjo*, *L. grippophytosa*, *L. bataviae*, *L. canicola*, *L. ictero*/*COP*, *L. australis*, *L. pyrogenes*, *L. bratislava*, *L. sejroe*, *L. icterohaemorrhagia*/*icterohaemorrhagia*, *L. javanica*, *L. szwajizak*, *L. saxkoebing*. Serologic testing for rabies virus was performed at Kansas State Veterinary Diagnostic Laboratory (Kansas State University, Manhattan, Kansas 66506, USA) using the rapid fluorescent focus inhibition test. Three of the maned wolves, CYB 1, CYB 2, and CYB 3, were tested for all the infectious agents listed above, whereas CYB 4 was only tested for the presence of antibodies to CAV, CDV, and CPV.

Fecal samples were examined by direct microscopic examination, sodium nitrate flotation, and sedimentation methods at the New York State Veterinary Diagnostic Laboratory. Adult ticks were identified on the basis of external morphology, using the keys of Jones et al.[22].

Frozen and formalin-fixed urine samples were analyzed at the School of Veterinary Medicine (University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA); these results will be reported elsewhere.

RESULTS

Table 1 summarizes the infectious disease agent serologic tests performed, methods used, level of titers defined as positive, and results for each test. All four maned wolves tested had positive antibody results to CAV and CPV and two had positive antibody results to CDV. Of the infectious diseases for which CYB 1, CYB 2, and CYB 3 were tested, none of them had antibodies to *B. canis* and CHV. All three of these maned wolves were antibody positive for one or more *L. interrogans* serovars including *L. ballum*, *L. grippophyfosa*, *L. icterohaemorrhagica*/*icterohaemorrhagica*, and *L. szwajizak*. One maned wolf (CYB 1) was antigen positive for *D. immitis* and one (CYB 3) had antibodies to coronavirus, rabies virus, and *T. gondii*.

Gastrointestinal parasites were identified in the

Table 1. Disease agent serologic tests performed, methods used, level of titers defined as positive, and results in the study for select infectious and parasitic disease agents in four free-ranging maned wolves (*Chrysocyon brachyurus*) in Noël Kempff Mercado National Park, Bolivia.

Disease agent (method used)[a]	Positive titer	CYB 1	CYB 2	CYB 3	CYB 4
Canine adenovirus (SN)	1:4	Positive 1:512[b]	Positive 1:512	Positive 1:384	Positive 1:512
Brucella canis (SlideAGG/AGID II)	NA	Negative	Negative	Negative	NA
Canine distemper virus (SN)	1:8	Negative	Positive 1:12	Negative	Positive 1:12
Canine herpesvirus (SN)	1:8	Negative	Negative	Negative	NA
Canine parvovirus (HAI)	1:10	Positive 1:10	Positive 1:10	Positive 1:10	Positive 1:20
Coronavirus (SN)	1:8	Negative	Negative	Positive 1:32	NA
Dirofilaria immitis (occult)	NA	Positive	Negative	Negative	NA
Rabies virus (RFFIT)	1:5	Negative	Negative	Positive 1:13	NA
Toxoplasma gondii (IHA)	1:64	Negative	Negative	Positive 1:128	NA
Leptospira interrogans 18 serovars (microagglutination)	1:100	*L. szwajizak* Positive 1:100	*L. ballum* and *L. icterohemorrhagia*/*icterohemorrhagia* Positive 1:200	*L. grippo* Positive 1:400; *L. icterohemorrhagia*/*icterohemorrhagia* Positive 1:100	NA

[a] SN, serum neutralization; Slide AGG/AGID II, slide agglutination/agar gel immunodiffusion test II; HAI, hemagglutination inhibition; RFFIT, rapid fluorescent focus inhibition test; IHA, indirect hemagglutination; NA, not applicable.

