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Pathogens of Wild Maned Wolves (*Chrysocyon brachyurus*) in Brazil

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ABSTRACT: The maned wolf, *Chrysocyon brachyurus*, is an endangered Neotropical canid that survives at low population densities. Diseases are a potential threat for its conservation but to date have been poorly studied. We performed clinical evaluations and investigated the presence of infectious diseases through serology and coprologic tests on maned wolves from Galheiro Natural Private Reserve, Perdizes City, Minas Gerais State, southeastern Brazil. Fifteen wolves were captured between 2003 and 2008. We found high prevalences of antibody to canine distemper virus (CDV; 13/14), canine parvovirus (CPV; 4/14), canine adenovirus type 2 (13/14), canine coronavirus (5/11), canine parainfluenza virus (5/5), and *Toxoplasma gondii* (6/8), along with Ancylostomidae eggs in all feces samples. Antibodies against *Leishmania* sp. were found in one of 10 maned wolves, and all samples were negative for *Neospora caninum*. Evidence of high exposure to these viral agents was also observed in unvaccinated domestic dogs from neighboring farms. High prevalence of viral agents and parasites such as CDV, CPV, and Ancylostomidae indicates that this population faces considerable risk of outbreaks and chronic debilitating parasites. This is the first report of exposure to canine parainfluenza virus in Neotropical free-ranging wild canids. Our findings highlight that canine pathogens pose a serious hazard to the viability of maned wolves and other wild carnivore populations in the area and emphasize the need for monitoring and protecting wildlife health in remaining fragments of the Cerrado biome.

Key words: Brazilian Cerrado, *Chrysocyon brachyurus*, conservation, disease, parasites, serologic survey, wildlife.

Canid ecology is characterized by some behaviors that are favorable to disease transmission, such as relatively large home ranges, omnivorous foraging habits, and scent marking. Wild canids are closely related to domestic dogs, *Canis lupus familiaris*, and are susceptible to many of their disease agents, including canine distemper virus and canine parvovirus. Such pathogens cause multisystemic infections, mortality, low birth rates, or even outbreaks in naïve, wild populations and represent serious threats for wild canid conservation (Laurenson et al., 2004).

Many factors, such as habitat loss and road kills, threaten population viability of the maned wolf, *Chrysocyon brachyurus*. Captive animals may be affected by canine distemper and parvovirus disease (Maia and Gouveia, 2002), but few have addressed this aspect in free-ranging maned wolves (Deem and Emmons, 2005; Curi et al., 2010).

Our aim was to provide long-term health data on the maned wolf population from Galheiro Natural Private Reserve (RPPNG), southeastern Brazil, as a part of a broader ecologic study, the Maned Wolf Project. The reserve (47°08′1″N, 19°14′06″W) encompasses an area of approximately 3,000 ha in the Cerrado Biome, Perdizes municipality, Minas Gerais State, southeastern Brazil. It is surrounded on one side by the Nova Ponte Hydroelectric Station Dam Lake and on the other by farms and small human settlements, where a large domestic dog population exists. Vagrant dogs are frequently seen in the region and inside the RPPNG. Maned wolves and other animals have full access to the reserve as RPPNG is fenced to exclude cattle only.

Humanely designed traps were employed under the approval of IBAMA (Brazilian Institute of Environment and

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Renewable Natural Resources; licenses 014/03 Fauna/MG, 173/03 Fauna/MG, and 084/05 NUFAS/MG) between 2003 and 2008. Captured animals were anesthetized with a combination of ketamine chlorhydrate (8 mg/kg) and xylazine chlorhydrate (2 mg/kg) and identified by microchips and hair painting (Curi and Talamoni, 2006). Additionally, radio collars helped to identify four wolves.

Blood samples from the cephalic vein and from the tip of the ear, feces, and ectoparasites were collected. Serum was extracted and stored at −20 C. Peripheral blood smears were obtained in the field, fixed with methanol, and stained with methylene blue. Feces were collected from the rectum of six captured maned wolves (all others had no feces at the moment of collection) and maintained refrigerated for up to 5 days. Ectoparasites were stored in 70% ethanol.

Blood samples from 20 adult dogs from farms around RPPNG were also collected. Vaccination against rabies is regularly conducted by the Brazilian government and achieves good coverage in this region. Most farmers around RPPNG do not vaccinate their dogs against other diseases. Even so, each owner was asked about their dog's vaccination status before sampling, to ensure the detection of exposure antibodies only. Clinical examinations revealed no significant alterations in the dog's health status at the time, but some owners reported past deaths among their dogs, apparently caused by infectious disease. Serum samples of dogs were processed as described for maned wolves.

