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Local threats and potential infectious hazards to maned wolves (*Chrysocyon brachyurus*) in the southeastern Argentine Chaco

Abstract: A near-threatened species, the maned wolf's (*Chrysocyon brachyurus*) range extends from central Brazil, northern and eastern Bolivia, and southeastern Peru to the southeast of the Gran Chaco ecoregion and adjacent scrubland in Argentina. Rural domestic dogs under ownership may pose threats to maned wolves as reservoir hosts of multiple pathogens. A serologic survey of rural domestic dogs and maned wolves was conducted to document exposure to canine pathogens in southeastern Santiago del Estero, Argentina, with a parallel questionnaire of knowledge, attitudes, and perception of local villagers in relation to maned wolves. The three maned wolves examined had evidence of exposure to canine adenovirus, canine distemper virus, canine parvovirus, *Leptospira interrogans* spp., and *Dirofilaria immitis*. Dogs had very high seroprevalence for *Neospora caninum* and canine coronavirus (67%), canine adenovirus (59%), *D. immitis* (58%), canine distemper virus (57%), *Toxoplasma gondii* (33%), and *L. interrogans* spp. (20%). Antibodies for canine parvovirus, *Brucella canis* and *Trypanosoma cruzi* were rare or absent. Multiple logistic regression analysis showed that dog seroprevalence was not significantly associated with age, sex, and function for any of the pathogens investigated. The frequency distribution of seroreactivity to specific pathogens per dog was highly aggregated. Villagers considered that illegal trade, hunting, vehicular collisions, and interactions with dogs were the most serious local threats for maned wolves. The multiple threats faced by the maned wolf populations in southeastern Santiago del Estero suggest that their populations are unlikely to remain viable unless specific protection measures are taken.

Keywords: carnivore conservation; *Chrysocyon brachyurus*; disease ecology; domestic dogs; infectious diseases.

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Introduction

Infectious diseases are a recognized threat to the long-term conservation of free-ranging wildlife (Daszak et al. 2000). Domestic animals can act as reservoir hosts of many pathogens and may transmit them to native wildlife populations and humans (Cleaveland and Dye 1995). Domestic dog populations have increased in size and spread to virtually all areas colonized by humans (Matter and Daniels 2000), increasing the chances of pathogen transmission between species, including wild canids (Woodroffe 1999, Woodroffe et al. 2012).

The maned wolf, *Chrysocyon brachyurus* (Illiger 1815), is listed as “Near Threatened” (IUCN Red List) (Rodden et al. 2008), whereas in Argentina, it is considered “Endangered” (Ojeda et al. 2012). Maned wolves inhabit Argentina, Bolivia, Brazil, Paraguay, Peru, and Uruguay (Ginsberg and Macdonald 1990) and number around 24,000 animals (Paula et al. 2008). Less than 1000 maned wolves are estimated to remain in Argentina (Beccaceci 1992, Pautasso 2009) where a little known population occupies marshlands on the edge of the Gran Chaco – an ecoregion that has suffered intense deforestation combined with expansion of cattle ranching and intensified agriculture over recent decades (The Nature Conservancy 2005). While the marshland population corresponds to one margin of its current distribution, historically, the maned wolf ranged to the 38th parallel in Argentina (Queirolo et al. 2011).

Common infectious diseases of domestic dogs have been shown to cause mortality in captive maned wolves including canine distemper virus (CDV), canine parvovirus (CPV), canine adenovirus (CAV), and rabies virus (Barbiers and Bush 1995, Maia and Gouveia 2002, Hammond 2012). In a preserved savanna in Bolivia, most of the

11 free-ranging maned wolves examined had seroreactivity to pathogens of domestic dogs, including CAV, CDV, CPV, canine coronavirus (CCV), rabies virus, *Leptospira interrogans* spp. (Stimson 1907), *Toxoplasma gondii* (Nicolle and Manceaux 1908), and *Dirofilaria immitis* (Leidy 1856) (Deem and Emmons 2005, Deem et al. 2008, Deem et al. 2012).

Assessing the prevalence of pathogens in maned wolf populations and sympatric domestic dog populations is relevant for the management and risk assessment of wild canids. In this study, we evaluated the health status and evidence of exposure to multiple infectious agents in free-ranging maned wolves and domestic dogs in a well-defined rural area in the southeastern Argentine Chaco, with a parallel survey of threats, knowledge, attitudes, and beliefs of local villagers in relation to maned wolves.

Materials and methods

Study area

This study was conducted in seven rural communities (Las Viboritas, El Aybal, El Oso, La Providencia, El Huaico, Ahi Veremos, and Los Porongos) in marshlands along the “Río Dulce” (29°34’S; 62°39’W), and in three neighboring urban communities (Argentina, Palo Negro, and Selva)

along Route 34, in southeastern Santiago del Estero Province, Argentina. The “Río Dulce” flows into the “Laguna de Mar Chiquita,” one of the largest saline lakes of the world (2000–6000 km²), located along the border with Cordoba province (Figure 1).

