Seroprevalence of pathogens in domestic carnivores on the border of Madidi National Park, Bolivia

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Abstract
The importance of diseases of domestic animals in the conservation of wildlife is increasingly being recognised. Wild carnivores are susceptible to many of the pathogens carried by domestic dogs and cats and some of these pathogens have caused disease outbreaks and severe population declines in threatened species. The risk of disease spillover from domestic to wild carnivores in South America has not been extensively investigated. This study examined the disease exposure of domestic carnivores living near a protected area in Bolivia. Forty dogs and 14 cats living in three towns on the eastern border of Madidi National Park were sampled. High levels of exposure to canine distemper virus, canine parvovirus, Sarcoptes scabiei and Toxoplasma gondii were found among domestic dogs, with similarly high levels of exposure to feline parvovirus, feline calicivirus and T. gondii being found among domestic cats. If contact occurs between domestic and wild carnivores, disease spillover may represent an important risk for the persistence of wild carnivores in the region. Additional research is therefore necessary to determine if wild carnivores living in proximity to these domestic carnivore populations are being exposed to these pathogens.

INTRODUCTION
The conservation community is increasingly aware of the disease risks to threatened wildlife (Woodroffe, 1999; Daszak, Cunningham & Hyatt, 2000, 2001; Deem et al., 2001; Funk et al., 2001). Domestic animals, and dogs (Canis familiaris) in particular, have been the proven or suspected reservoir for infectious agents that have led to numerous epizootics in a variety of wild carnivore species (Sillero-Zubiri, King & MacDonald, 1996; Cleaveland et al., 2000). African lions (Panthera leo: Roelke-Parker et al., 1996), Ethiopian wolves (Canis simensis: Laurenson et al., 1998), and African hunting dogs (Lycaon pictus: Gascoyne et al., 1993), for instance, have suffered population declines as a result of disease spillover from domestic dogs.

Populations of domestic carnivores, especially dogs, act as ideal disease reservoirs; they may travel large distances into wildlife habitats and, in some areas, high resident population densities of feral or unvaccinated animals allow even very virulent pathogens to persist in the broader carnivore population (Gottelli & Sillero-Zubiri, 1992; Cleaveland & Dye, 1995; Cleaveland, Laurenson & Taylor, 2001). As habitat fragmentation increases (Skole & Tucker, 1993), the frequency and intensity of interactions between domestic and wild carnivores will also probably increase (Laurance, Vasconcelos & Lovejoy, 2000). Even in the absence of direct contact between domestic and wild carnivores, the ability of some pathogens to remain viable in the environment for extended periods of time (Table 1) means that domestic and wild carnivore sympathy may be sufficient for disease transmission. There are many examples from Africa (Ginsberg, Mace & Albon, 1995; Laurenson et al., 1998) and North America (Thorne & Williams, 1988; Valenzuela, Ceballos & Garcia, 2000; Miller et al., 2002) in which diseases of domestic animals caused morbidity and mortality in endangered carnivore populations. Therefore, knowledge of the diseases present in the domestic carnivore populations in close proximity to wildlife is essential for conservation planning.
Table 1. Major modes of transmission, infectious materials and length of persistence in environment for carnivore pathogens