[b] Positive titer.

feces of all four maned wolves and included *Ancylostoma* sp., *A. caninum*, *Capillaria* sp., *C. aerophilia*, *Gnathostoma* sp., *Isospora* sp., *Physaloptera* sp., *Strongyles* spp., *Toxocara canis*, *Trichuris* sp., and *Uncinaria* sp.

Ticks collected from all four maned wolves were *Ambylomma* spp. The most prevalent species, *A. tigrinum*, was present on all four maned wolves. A few *A. ovale* ticks were found on CYB 1 and CYB 2. Of the two maned wolf urine samples evaluated in the field, one (CYB 1) was observed to have *D. renale* ova on urine sedimentation. The other (CYB 2) was negative for this parasite.

DISCUSSION

The serologic tests used in this study document exposure but not disease by the detection of antibodies to infectious agents and not the agents themselves. The occult heartworm test is an exception because the test detects the causative agent, *D. immitis*. Therefore, the serologic portion of this study was used to determine whether these maned wolves were exposed to a select number of infectious and parasitic agents known to be of concern for wild carnivore conservation.[17] Disadvantages to serology are the possibility of false positives, because of cross-reaction with other agents, and false negatives. Moreover, the tests used in this study have not been validated for use in maned wolves because there is possible inaccuracy or cross-reacting substances in the host.[21] Currently, there are no serologic tests validated for maned wolves, but the tests developed for domestic dogs, which were used in this study, are widely used in the testing of captive maned wolves.

Results suggest that free-ranging maned wolves in the NKMNP have been exposed to pathogens, which are known to cause high morbidity and mortality in captive maned wolves and other carnivores. This is of particular interest because the NKMNP is located in a relatively isolated area of northeastern Bolivia, far from urban centers and large populations of domestic dogs. We believe that the most likely route of pathogen exposure for these maned wolves stems from domestic dogs, which live in villages and on ranches surrounding the park. Alternatively, these disease agents may be self-sustaining in free-ranging maned wolves and other carnivores in the NKMNP.

Evidence suggests two possible disease epidemics in the wild carnivore populations of the NKMNP during the years we have been working in the park. The first maned wolf (CYB 1) died 8 mo after it was radiocollared. Its remains were found at a time when fox activity was appreciably diminished in the park. In 2003, three pups and an unmarked mother maned wolf disappeared between July and September 2003. Based on reports from

park guards, road kill, shooting, and habitat changes were ruled out as causes of their disappearance. Disease is the most likely cause of the disappearance of CYB 1 and the other four maned wolves that disappeared. It is noteworthy that maned wolf CYB 3, antibody positive for CAV, CPV, coronavirus, rabies virus, *T. gondii*, and *L. interrogans* spp., was sampled in September 2003 after the disappearance of this family group, with which it was known to be in contact. Genetic evaluation of the maned wolf population in the NKMNP has yet to be finished, but it is known that CYB 1 was unrelated to the other maned wolves in this study and that CYB 2–CYB 4 were all related and in documented close contact with each other.

The evidence of exposure in these maned wolves to CDV (two of four) and CPV (four of four) is of greatest concern. CDV has caused serious epidemics and population declines in wild carnivores[11] and is known to cause mortality in captive maned wolves.[7,36] It is most commonly spread by close contact with infectious carnivores through aerosolized respiratory secretions but can remain viable in the environment for weeks under the proper conditions.[19] Parvovirus has been reported as a cause of morbidity and mortality in a number of free-ranging canid species[4] and has caused mortality in captive maned wolves.[13,16,27] Transmitted by the fecal–oral route, CPV can survive for months in the environment and does not require close contact for transmission.

All four of the maned wolves tested were positive for CAV. CAV likewise has been shown to cause mortality in captive neonatal maned wolves,[3] although it is probably of less pathogenic significance than CDV and CPV.