The area lies in the ecotone between the dry Chaco and scrub (“Espinal”) and has marked seasonal variations in temperature (monthly means ranging between 15°C and 28°C). The vegetation is mostly xerophytic with savannah remnants dominated by *Spartina argentinensis* Parodi (1919) and the African grass, *Elionorus muticus* (Spreng) Kuntze 1898. Soils are predominantly alkaline, with low permeability and frequent floods. Climatic variation associated with El Niño events and a large expansion of “Laguna Mar Chiquita” has occurred in the area since 1990. A long-lasting dry period spanned from 1999 to 2008 with the exception of 2005 being a wet year.

All rural communities have suffered extensive deforestation since 1980 (UMSEF, Dirección de Bosques, SayDS), but Las Viboritas (comprising 22 houses), El Aybal (8 houses), El Oso (2 houses), and La Providencia (13 houses) have been affected more than El Huaico (16 houses), Ahi Veremos (4 houses), and Los Porongos (20 houses), the latter of which can only be reached by small boat. The mean number of people per household ranged from 2.2 (El Aybal and El Oso) to 3.3 (El Huaico). Most of the local economy is based on cattle ranching (45% of total population) and subsistence farming (35%). The other

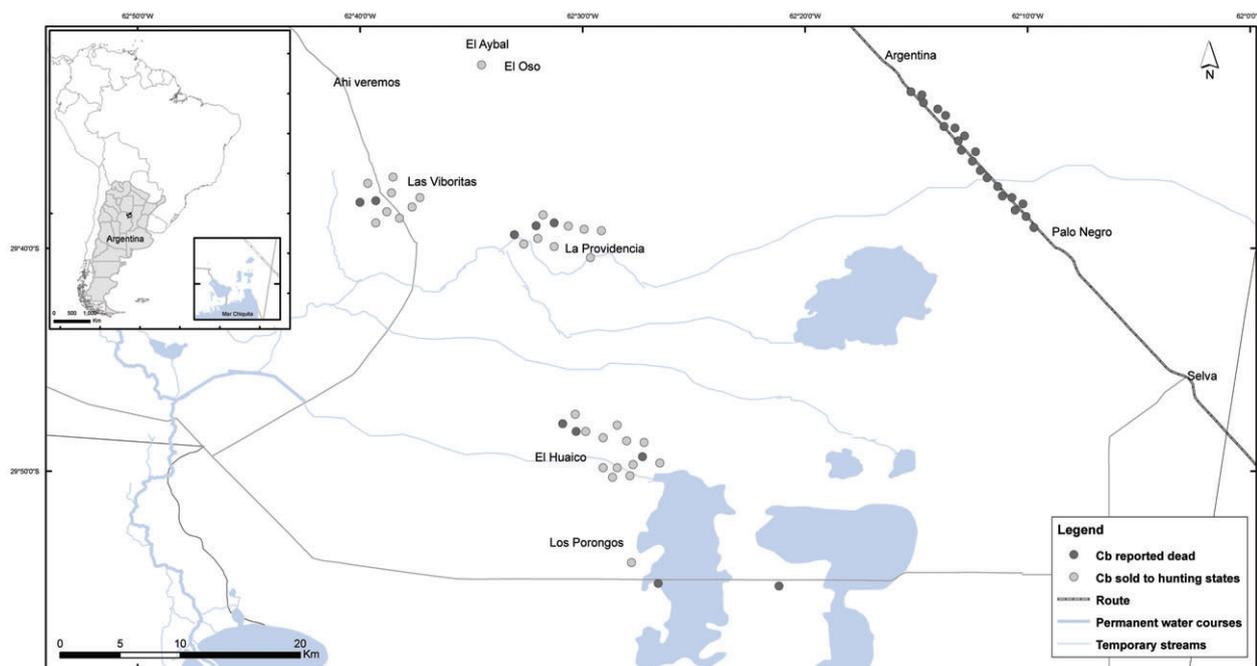


Figure 1 Study area in southeastern Santiago del Estero, Argentina, and reported sightings of maned wolves found dead or sold illegally between 1995 and 2005 in southeastern Santiago del Estero, Argentina.

villagers are involved in education, subsistence trade, and other occupations (20%). Unlike the rural villages, the three urban communities have electricity, potable water, and direct access from the road.

Field methods

The head of each household in the seven rural communities (aged >18 years old) was interviewed in April 2005 to explore their knowledge, attitudes, and practices regarding maned wolves, with the intent of identifying local threats and other aspects of the maned wolf-human relationship in rural settings. Villagers were asked about the history of local occurrence of maned wolves; general knowledge about them; whether they have held maned wolves in captivity; sightings, activities, and responses of maned wolves when encountering villagers, and location of encounters, including approximate dates and times. The interviews were verbal and included open-ended questions. We also interviewed five key local citizens (e.g., people living near the route, hunters, workers involved in road shoulder cleanup).