<table>
<thead>
<tr>
<th>Disease agent</th>
<th>Method of transmission</th>
<th>Infectious material</th>
<th>Environmental persistence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canine distemper virus</td>
<td>Oronasal exposure to aerosolised body fluids</td>
<td>Respiratory secretions, urine, tissues</td>
<td>Hours in warm temperatures; weeks in cold temperatures Months</td>
</tr>
<tr>
<td>Canine parvovirus</td>
<td>Oronasal exposure to faeces</td>
<td>Faeces</td>
<td>Hours in warm temperatures Probably not</td>
</tr>
<tr>
<td>Canine coronavirus</td>
<td>Ingestion</td>
<td>Faeces</td>
<td>Days in warm temperatures; months in cold temperatures Probably not</td>
</tr>
<tr>
<td>Canine herpesvirus</td>
<td>Oronasal exposure to body fluids; <em>in utero</em> exposure</td>
<td>Respiratory secretions, genital lesions</td>
<td>Up to hours</td>
</tr>
<tr>
<td>Rabies virus</td>
<td>Oronasal exposure to body fluids; possibly also via ectoparasites</td>
<td>Saliva, urine, faeces, tissues</td>
<td></td>
</tr>
<tr>
<td>Feline leukaemia virus</td>
<td>Bite wounds</td>
<td>Saliva, tissues</td>
<td></td>
</tr>
<tr>
<td>Feline immunodeficiency virus</td>
<td>Oronasal exposure to saliva or other bodily fluids</td>
<td>Saliva, blood, tissues</td>
<td></td>
</tr>
<tr>
<td>Feline panleukopaenia virus</td>
<td>Bite wounds</td>
<td>Blood and saliva</td>
<td>No</td>
</tr>
<tr>
<td>Feline herpesvirus</td>
<td>Inhilation of oronasal and ocular secretions</td>
<td>Urine, faeces, all other secretions</td>
<td>Months to a year</td>
</tr>
<tr>
<td>Feline calicivirus</td>
<td>Inhilation of oronasal and ocular secretions</td>
<td>Respiratory and oral secretions</td>
<td>Probably not</td>
</tr>
<tr>
<td>Toxoplasma gondii</td>
<td>Oronasal exposure to faeces or body fluids; <em>in utero</em> exposure</td>
<td>Faeces, saliva, urine, respiratory secretions</td>
<td>Hours to weeks</td>
</tr>
<tr>
<td><em>Dirofilaria immitis</em> (heartworm disease)</td>
<td>Inhilation of infected tissues or oocysts from faeces; <em>in utero</em> exposure</td>
<td>Respiratory and oral secretions; faeces</td>
<td>Up to a week</td>
</tr>
<tr>
<td><em>Sarcopes scabiei</em> (sarcoptic mange)</td>
<td>Direct and indirect contact</td>
<td>Facess of cats; tissues of intermediate hosts</td>
<td>Months</td>
</tr>
<tr>
<td><em>Leptospira interrogans</em></td>
<td>Bite wounds, contact with or ingestion of tissues, urine or urine-contaminated water</td>
<td>Urine, tissues</td>
<td>Days in standing water</td>
</tr>
</tbody>
</table>

Source: Samuel, Pybus & Kocan (2001) and Greene (1998c). N/A, not applicable.

While numerous reports of disease spillover exist in the literature, these events are not distributed evenly across the globe. Well-coordinated and long-standing domestic dog vaccination programmes in much of North America and Europe have made disease spillover from domestic carnivores to wildlife rare; in fact, in the case of rabies, it is spillover from wildlife into domestic populations that is more of a concern (Rupprecht et al., 1995; Chang et al., 2002). In the developing world, most of the well-documented occurrences of spillover have been in Africa (Gascoune et al., 1993; Sillero-Zubiri et al., 1996; Nel et al., 1997; Cleaveland et al., 2000). This may be an artifact of the fact that many African carnivores inhabit open savannahs where they are easily monitored, or it may be simply an artifact of the lack of studies in other regions. In places where studies have been conducted, the potential for disease spillover has been documented. For instance, Mainka et al. (1994) identified antibodies to canine distemper virus (CDV) and canine parvovirus (CPV) in domestic dogs inhabiting the Wolong Reserve in China, indicating the potential for spillover from dogs to giant pandas (*Ailuropoda melanoleuca*) and other wild carnivores. Disease studies from Latin America are few, but as the human population there increases, it is likely that spillover events will, or already have, occurred. The present study aims to increase our knowledge of disease exposure in domestic carnivores living close to a large protected area, the Madidi National Park (MNP) and Integrated Management Area (IMA), in Bolivia.

Numerous species of canids and felids inhabit the park, including jaguars (*Panthera onca*), pumas (*Puma concolor*), ocelots (*Leopardus pardalis*), bush dogs (*Speothos venaticus*), margays (*Leopardus wiedii*), Geoffroy’s cats (*Oncifelis Geoffroyi*) and jaguarundis (*Herpailurus yaguarondi*) (WCS-Bolivia, 2002). Captive animals of many of these species have been infected with domestic carnivore disease agents, indicating their susceptibility to these pathogens (Table 2). All but the puma are considered to be threatened species by the World Conservation Union (IUCN) and are listed on Appendix I or II of the Convention on International Trade in Endangered Species (CITES).