Serologic testing was chosen to evaluate exposure to canine pathogens in maned wolves and dogs. Serum samples of maned wolves were tested for antibodies against canine distemper virus (CDV), canine parvovirus (CPV), canine adenovirus type 2 (CAV-2), canine coronavirus (CCV), canine parainfluenza virus (CPIV), *Toxoplasma gondii*, *Leishmania* sp., and *Neospora caninum*. Serologic methods and corresponding references are listed in Table 1. All tests were performed after pilot trials and standardization of protocols. Samples were analyzed in duplicate, using negative and positive controls from a previously tested dog serum bank, to minimize the possibility of false negatives. Twenty domestic dog samples were tested for *Leishmania* sp., and, of these, 11 samples (unvaccinated) were tested for CDV, CPV, CAV-2, and CPIV. Discrepancies in numbers of samples tested are due to a lack of serum for some individuals and irregular availability of test protocols and materials through the years of the study. Routine coproparasitologic analyses (fluctuation-Willis, sedimentation-Hoffman, Pons, and Janer (HPJ), and Baermann modified tests) were performed. Blood smears microscopically searched for hemoparasites.

Results were interpreted as apparent prevalence, or the number of positive samples divided by the total number of samples (Gardner et al., 1996; Bush et al., 1997). The chi-square test was used to detect differences in antibody prevalence for each agent between male and female, and also between adult and subadult maned wolves.

Fifteen individuals were sampled between 2003 and 2008, comprising eight female and seven male maned wolves. These were 10 adults and five subadults (estimated age of 5 mo to 1 yr). No signs of disease or clinical infection were found in most maned wolves, except for an adult female (AF4; Table 2), which showed emaciation and ocular discharge. The serologic examination revealed high apparent prevalence of antibody to CDV, CPV, CAV-2, CPIV, and *T. gondii* (Table 2), and antibody for seven of eight diseases tested. All samples had antibodies for at least one agent surveyed, but all were negative for antibody to *N. caninum*. Only one sample had detectable antibody to *Leishmania* sp. One female (AF3) was trapped twice and had similar antibody titers to CDV (64 in 2006; 32 in 2008) and decreasing titers for CAV-2 (>1,256 in 2006; 64 in 2008). Samples from this individual remained

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TABLE 1. Agents surveyed and serologic tests performed in maned wolves (*Chrysocyon brachyurus*) and domestic dogs (*Canis lupus familiaris*) from Galheiro Natural Private Reserve, Minas Gerais, Brazil.

Agent[a]	Test	Cutoff point[b]	References
CDV	Serum neutralization	8	Appel and Robson (1973)
CPV	Hemagglutination inhibition	20	Senda et al. (1986)
CAV-2	Serum neutralization	16	Appel et al. (1975)
CCV	Serum neutralization	2	Mochizuki et al. (1987)
CPIV	Hemagglutination inhibition	16	Mouzin et al. (2004)
Toxoplasma gondii	Modified agglutination test	25	Dubey et al. (2007a)
Neospora caninum	Indirect fluorescent antibody test	50	Dubey et al. (2007b)
Leishmania sp.	Immune enzyme assay and indirect immune fluorescence reaction with anti-dog conjugate	40	Curi et al. (2006)

[a] CDV = canine distemper virus; CPV = canine parvovirus; CAV-2 = canine adenovirus type 2; CCV = canine coronavirus; CPIV = canine parainfluenza virus.

[b] Minimum titer considered positive expressed as reciprocal of the highest dilution.

steady for CPV, with titers of 160 in both time periods. This individual was found dead in 2009, but we could not assess the causes. The female AF4 showed high antibody titers, especially for CAV-2, which may be compatible with her clinical signs (see above and Table 2). Differences in prevalence between sexes or ages were not statistically significant ($P>0.05$). Domestic dog samples revealed high prevalence for CDV, CPV, and CPIV antibodies (Table 3).

The gastrointestinal parasite taxa included Trichiuridae (3/6), Ancylostomidae (6/6),

TABLE 2. Antibody titers and prevalence for selected infectious disease agents in maned wolves (*Chrysocyon brachyurus*) captured 2003–08 in Galheiro Natural Private Reserve, Minas Gerais, Brazil. Titers are expressed as the reciprocal of the highest dilution positive.[a]