Maned wolves from southeastern Santiago del Estero and along the border with Córdoba and Santa Fe provinces were examined between 2005 and 2009. We recorded opportunistically the presence of tracks and signs of maned wolves in four fields of southeastern Santiago del Estero during 2005, and efforts to capture maned wolves were made during March and April 2006. Capture procedures used were the same as described in Deem et al. (2012). Maned wolves found recently dead (maximum 4 h) on routes and live animals sampled during provincial rescue procedures were also examined. Capture and transit permits were obtained from the provincial government.

One maned wolf was subjected to a physical examination and was given parenteral anesthesia for induction and inhalatory anesthesia for maintenance. Parenteral anesthesia was performed with ketamine hydrochloride (Vetaset®, Fort Dodge, La Vall De Bianya, Girona, 17813, Spain, 10 mg/kg, i.m.) combined with xylazine (Rompún®, Bayer, Leverkusen, 51373, Germany, 2 mg/kg, i.m.) delivered via blowpipe. Inhalatory anesthesia with Isoflurane® (Forane®, Abbott Laboratories Argentina S.A., Ing Butty 240, C1001AFB, Capital Federal, Argentina) was delivered with a vaporizer and medicinal O₂ (0.25–3 l/min). The xylazine was reversed with yohimbine (Wildlife Pharmaceuticals, Inc., Fort Collins, CO 80522, USA; 0.125 mg/kg, i.m.). All procedures were carried out by veterinarians and personnel from Fundación Temaikèn. One young maned

wolf was examined under manual restraint and handled with the help of local ranch personnel.

The general condition and sex of each maned wolf was determined. Blood samples were drawn by venipuncture from the jugular vein and allowed to clot at ambient temperature. The serum was separated after centrifugation at 3000 rpm during 15 min (~1500×g) and was frozen in duplicate vials at -160°C in liquid nitrogen. Different aliquots of each serum sample were sent on wet ice to reference laboratories for serologic diagnosis, accompanied with transit and export permits.

A demographic and serologic survey of domestic dogs from four rural communities (Las Viboritas, El Aybal, La Providencia, and El Huaico, totaling 59 houses) was undertaken in July–August 2005. The head of each household was interviewed, and a standard demographic questionnaire was completed for each dog, including name, age, sex, place of birth, and function (pet, hunting, and herding) (Cardinal et al. 2007).

In total, 107 dogs were given a physical examination to detect clinical signs supportive of infectious or parasitic disease. The weight of each dog was estimated. Dogs were handled with the assistance of their owners. Only animals 4 months of age or older were included in the serosurvey to avoid measuring residual maternal antibodies (Maclachlan and Dubovi 2010). Approximately 5–10 ml of blood was drawn from each dog. Blood samples were centrifuged, and the serum was separated in duplicates, stored in a liquid nitrogen tank, and thereafter kept at -70°C at the laboratory. All animal processing was conducted according to protocols approved by the Argentinean “Dr. Carlos Barclay” Ethical Committee.

Laboratory methods

Serologic tests and cutoff values are given in Table 1. Sera with titers that met or exceeded the cutoff values were considered indicative of prior exposure to the tested pathogens. Serologic assays for CDV, CPV, CAV, CCV, *Neospora caninum* (Bjerkas and Presthus 1988), *Brucella canis* (Carmichael and Bruner 1968), and *Leptospira interrogans wolffi*, *L. interrogans pyrogenes*, *L. interrogans pomona*, *L. interrogans grippotyphosa*, *L. interrogans canicola*, *L. interrogans copenhageni*, *L. interrogans tarassovi*, and *L. interrogans castellanis* were conducted at the Rosenbusch Institute (Buenos Aires); serologic assays for CDV, CAV, CCV (serum neutralization), and CPV (hemagglutination inhibition) for one maned wolf were conducted at Cornell University Animal Health Diagnostic Center; toxoplasmosis testing was conducted at the Faculty of

Table 1 Serologic test methodologies and cutoff values used for detection of exposure to canine adenovirus, canine parvovirus, canine distemper virus, canine coronavirus, *Leptospira interrogans* spp., *Toxoplasma gondii*, *Neospora caninum*, *Trypanosoma cruzi*, *Brucella canis*, and *Dirofilaria immitis*.

Infectious agent	Methodology	Cutoff	
		Dogs	Maned wolf
Canine adenovirus	Hemagglutination inhibition	40	40 ^a
Canine parvovirus	Hemagglutination inhibition	20	10
Canine distemper virus	Serum neutralization	8	8 ^b
Canine coronavirus	Serum neutralization	8	8
<i>Leptospira interrogans</i> spp.	Microagglutination	100	100
<i>Toxoplasma gondii</i>	Indirect fluorescent	50	50
<i>Neospora caninum</i>	Indirect fluorescent	200	200
<i>Trypanosoma cruzi</i>	Enzyme-linked immunosorbent assay	≥0.2	≥0.2
<i>Brucella canis</i>	Slide agglutination	n/a	n/a
<i>Dirofilaria immitis</i>	Immunochromatography ^c	n/a	n/a

^a*Cb1* was also tested by serum neutralization (SN) (cutoff=8). ^b*Cb1*, *Cb2*, and *Cb3* were tested by indirect fluorescent antibody test (IFA) (cutoff=40) and *Cb1* also by SN. ^cSpeed Diro test (BVT, Virbac). n/a, not applicable (no cutoff value, test results scored as either positive or negative).