Our primary objective was to determine the seroprevalence of antibodies to a variety of pathogens in domestic carnivores inhabiting a region of high priority for
Table 2. Selected reports of exposure to or infection with disease agents in Latin American non-domestic carnivores

<table>
<thead>
<tr>
<th>Disease agent</th>
<th>Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canine distemper virus</td>
<td>Crab-eating fox, maned wolf, crab-eating fox, jaguar, maned wolf, bush dog</td>
<td>Mann et al. (1980); Appel et al. (1994); Cubas (1996); Murray et al. (1999)</td>
</tr>
<tr>
<td>Canine parvovirus</td>
<td>Puma</td>
<td>Cubas (1996)</td>
</tr>
<tr>
<td>Rabies virus</td>
<td>Puma</td>
<td>Murray et al. (1999); Jessup et al. (1993); Murray et al. (1999)</td>
</tr>
<tr>
<td>Feline leukemia virus</td>
<td>Puma, jaguar, ocelot, margay, Geoffroy's cat, jaguarundi, others</td>
<td>Olmsted et al. (1992); Brown, Mitthiapala &amp; O'Brien (1993); Carpenter &amp; O'Brien (1995)</td>
</tr>
<tr>
<td>Feline immunodeficiency virus</td>
<td>Puma, jaguar, ocelot, margay, puma</td>
<td>Paul-Murphy et al. (1994); Cubas (1996)</td>
</tr>
<tr>
<td>Feline panleukopenia virus</td>
<td>Jaguar, margay, ocelot, puma</td>
<td>Paul-Murphy et al. (1994); Murray et al. (1999)</td>
</tr>
<tr>
<td>Feline herpesvirus</td>
<td>Puma</td>
<td>Paul-Murphy et al. (1994)</td>
</tr>
<tr>
<td>Feline coronavirus</td>
<td>Puma</td>
<td>Murray et al. (1999)</td>
</tr>
<tr>
<td>Feline calicivirus</td>
<td>Puma</td>
<td>Paul-Murphy et al. (1994); Murray et al. (1999)</td>
</tr>
<tr>
<td>Toxoplasma gondii</td>
<td>Puma, jaguar</td>
<td>Otto (1974)</td>
</tr>
<tr>
<td>Dirofilaria immitis (heartworm</td>
<td>Jaguar, jaguarundi</td>
<td>Deem et al. (2002)</td>
</tr>
<tr>
<td>disease)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoptes scabiei (mange)</td>
<td>Pampas fox</td>
<td></td>
</tr>
</tbody>
</table>

1 Indicates that clinical signs were observed.
2 Indicates a species whose range includes the Madidi National Park.

Carnivore conservation. We sampled owned, free-roaming domestic carnivores living in communities situated on the borders of the MNP/IMA and performed serological testing for numerous disease agents, including viral, bacterial, parasitic and protozoal pathogens. Serological assays are commonly used for epidemiological studies (Greiner & Gardner, 2000b), even though they cannot distinguish between active and previous infection, or infection and disease (Barr, 1996; Evermann & Eriks, 1999). While the limitations of serology can be problematical for determining the disease status of an individual animal, in this case we are using it to screen for exposure on a population level (Christensen & Gardner, 2000). For the purposes of this study, it is not important if a given dog was ill as a result of infection with canine distemper; instead, it is important to know if dogs in the population are commonly infected with this virus, regardless of its clinical effect on individuals.