Animal[b]	Trapping year	CDV	CPV	CAV-2	CCV	CPIV	*Leishmania* sp.	*Toxoplasma gondii*	*Neospora caninum*
AF1	2003	32	80	64	8	–	Neg	Neg	Neg
AM1	2003	32	80	128	16	–	–	500	Neg
AM2	2003	16	–	128	Neg	–	Neg	Neg	Neg
SF1	2004	–	320	64	Neg	–	40	–	–
SF2	2005	32	160	128	8	–	Neg	50	Neg
AM3	2005	64	320	128	Neg	–	Neg	50	Neg
AM4	2005	16	160	32	Neg	–	Neg	–	–
AM5	2006	64	160	128	Neg	–	Neg	50	Neg
AF2	2006	64	320	Neg	Neg	–	Neg	25	Neg
AF3	2006	64	160	>1,256	2	–	Neg	50	Neg
SF3	2007	2	20	–	–	–	Neg	–	–
AM6	2007	8	20	64	–	40	–	–	–
SM1	2007	32	40	128	–	160	–	–	–
AF4	2008	64	80	>1,256	8	160	–	–	–
AF3*	2008	32	160	64	–	160	–	–	–
SF4	2008	16	40	128	–	160	–	–	–
Apparent prevalence (%)		13/14 (93)	14/14 (100)	13/14 (93)	5/11(45)	5/5(100)	1/10(10)	6/8 (75)	0/8(0)

[a] CDV = canine distemper virus; CPV = canine parvovirus; CAV-2 = canine adenovirus type 2; CCV = canine coronavirus; CPIV = canine parainfluenza virus; – = not tested; Neg = negative result.

[b] A = adult; S = subadult; F = female; M = male; * = resampling.

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TABLE 3. Prevalence of antibody to selected infectious disease agents in domestic dogs (*Canis lupus familiaris*) sampled around Galheiro Natural Private Reserve, Minas Gerais, Brazil, expressed as number of positives/number analyzed (apparent prevalence). Titers are expressed as the reciprocal of the highest dilution positive.

Agent tested[a]	Apparent prevalence (%)	Titer frequency distribution
CDV	11/11 (100)	8 (1), 16 (1), 32 (2), 64 (2), 128 (5)
CPV	11/11 (100)	40 (1), 80 (3), 160 (7)
CAV-2	4/11 (36)	2 (1), 16 (1), 64 (2), 128 (1)
CPIV	10/11 (91)	40 (5), 80 (3), 160 (2)
Leishmania sp.	0/20 (0)	–

[a] CDV = canine distemper virus; CPV = canine parvovirus; CAV-2 = canine adenovirus type 2; CCV = canine coronavirus; CPIV = canine parainfluenza virus.

Physalopteridae (3/6), Hymenolepidae (3/6), and Acanthocephala (3/6). Ectoparasites included *Amblyomma* sp., *Amblyomma cajennense*, and *Amblyomma tigrinum* ticks. All animals were parasitized by *A. cajennense*, but only one adult female had a severe tick burden. *Amblyomma tigrinum* ticks were found in two adult male maned wolves. No hemoparasites were found in the blood smears.

Positive serology and high evidence of exposure were found for CDV, CPV, CAV-2, CCV, and CPIV, which indicated that the animals had contact with these agents and that these viruses are present in the study area. Persistence of pathogens in an area is a critical issue for canid conservation and is best determined by longitudinal studies (Laurenson et al., 2004). Persistence of antibodies in samples taken in different time periods indicates probable prolonged exposure to viral agents tested. To our knowledge, this is the first report describing the exposure of free-ranging maned wolves to infectious agents over multiple years.

Domestic dogs are likely the source of infections to maned wolves. This is further supported by the high prevalence for CDV, CPV, CAV-2, and CPIV found in these populations of maned wolves and domestic dogs, and the well-known role of dogs as reservoirs and sources of pathogens for wild canids (Laurenson et al., 2004).

Canine distemper may cause high mortality (Deem et al., 2000), and parvovirus infection can reduce reproductive output of wild canids by causing pup mortality (Mech et al., 2008). Therefore, the persistence and high apparent prevalence of these diseases may have a strongly negative impact on the maned wolf population of RPPNG. Other studies have also shown evidence of CDV, CPV, CAV, and CCV infection in wild canids from South America (Deem and Emmons, 2005; Curi et al., 2010), and recent reports identified genetically similar CDV in domestic dogs and sick wild canids from Brazil (Megid et al., 2010). To our knowledge, this is the first evidence of CPIV infection in free-ranging Neotropical wild canids.

The finding of anti-*Leishmania* antibodies in only one maned wolf (a subadult female), the negative results for domestic dogs, and the fact that the studied region is not endemic for leishmaniasis suggest that the result is a false positive. Alternatively, the disease could be maintained at a very low prevalence in our study area. The high proportion of antibody-positive maned wolves demonstrates that *T. gondii* is strongly present in the RPPNG area. Gastrointestinal and ectoparasite taxa were previously reported (Curi et al., 2010).

Based on our findings, the diseases in the domestic dog population around RPPNG pose a significant health threat to the maned wolf population. Dog population control is imperative and a key to wildlife protection in some reserves (Lacerda et al., 2009) and should be implemented in this

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case. Simultaneously, domestic dogs from the surrounding area should be vaccinated against the diseases identified as possible threats (see Laurenson et al., 2004). Finally, more research is needed to elucidate disease impacts and dynamics, and to guide management actions for the conservation of Neotropical wild canids.

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