Veterinary Sciences (Universidad Nacional de La Plata), and the enzyme-linked immunosorbent assay (ELISA) for *Trypanosoma cruzi* (Chagas 1909) and an immunochromatography test (Speed Diro Test, BVT, Virbac) for canine heartworm diagnosis (a qualitative test without titers) were conducted at the Laboratory of Eco-Epidemiology (UBA).

Statistical analysis

To assess the relationship between potential risk factors and specific antibody responses to the set of pathogens investigated, we conducted maximum-likelihood logistic multiple regression analysis with robust standard errors (Stata 12, StataCorp, College Station, TX, USA), with nominal significance levels set at 5%. The demographic variables considered were age (using four levels: 4–11 months (except for demographic analysis where the level include dogs aged <4 months old), 1–2 years, 3–5 years, and ≥6 years of age); sex (male:female) and function (pet and working (i.e., hunting or herding)).

Results

Knowledge, attitudes, and perception of maned wolves

Householders reported 138 sightings of maned wolves between 1995 and 2005. Maned wolves were reported near

rural villages along the riverbanks since 1990. Most sightings occurred during the long drought that persisted until 2005. In urban villages, an increase in the local presence of maned wolves was reported since 2000. Of all sightings, four (3%) were vehicular collisions along Route 34, and the remainder (97%) were live animals. Among the latter, 75 (56%) wolves were only sighted, 30 (22%) were hunted by villagers and sold to game ranches, 10 (8%) were hunted by villagers and killed or kept in captivity until they died, and 19 (14%) were captured and then escaped or were released. Nearly 20% of all sightings occurred near houses, whereas only 6% were recorded close to Route 34; the remainder (74%) occurred in grasslands and along trails. Of all sightings by local villagers, 26% involved close contact between maned wolves and dogs. Villagers frequently reported that dogs encountered sleeping maned wolves in *Spartina* grasslands during the daytime and that they encouraged their dogs to chase maned wolves to capture or kill them.

Key local citizens provided further information on 44 additional sightings of maned wolves between 1995 and 2005, of which 17 dead and 3 live animals (46%) were registered along Route 34. Among all sightings (i.e., householders and key local citizens), 31 maned wolves were reported dead (21 along Route 34 and 10 killed by villagers), and 30 animals were sold to game ranches in a 10-year period (Figure 1). The main local threats for maned wolf conservation perceived by local villagers included illegal trade for game ranches (32%), drought (8%), dogs (7%), and vehicular collisions along Route 34 (5%). Nearly half (48%) of the respondents were unable to identify local threats to maned wolves.

Dog demography

The dog population numbered 136 dogs (120 males and 16 females) owned by 40 households. The mean number per household was 3.4 dogs (SD=1.7) and 1.1 cats (SD=1.2). Most of the dogs were of a mixed breed (94%), and only 6% were “greyhound.” The median age of the dog population was 3.9 years (first-third quartiles, 1.5–5.0), with 13.2% of dogs aged ≤ 11 months, 20.6% between 1 and 2 years, 50.7% 3–5 years, and 15.5% ≥ 6 years old. The sex distribution of dogs was highly skewed toward males (88%), as female dogs are often killed by rural villagers as a means of population regulation.

All dogs were associated with a household. In general, their movements were not restricted or supervised unless they took part in a hunting trip. More than half (56%) of the dogs were used for herding; 29% were reported as pets, and the remainder (15%) were hunting dogs. Most dogs (92%) were assessed to be underweight. On average, 74% of the dogs partly ate homemade food and partly hunt leftovers, whereas the remainder only ate homemade food. Also, 74% of dogs were reported to search for food in natural habitats. Over half (58%) of the dogs examined had ectoparasites (usually ticks) and were reported to shed worms (49%). Only three dogs in one household received sporadic veterinary treatment, and no dog was reported to have ever been vaccinated before our survey.

Maned wolf capture and sampling

Three maned wolves were examined in the study area. *Chrysocyon brachyurus 1* (Cb1, adult of undetermined sex) was found dead on Route 34 in 2005. *C. brachyurus 2* (Cb2, a juvenile female) was caught alive in northern Santa Fe province in 2006. This maned wolf had been rescued from illegal trafficking and later was held in a local field for recovery. *C. brachyurus 3* (Cb3, an adult male) was captured during a rescue procedure conducted by Agencia Córdoba and Fundación Temaikèn in northwestern Córdoba province in 2009. Cb3 was fitted with a VHF radio collar and then released and monitored by local rangers and personnel from Agencia Córdoba Ambiente and Fundación Temaikèn.

No maned wolf was captured with traps in southeastern Santiago del Estero in 2006. Unlike other campaigns, we found few signs of the presence of maned wolves in the study area. Two local villagers reported seeing maned wolf pups 1 month before our arrival, whereas other key local citizens suggested a significant decrease in maned wolf sightings over the previous 3 months.