STUDY SITE AND METHODS

We sampled in several locations to increase the number of animals represented and assess the heterogeneity in disease exposure among closely situated communities. Three towns on the eastern border of the MNP/IMA were chosen for sampling on the basis of ease of access for the field researchers and proximity to the park (Fig. 1). Madidi is a relatively new park of over 18,000 km² in north-western Bolivia. It consists of tropical rainforest and cloud forest habitat. San Buenaventura, which houses the main offices of the park, has a population of about 6200. It is situated on the Río Beni, directly across the river from Rurrenabaque, a larger town (pop. 7000) with an active tourist industry. Therefore, dogs in San Buenaventura are probably exposed to dogs from Rurrenabaque and other nearby towns and villages. Tumupasa, however, is an isolated village of just under 1000 people, situated about 60 km north-west of San Buenaventura. Ixiamas is about 30 km north-west of Tumupasa; it has a population of about 5600 and has grown rapidly over the last few years. The primary economic activities in the area are subsistence hunting and agriculture. Ixiamas has a commercial dairy farm; dogs from this town might therefore be more likely to be exposed to livestock pathogens. None of the towns (including Rurrenabaque) has a resident veterinarian, although rabies vaccination campaigns are periodically operated by the government. Ixiamas and Rurrenabaque both have small medical clinics and several pharmacies, but Tumupasa has neither.

We obtained permission from the municipal governments of each town prior to sampling and in both San Buenaventura and Ixiamas, a town resident was assigned to work with our team during sample collection. Although we tried to recruit dogs and cats from all of the neighborhoods in each village, no attempt was made to randomise the subjects. In fact, an effort was made to include hunting dogs, which are the most likely to have contact with wildlife. We went from house to house, discussing our project with the residents and asking for their consent to sample their pets. Dog owners were asked to provide information on age and vaccination status of their animal and to indicate whether the dog was purchased specifically for hunting. Unfortunately, conversations with residents revealed that although some dogs were designated as 'hunting dogs,' categorising dogs by hunting status was not realistic because most dogs not specifically identified as hunting dogs nevertheless participated in hunting activities. Only dogs 5 months of age and older were sampled, to ensure that the antibodies detected were endogenous and not of maternal origin (Greene, 1998). Using a muzzle and manual restraint,
blood was drawn from the jugular or cephalic vein. Three dogs required 1 mg of acepromazine maleate (Boehringer-Ingelheim, Ingelheim, Germany) intramuscularly to facilitate sampling.

Cats (*Felis catus*) are infrequently kept as pets in this area, consequently our sample size was smaller than for dogs. Owners were often unsure of the age of their cats, but all the cats we sampled had adult dentition, indicating that they were at least 6 months of age. All cats required chemical restraint for sampling. Each cat was given an intramuscular injection of 10 mg of a premixed combination of equal parts tiletamine hydrochloride and zolazepam (Telazon®, Fort Dodge Laboratories, Fort Dodge, IA) either alone or with 0.1 mg of acepromazine. On a few occasions, additional ketamine hydrochloride (Ketaset®, Fort Dodge Laboratories, Fort Dodge, IA) was administered to effect sufficient immobilisation. Blood was collected from the medial femoral or jugular vein. Sterile ophthalmic ointment was applied to the eyes; temperature, heart rate, respiratory rate and pulse quality were monitored every 3–5 min until the cats were ambulatory.

Serum was separated from the cellular components within a few hours of collection and stored on ice. Sera were transported to the USA on ice and then stored in a freezer at −70°C prior to submission to diagnostic laboratories in the USA and Switzerland. The diagnostic methodology for each test is listed in Table 3. *Dirofilaria immitis* and the feline leukaemia virus (FeLV) assays detect antigen in blood and so generally indicate an active or very recently cleared infection (Barr, 1996; Goodwin, 1998). The remainder of the tests detect antibodies to the disease agent in question.

Chi-square tests were used to determine if there were statistically significant differences in the prevalences of each disease between the three towns; in some cases, small sample sizes required use of the more conservative G-test. Analyses of variance (ANOVA) were used to test for interactions between vaccination status, town and positive titres to different pathogens.
Table 3. Methodologies and positive cut-off values used by laboratories to detect exposure to disease agents