Only one of the maned wolves was antibody positive to coronavirus, rabies virus, and *T. gondii*. All these agents can cause morbidity and mortality in free-ranging canids and have been implicated as a threat to the conservation of a number of carnivore populations.[17] Rabies virus has caused disease in captive maned wolves.[36] Although not a significant pathogen alone, coronavirus infections concurrent with other viral or bacterial agents are likely to increase morbidity and mortality.[14,29] Exposure to *T. gondii* in this maned wolf was most likely through the ingestion of raw meat (i.e., eating small mammals), and thus, probably does not suggest transmission from domestic dogs or other carnivores in the region.

Leptospirosis is a zoonotic bacterial disease commonly associated with fever, sepsis, kidney failure, and reproductive abnormalities in a number of animal species and humans.[20] Many free-ranging canids are seropositive to various *L. interrogans* serovars without showing illness or functioning as important reservoirs.[23] The significance of the findings of positive antibodies to a few *L. interrogans* serovars in the maned wolves is not known.

One of the three maned wolves tested was positive for *D. immitis*, the causative agent of canine heartworm. This is a potentially fatal, mosquito-borne disease of domestic and wild carnivores. In captivity, maned wolves are often maintained on a heartworm prophylactic because of the devastating effect of this parasite. The role of *D. immitis* in morbidity and mortality of free-ranging maned wolves is not known.

The finding of *D. renale* ova was not surprising because this parasite is cited as a common pathogen of recently captive maned wolves.[25] *Dioctophyme renale* is often associated with a hypoplastic right kidney in infested maned wolves and could contribute to mortality of wolves especially with concurrent disease.[25,28]

Free-ranging canid species usually harbor enteric parasites, including those found in the maned wolves in this study.[23] These parasites are not often present in high numbers and do not cause a clinical problem in adult free-ranging canids. However, in animals immunocompromised because of factors such as concurrent disease or physiologic stress related to habitat or population modifications, enteric pathogens may result in disease. The lungworm *Capillaria aerophila*, detected in the feces of two maned wolves in the study, can cause clinical signs associated with bronchitis and pneumonia, but these animals had no overt respiratory signs. *Amblyomma* spp. ticks have been collected from free-ranging maned wolves before this study;[12] however, this was the first documented *A. ovale* record from maned wolves, as previously reported.[30]

Recently, disease has become a recognized threat to the long-term conservation of free-ranging wildlife.[8–10,38] An increased prevalence of disease is most likely associated with global scale anthropogenic changes, which include human population growth, habitat fragmentation and degradation, the isolation of populations of species, and an increased proximity of humans (and their domestic animals) to wildlife;[10] all these appear to be present in Bolivia. Studies to determine pathogen exposure and disease prevalence in both the threatened wildlife and the domestic animals, which share the habitats with these animals, are imperative to minimize the effect of disease on the conservation of wildlife species. For example, a study on the border of the Madidi National Park in northwestern Boliva showed a high prevalence of exposure of domestic

dogs and cats to pathogens, which may infect wild carnivores in that park.[15]

The data presented in this study have been the basis for formulating a large-scale study of the disease ecology of domestic dogs, sympatric crab–eating foxes (*Cerdocyon thous*), and maned wolves in and on the perimeter of the NKMNP. The population of maned wolves in the park has been estimated at 120 breeding pairs.[34] If so, NKMNP is one of the most important remaining protected areas for free-ranging maned wolves. Determining the risk of infectious disease exposure to the whole population will be important to help park managers as they develop policies to protect maned wolves and other carnivores in the park.

Control of disease in domestic and feral domestic animals is likely to become an increasingly important part of protected area management. Although already followed by many National Park systems, we recommend that two rules be universally followed in protected areas: 1) prohibit the release of individuals of wild species from captivity into wild populations, unless the wild populations are threatened and in need of augmentation for survival, and strict health and genetic evaluations have been performed before release[37] and 2) exclude all pets and other domestic animals from parks. If parks include human settlements with domestic animals, we recommend that these are vaccinated and monitored for disease. These measures have been instituted in the NKMNP, elsewhere in Bolivia, and at many other sites around the world.

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