Seroprevalence of infectious diseases

The three maned wolves examined had evidence of exposure to 5, 7, and 6 of the study pathogens, respectively (Table 2). All three had serologic evidence of exposure to CAV (with high titers) and CPV (two with titers of 80, higher than those found in CPV-positive dogs). For *Leptospira interrogans*, the serovar *grippotyphosa* was detected in the three individuals, whereas serovars *wolffi*, *copenhageni*, and *tarassovi* occurred in two maned wolves. *Dirofilaria immitis* antigens were found in two maned wolves. For CDV, only one maned wolf presented a low titer. Very low titers may correspond to inhibitory unspecific substances in blood samples, cross-reactivity to other wild viral strains, past exposure, or waning antibodies (Appel et al. 1984, Greene and Appel 2008). No antibody responses to CCV, *Toxoplasma gondii*, *Neospora caninum*, *Brucella canis*, and *Trypanosoma cruzi* were found.

A blood sample was drawn from 107 (79%, 94 males and 13 females) of 136 dogs. Thirteen serum samples from dogs were of insufficient volume to test for CCV and CDV antibodies, and five samples were insufficient to test for toxoplasmosis. The study dogs had antibodies to all the

Table 2 Seroprevalence of *Dirofilaria immitis* antigens and antibodies to *Neospora caninum*, canine adenovirus, canine coronavirus, canine distemper virus, *Toxoplasma gondii*, *Leptospira interrogans* spp., canine parvovirus, *Brucella canis*, and *Trypanosoma cruzi* in three maned wolves from “Bañados de los Ríos Dulce y Salado,” Argentina.

Infectious agent	<i>Chrysocyon brachyurus 1</i>	<i>C. brachyurus 2</i>	<i>C. brachyurus 3</i>
<i>N. caninum</i>	Negative	Negative	Negative
Canine adenovirus	768 ^a	80	80
Canine coronavirus	<4	Not tested	Not tested
<i>D. immitis</i>	Positive	Positive	Negative
Canine distemper virus	40 ^b –6 ^a	<10	<10
<i>T. gondii</i>	Negative	Negative	Negative
<i>L. interrogans</i> spp.			
<i>copenhageni</i>	Negative	200	800
<i>canicola</i>	Negative	Negative	Negative
<i>grippotyphosa</i>	200	200	200
<i>wolffi</i>	Negative	200	400
<i>tarassovi</i>	Negative	200	200
<i>pomona</i>	Negative	Negative	Negative
<i>castellonis</i>	Negative	Negative	Negative
<i>pyrogenes</i>	Negative	Negative	Negative
Canine parvovirus	20	80	80
<i>B. canis</i>	Negative	Negative	Negative
<i>T. cruzi</i>	Negative	Negative	Negative

^aAssessed by SN.

^bAssessed by IFA.

pathogens tested except *Trypanosoma cruzi*, in which absorbance values <0.15 were clearly negative. Overall seroprevalence was high for *Neospora caninum* (67%; 95% confidence interval (CI), 58.4–76.2%), CCV (67%; 95% CI, 57.5–76.5%), CAV (59%; 95% CI, 49.5–68.2%), *Dirofilaria immitis* (58%; 95% CI, 48.6–67.3), and CDV (57%; 95% CI, 47.4–67.4) and was lower for *Toxoplasma gondii* (33%; 95% CI, 28.2–42.5) and *Leptospira interrogans* spp. (20%). Only two (2%; 95% CI, 12.1–27.2) dogs had low antibody titers for CPV (both were at the same village, La Providencia), and only one dog was seropositive for *Brucella canis*; all of these dogs were adults ≥ 4 years of age (Figure 2). The evaluated animals did not show any clinical sign compatible with the diseases under study.

Multiple logistic regression analysis showed that dog seroprevalence was not significantly associated ($p > 0.1$) with age class, sex, and function for any of the pathogens investigated. The seroprevalence of *Neospora caninum* and CDV in the 4- to 11-month age class was 82% and 64%, respectively (Table 3). Antibody prevalence increased with age in *Leptospira interrogans* spp. and in CCV decreased from 91% in dogs aged between 4 and 11 months to 56% in dogs ≥ 6 years, although nonsignificantly so (Table 3). Toxoplasmosis antibody prevalence increased slightly with age from 18% to 38%. Dogs had serum titers for *N. caninum* >800 , and seroreactivity varied from 47% to 76% among the four villages (Las Viboritas, 71%; El Huaico, 76%; El Aybal, 64%, and La Providencia, 47%).