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Methodology</th>
<th>Positive cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canine adenovirus</td>
<td>Antibody SN</td>
<td>1:4</td>
</tr>
<tr>
<td>Canine coronavirus</td>
<td>Antibody SN</td>
<td>1:8</td>
</tr>
<tr>
<td>Canine distemper virus</td>
<td>Antibody SN</td>
<td>1:8</td>
</tr>
<tr>
<td>Canine herpesvirus</td>
<td>Antibody SN</td>
<td>1:8</td>
</tr>
<tr>
<td>Canine parvovirus</td>
<td>Antibody HAI</td>
<td>1:10</td>
</tr>
<tr>
<td><em>Dirofilaria immitis</em></td>
<td>Antigen ELISA</td>
<td>P/N</td>
</tr>
<tr>
<td><em>Sarcopes scabiei</em></td>
<td>Antibody ELISA</td>
<td>P/N</td>
</tr>
<tr>
<td>Feline calicivirus</td>
<td>Antibody SN</td>
<td>1:8</td>
</tr>
<tr>
<td>Feline coronavirus</td>
<td>Antibody KELA</td>
<td>1:8</td>
</tr>
<tr>
<td>Feline herpesvirus</td>
<td>Antibody SN</td>
<td>1:8</td>
</tr>
<tr>
<td>Feline immunodeficiency virus</td>
<td>Antibody ELISA</td>
<td>P/N</td>
</tr>
<tr>
<td>Feline immunodeficiency virus confirmatory</td>
<td>Western blot</td>
<td>P/N</td>
</tr>
<tr>
<td>Feline leukaemia virus</td>
<td>Antigen ELISA</td>
<td>P/N</td>
</tr>
<tr>
<td>Feline panleukopenia virus</td>
<td>Antibody HAI</td>
<td>1:10</td>
</tr>
<tr>
<td><em>Leptospira interrogans</em></td>
<td>Antibody MA</td>
<td>1:100</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em> – canine</td>
<td>Antibody IHA</td>
<td>1:64</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em> – feline</td>
<td>Antibody KELA</td>
<td>1:48</td>
</tr>
</tbody>
</table>

Analyses were performed on serum at the Cornell University Veterinary Diagnostic Lab, Ithaca, NY except the *S. scabiei* assay, which was performed at Labor Laupeneck, Switzerland.

SN, serum neutralisation; HAI, haemagglutination-inhibition; ELISA, enzyme-linked immunosorbent assay; KELA, kinetic ELISA; IHA, indirect haemagglutination; MA, micro-agglutination; P/N, test scored as positive or negative.

RESULTS

Sufficient quantities of serum for at least one test were collected from 14 dogs and three cats from San Buenaventura, 13 dogs and five cats from Ixiamas and 13 dogs and six cats from Tumupasa. Twenty-nine dogs and three cats had been previously vaccinated against rabies; the remainder were either never vaccinated or had an unknown vaccination status. Five dogs were identified as purpose-bred hunting dogs, although many other dogs participated in hunting activities. The majority of dogs were between 6 months and 3 years of age.

Seroprevalence for each disease agent in dogs is given in Table 4. Antibodies to canine distemper virus (CDV) and canine parvovirus (CPV) were very common; over 90% of the dogs were positive. Canine adenovirus (CAV) antibodies were found in over three-quarters of the dogs, while canine herpesvirus (CHV) and canine coronavirus (CCV) were less common, each of the latter occurring in about one-third of the dogs sampled. Antibodies to one or two serovars of *Leptospira interrogans* were found in about one-fifth of dogs. These serovars were identified as *hardjo* and *canicola* (Table 5). Antibodies to *Toxoplasma gondii* were found in 62% of dogs, of which six (36%) had high titres (≥1:1024) indicative of active infection (Fig. 2).

Table 4. Number of dogs in each town with positive serological results for each pathogen over the number of dogs tested