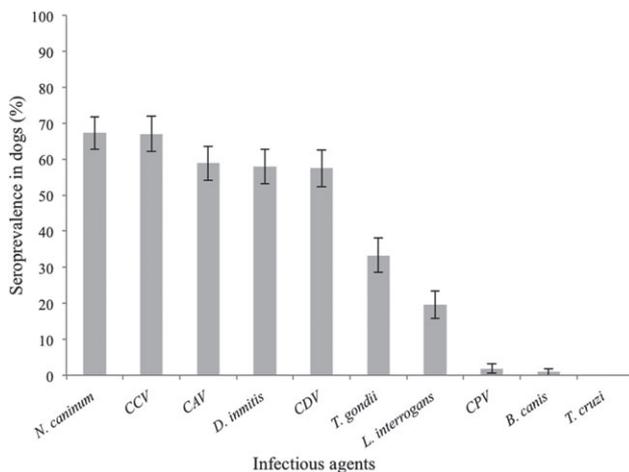


Figure 2 Seroprevalence of antibodies to *Neospora caninum*, canine coronavirus, canine adenovirus, canine distemper virus, *Toxoplasma gondii*, *Leptospira interrogans* spp., canine parvovirus, *Brucella canis*, and *Trypanosoma cruzi*, and of *Dirofilaria immitis* antigens in four rural dog populations from southeastern Santiago del Estero, Argentina, July–August 2005. Total number of dogs examined: 107, except for CDV and CCV ($n=94$) and for *T. gondii* ($n=102$).

The prevalence of antibodies to CCV in dogs was very high (56–77%) in all villages. Positive serum titers were 8 (in 44% of dogs) and 16 (in 23% of dogs); no dog had a titer ≥ 32 . Seroprevalence for CAV ranged from 36% at El Aybal to 59–69% in other villages, and antibody titers were all 40. CDV antibody prevalence ranged from 45% to 76% among villages; positive serum titers were 8 in 44% of the dogs, 16 in 11%, and 32 in only 3% of dogs. The seroprevalence and titers of CPV were low and occurred in only one village (La Providencia). The prevalence of *Toxoplasma gondii* was higher at El Aybal (57%) than in the other villages (range, 19–33%). *Dirofilaria immitis* prevalence was highest at Las Viboritas and La Providencia (57–59%).

Leptospira interrogans spp. antibody titers were highest in El Huaico (30%) where one dog was positive for seven serovars and varied from 9% to 18% in the rest of the villages. In dogs, the most frequent serovar detected was *L. interrogans wolffi* (13 dogs) followed by *L. interrogans pyrogenes* (6 dogs) and *L. interrogans pomona* (5 dogs). Other serovars detected were *L. interrogans grippotyphosa*, *L. interrogans canicola*, *L. interrogans copenhageni*, *L. interrogans tarassovi*, and *L. interrogans castellanis*, each of them in two dogs.

The frequency distribution of seroreactivity to specific pathogens per individual dog ranged from 0 to 8. Among the 91 dogs tested for all the 10 study pathogens, only 1 (1.1%) dog was seronegative for all pathogens, 3 (3.3%) dogs were positive for 1 pathogen, 10 (11.0%) dogs for 2, 27

Table 3 Age-specific prevalence of antibodies to *Neospora caninum*, canine coronavirus, canine adenovirus, canine distemper virus, *Toxoplasma gondii*, *Leptospira interrogans* spp., canine parvovirus, *Brucella canis*, and *Trypanosoma cruzi*, and *Dirofilaria immitis* antigens in four rural dog populations from southeastern Santiago del Estero, Argentina.

Infectious agent	% Seropositive by age class ^a			
	4–11 months	1–2 years	3–5 years	≥ 6 years
<i>N. caninum</i>	82	52	71	67
Canine coronavirus	91	65	66	56
Canine adenovirus	82	48	55	72
<i>D. immitis</i>	45	70	56	56
Canine distemper virus	64	50	62	50
<i>T. gondii</i>	18	29	37	38
<i>L. interrogans</i> spp.	0	22	18	33
Canine parvovirus	0	0	2	6
<i>B. canis</i>	0	0	2	0
<i>T. cruzi</i>	0	0	0	0

^aNumber of dogs examined per age class were 23, 24, 42, and 18, respectively, except for canine distemper virus and canine coronavirus (21, 21, 36, and 16, respectively).

(29.7%) for 3, 23 (25.3%) for 4, and 27 (29.7%) dogs for 5 or more pathogens. The frequency distribution of pathogen exposures differed significantly from a random (Poisson) distribution ($\chi^2=36.9$, $p<0.0001$; $df=5$), with more dogs having been exposed to multiple pathogens than expected by chance alone. The distribution of pathogen exposure was highly aggregated and fit a negative binomial distribution ($p=0.125$; $df=5$).

Discussion and conclusion

This study confirmed the occurrence of infectious diseases as potential hazards to maned wolves from domestic dogs, and identified illegal trade, hunting, drought, dogs, and vehicular collisions as community-perceived risks for maned wolves. Vehicular collisions of *Chrysocyon brachyurus* were also frequent in neighboring provinces (Pautasso 2009) and have been considered one of the most important causes of maned wolf decline in Argentina (Paula et al. 2008). Climatic cycles with wet and dry periods related to El Niño and La Niña events probably enhanced the long-distance dispersal of maned wolves, with more frequent sightings of maned wolves occurring during the multiyear dry period up to 2005. In the dry period, wolves were sighted in the vicinities of Argentina and Palo Negro villages, where Route 34 is crossed by a temporary tributary of the “Río Dulce” that may serve as a corridor along most of the study villages. Displacement of maned wolves likely enhanced their frequency of contact with rural villagers and mortality caused by hunting and collision with vehicles. In our study area, hunting of maned wolves was stimulated by sustained demand from a local game ranch. The multiple threats faced by the maned wolf populations in southeastern Santiago del Estero suggest that their populations are unlikely to remain viable unless specific protection measures are taken.

The current status of maned wolves in the study region may render them highly vulnerable to threats derived from frequent contact with domestic dogs. Reports from local rural villagers and key local citizens clearly indicated: i) the occurrence of overlapping habitat use between wild and domestic carnivores, which may enhance contact rates across species; ii) maned wolves kept in captivity for extended periods had increased chances of direct contact with local dogs; iii) maned wolves sold illegally to game ranches and confined in large stretches of land or often in poor installations (where animals often are kept in overcrowded conditions), had enhanced contact with dogs and wildlife, and iv) maned wolves frequently

approached rural houses had close contact with domestic dogs and frequently were attacked by them. Although these encounters most frequently resulted in the death of maned wolves, the few animals that reportedly escaped had been in contact with dogs and their secretions, thus increasing the risk of pathogen transmission between species.

Our study shows that numerous pathogens in rural dog populations pose a sizable threat to maned wolves and other carnivores, especially *Neospora caninum*, CCV, CAV, CDV, CPV, several *Leptospira interrogans* serovars, and *Dirofilaria immitis*. All of these (except *N. caninum*, *T. Toxoplasma gondii*, and CCV) were detected in local maned wolves. The frequent use of dogs for herding and hunting, along with their frequent foraging in natural habitats, could favor direct or indirect transmission of multiple pathogens.

CDV and CPV may exert substantial mortality on wild canids (Woodroffe et al. 2012) and may have caused the disappearance of various maned wolves in the Bolivian Cerrado (Deem and Emmons 2005, Deem et al. 2008). Although CDV infection in maned wolves was confirmed elsewhere in Argentina and Brazil (Maranho et al. 1991, Lertora et al. 2008), there was no exposure in the studied maned wolves. In dogs, however, CDV was highly prevalent (with low titers) and fairly homogeneous among age classes and villages, suggesting viral transmission throughout the area or the occurrence of previous outbreaks. CDV may cause high mortality in susceptible dogs, but 25–75% of dogs may develop a subclinical infection and spread the virus transiently (Greene and Appel 2008), which can be actively maintained by the domestic dog population (Muir and Emmons 2012).

CPV was rare in the study dogs, unlike in the three maned wolves and in the Bolivian Chaco and Cerrado (Fiorello et al. 2004, 2006, Deem and Emmons 2005, Bronson et al. 2008, Deem et al. 2008). The low antibody prevalence to CPV in the study dogs may be explained by the high case fatality rate in unvaccinated pups during an outbreak. Dog populations with a large fraction of susceptible hosts, such as the study population, may pose a threat to maned wolves and other susceptible wildlife if the virus is introduced from elsewhere and causes an outbreak.

The high seroprevalence to CAV with low antibody titers in the study dogs suggests previous exposure, or less likely, subclinical infection with frequent exposure. CAV prevalence was high at 59% compared to 18% in the domestic dogs around Noel Kempff Mercado National Park in Bolivia (Bronson et al. 2008). Antibodies to CAV were found in the three maned wolves of this study (with higher titers than in dogs), in coincidence with seroprevalence of 100% found

in asymptomatic adult maned wolves from Bolivia (Deem et al. 2008). CAV could be endemic in the maned wolf population, as in Bolivia (Muir and Emmons 2012). CAV is associated with neonatal mortality in maned wolves (Barbiers and Bush 1995) and also could be associated with loss of litters (Deem et al. 2012). Additional studies are needed to investigate the variations of seroprevalence of CAV over time and compare these results with the variable patterns found elsewhere (Deem et al. 2012).

The local seroprevalence of CCV in dogs was high, and antibody titers were predominantly low. Most (81%) of the young dogs had already been exposed, whereas the older dogs with lower seroprevalence may have lost their specific antibody response. These findings are consistent with CCV usually causing a benign disease with short-lived antibody titers (McCaw and Hoskins 2008), and prevalences higher than 80% in unvaccinated dog populations (Binn et al. 1981). The only maned wolf examined for CCV showed a titer <4 by SN and therefore was seronegative. Different results were found in Bolivia, where seroprevalence was low in dogs (Bronson et al. 2008) and maned wolves (Deem et al. 2012). Local dog populations could play an important role in the persistence of CCV and imply a risk to susceptible wildlife, especially in association with other infectious disease agents (Pratelli et al. 2001).