<table>
<thead>
<tr>
<th>Town</th>
<th>Canine distemper virus</th>
<th>Canine herpesvirus</th>
<th>Canine adenovirus</th>
<th>Canine coronavirus</th>
<th>Canine parvovirus</th>
<th>Leptospirosis</th>
<th>Toxoplasmosis</th>
<th>Dirofilaria</th>
<th>Sarcoptes</th>
</tr>
</thead>
<tbody>
<tr>
<td>San Buenaventura</td>
<td>5/5 (100)</td>
<td>3/5 (60)</td>
<td>3/5 (60)</td>
<td>2/5 (40)</td>
<td>5/5 (100)</td>
<td>1/5 (20)</td>
<td>3/5 (60)</td>
<td>5/6 (83)</td>
<td>6/14 (43)</td>
</tr>
<tr>
<td>n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumupasa n (%)</td>
<td>8/9 (89)</td>
<td>2/9 (22)</td>
<td>5/9 (56)</td>
<td>3/9 (33)</td>
<td>7/9 (78)</td>
<td>2/9 (22)</td>
<td>6/9 (67)</td>
<td>4/10 (40)</td>
<td>12/13 (92)</td>
</tr>
<tr>
<td>Ixiamas n (%)</td>
<td>11/12 (92)</td>
<td>4/12 (33)</td>
<td>12/12 (100)</td>
<td>3/12 (25)</td>
<td>12/12 (100)</td>
<td>5/12 (42)</td>
<td>7/12 (58)</td>
<td>2/12 (17)</td>
<td>4/13 (31)</td>
</tr>
<tr>
<td>Total n (%)</td>
<td>24/26 (92)</td>
<td>9/26 (35)</td>
<td>20/26 (77)</td>
<td>8/26 (31)</td>
<td>24/26 (92)</td>
<td>8/26 (31)</td>
<td>16/26 (62)</td>
<td>11/28 (39)</td>
<td>22/40 (55)</td>
</tr>
</tbody>
</table>

The figures in brackets are the % of dogs that had a positive serological result for the pathogen being tested for.

Table 5. Titres of the five dogs from Ixiamas testing positive for exposure to two *Leptospira interrogans* serovars

<table>
<thead>
<tr>
<th>Individual</th>
<th>canicola</th>
<th>hardjo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog 16</td>
<td>1:3200</td>
<td>Neg</td>
</tr>
<tr>
<td>Dog 18</td>
<td>1:400</td>
<td>Neg</td>
</tr>
<tr>
<td>Dog 19</td>
<td>1:1600</td>
<td>Neg</td>
</tr>
<tr>
<td>Dog 23</td>
<td>Neg</td>
<td>1:800</td>
</tr>
<tr>
<td>Dog 27</td>
<td>1:400</td>
<td>1:100</td>
</tr>
</tbody>
</table>

Titres ≥1:1600 are considered suggestive of recent infection with that serovar, neg, negative.

Fig. 2. Frequency of titres to *Toxoplasma gondii* found in dogs using indirect haemagglutination. Titres ≥1:1024 are considered to be indicative of active or recent infection.
Table 6. Number of cats in each town with positive serological tests for each pathogen over number tested

<table>
<thead>
<tr>
<th>Town</th>
<th>Feline parvovirus</th>
<th>Feline leukaemia virus</th>
<th>Feline calicivirus</th>
<th>Leptospirosis</th>
<th>Toxoplasmosis</th>
<th>Dirofilaria</th>
</tr>
</thead>
<tbody>
<tr>
<td>San Buenaventura n (%)</td>
<td>3/3 (100)</td>
<td>0/3</td>
<td>3/3 (100)</td>
<td>1/3 (33)</td>
<td>3/3 (100)</td>
<td>0/3</td>
</tr>
<tr>
<td>Tumupasa n (%)</td>
<td>6/6 (100)</td>
<td>0/6</td>
<td>5/6 (83)</td>
<td>0/6</td>
<td>6/6 (100)</td>
<td>1/6 (17)</td>
</tr>
<tr>
<td>Iximas n (%)</td>
<td>5/5 (100)</td>
<td>1/5 (20)</td>
<td>5/5 (100)</td>
<td>0/5</td>
<td>4/5 (80)</td>
<td>0/5</td>
</tr>
<tr>
<td>Total n (%)</td>
<td>14/14 (100)</td>
<td>1/14 (7)</td>
<td>13/14 (93)</td>
<td>1/14 (7)</td>
<td>13/14 (93)</td>
<td>1/14 (7)</td>
</tr>
</tbody>
</table>

Antibodies to feline herpesvirus, feline coronavirus, and feline immunodeficiency virus were not found in any cats. The figures in brackets are the % of cats that had a positive serological result for the pathogen being tested for.