Leptospira interrogans spp. occurred throughout the area and peaked at El Huaico – a village isolated by water during half of the year. Various serovars (e.g., *L. interrogans wolffi*, *L. interrogans pyrogenes*, *L. interrogans pomona*) were highly prevalent in local dogs and maned wolves although with low antibody titers. Working dogs had a higher seroprevalence than pet dogs, as recorded elsewhere (Ward et al. 2002). *L. interrogans* spp. may cause acute, chronic, or subclinical infections in dogs (Leighton and Kuiken 2001), and specific antibodies are often short-lived (Greene et al. 2008). Rural dogs and many wildlife species are susceptible to *L. interrogans* spp., especially in suitable habitats such as wetlands (Ghneim et al. 2007). The climatic and environmental conditions of our study area are ideal for the survival and transmission of *L. interrogans* spp. (Ward et al. 2004, Sykes et al. 2011).

Maned wolves are susceptible to the filarid *Dirofilaria immitis* (Carvalho and Vasconcellos 1995) transmitted by *Culex*, *Aedes*, and *Anopheles* mosquitoes (Ludlam et al. 1970), which are extremely abundant along the “Río Dulce.” Dogs showed significant prevalence to *D. immitis* (58%), and filarial antigens were detected in two of the wolves examined. Thus, *D. immitis* could be a potential threat to maned wolves in the Chaco region as in Cerrado habitats in Bolivia, where 85% of samples were antigen positive and increased to 100% adult prevalence during

the 10-year study (Deem et al. 2012). Further studies are needed in the maned wolves of the Gran Chaco.

Wetland habitats combined with warm climatic conditions are suitable for the survival and dissemination of the coccidia *Toxoplasma gondii* and *Neospora caninum* (Dubey et al. 2007, Alfonso et al. 2010). Although exposure of maned wolves to both coccidia has been documented (Vitaliano et al. 2004, Deem and Emmons 2005), they were not detected in the three maned wolves in this study. In contrast, the high seroprevalence of *N. caninum* in dogs is consistent with cattle-ranching activities and frequent use of dogs for herding, as in other rural areas with history of bovine abortion (Dubey et al. 2007, Nogueira et al. 2013). Subclinical infections may be frequent, as serological findings are usually much more prevalent than clinical findings (Trees et al. 1993). Because susceptible hosts become infected through food or water contaminated with oocysts of *N. caninum*, the local risk of infection with *N. caninum* could be sizable during the wet, warm season. In reference to *T. gondii*, wild and domestic felines are definitive hosts and represent a source of infection for humans, dogs, herbivores, and other wildlife. Domestic cats from rural areas usually roam in natural habitats and may disseminate oocysts in their feces. *T. gondii* was less seroprevalent in dogs than other pathogens and did not occur in local maned wolves, unlike in Bolivia where 80% of maned wolves and domestic dogs were seropositive (Bronson et al. 2008, Deem et al. 2012). The occurrence of *T. gondii* could pose an important risk to local carnivorous species that share habitats with rodents and wild or domestic felids (Deem et al. 2012).

The absence of *Trypanosoma cruzi* infections in the study dogs contrasts with the large seroprevalence recorded elsewhere in the same province (Gürtler et al. 2007). Such apparent disagreement may be explained by the impact of past insecticide spraying campaigns combined with the less suitable humid environment. Both factors lead to the very low indices of house infestation with the vector *Triatoma infestans* ($<4\%$) in 2008 and to the recent interruption of vector-borne transmission of human *T. cruzi* infection at department level (PAHO 2012).

Our study had some limitations: i) The few maned wolves examined for antibodies are related to their very low abundance after a long dry period and the large capture efforts needed. Although maned wolves were apparently easily caught by rural villagers to sell them to the local game ranch, it is more difficult to catch wolves with safe methods in a context of large hunting pressure; ii) Whether antibody presence represents past exposure with or without illness followed by recovery or active infection is uncertain, and iii) Serologic tests have not

been validated for maned wolf diagnostics. Although we have not attempted to isolate pathogens from either host, various lines of evidence support that dogs have plenty of opportunities to share pathogens with maned wolves. Several demographic characteristics of rural dog populations and their shared habitat use with wild carnivores create conditions for disease spillover, as in the Bolivian Chaco (Fiorello et al. 2006). Infectious diseases are a component affecting the survival of wildlife populations, especially of rare and endangered species, which may be driven to extinction by generalist pathogens that “spill over” from other host species, often domestic animals (Cleaveland et al. 1995, Woodroffe 1999, Daszak et al. 2000, Taylor et al. 2001).

The design of conservation strategies is often hampered by the lack of basic information about pathogens that represent serious threats, their epidemiology in multiple host systems, and treatment protocols that are likely to be most effective under field conditions (Woodroffe 1999). Our findings suggest that conservation planning needs to consider infectious diseases in dog-wolf interactions. We recommend further longitudinal studies and specific molecular diagnosis to determine the relationship between the infectious agents found in maned wolves and domestic dogs. Additional studies are required to determine whether the risks are large enough to warrant different conservation actions.

Legal actions to prevent illegal trade and hunting, education activities, and the construction of viaducts and barriers to protect maned wolves from collisions along

main roads would increase the viability of populations in Argentina. The available preventive methods involve promoting responsible dog ownership and awareness of the conservation status of maned wolves, including vaccination and treatment of domestic dogs.

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