Dogs with high *T. gondii* titres were found in all three towns; one from San Buenaventura, three from Iximas and two from Tumupasa. Heartworm antigen was found in 39% of dogs and antibodies to *Sarcocystis scabies* were present in 55% of dogs.

All cats had antibodies to feline parvovirus and over 90% had antibodies to feline calicivirus (Table 6). Antibodies to feline immunodeficiency virus, feline coronavirus, and feline herpesvirus were not detected in any cats (n = 14). One cat was positive for FeLV antigen. *Leptospira interrogans* antibodies were found in one cat; these were against the *canicola* serovar. Most cats (93%) had antibodies to *T. gondii*, although none had titres high enough to suggest recent infection (Fig. 3). One cat was positive for heartworm antigen.

Heartworm disease in dogs was more common in San Buenaventura (G-test; *P* = 0.0198), while sarcoptic mange in dogs was more common in Tumupasa (G-test; *P* = 0.0016). No other significant differences were found (see Table 4). There were also no significant differences between villages in the expected prevalences of any of the pathogens between dogs that were and were not vaccinated against rabies. Sample sizes were insufficient to explore differences between rabies-vaccinated and unvaccinated cats.

DISCUSSION

This survey indicates that the potential for spillover of pathogens from domestic to wild carnivore populations in this region of Bolivia is real. Exposure to several disease agents was documented at relatively high prevalences, including CDV, CPV, feline parvovirus, and *T. gondii*. It is possible for spillover of these pathogens from regionally large domestic populations to less dense wild populations to result in disease epidemics in populations of endangered carnivores (e.g. Gascoyne et al., 1993; Roeke-Parker et al., 1996; Laumen et al., 1998). Whether this will occur depends on the exposure and immunological status of the wild populations, which we did not investigate, but our evidence does show that domestic carnivores are exposed to disease agents to which wild species are susceptible.

Of the disease agents we identified, CDV and CPV are perhaps of greatest concern. CDV is a generalist pathogen that has been responsible for serious epidemics and population declines in wild carnivores (Alexander & Appel, 1994; Roeke-Parker et al., 1996; Cleveland et al., 2000; Kennedy et al., 2000). It is spread most commonly by close contact between individuals via aerosolised respiratory secretions, although it can remain viable in the environment for hours or even weeks in cool temperatures (Greene & Appel, 1998: see Table 1). The climate of the Amazon basin probably precludes environmental survival, leaving direct contact as the probable means of transmission. CPV, however, is transmitted via the faecal–oral route and can survive for months in the environment (Mann et al., 1980; Hoskins, 1998: see Table 1). Although natural parvovirus infections of wild canids have been reported (Hoskins, 1998; Peterson et al., 1998), the potential effects on wild populations are poorly understood (Mech & Goyal, 1993; Johnson, Boyd & Fletscher, 1994).

Over 90% of the domestic dogs we sampled were seropositive for CDV and CPV, indicating that these viruses are endemic in this population (Table 4). The level of prevalence found in this study differs sharply from that found in a recent study in the Amazon basin of Brazil. Using similar methodologies, Courtenay, Quinnell & Chalmers (2001) found CDV and CPV antibodies in only 9% and 13%, respectively, of 23 dogs. Those authors

![Fig. 3. Toxoplasma gondii titre for each cat sampled, using kinetic enzyme-linked immunosorbent assay (ELISA). Titres 1:750 are considered to be indicative of active or recent infection. Open bars, Tumupasa; cross-hatched bars, Iximas; solid bars, San Buenaventura.](image-url)
also sampled sympatric crab-eating foxes (*Cerdocyon thous*). Despite a large amount of peridomestic activity by the foxes, documented with radiotelemetry, none of the 24 foxes tested had antibodies to either virus (Courtenay et al., 2001). While these findings might seem reassuring, it is not surprising given the low prevalence of these diseases in that domestic dog population.

The other canine viruses we investigated occurred at moderate to high seroprevalence, but are less likely to be of epidemic significance in wild carnivores (Holzman, Conroy & Davidson, 1992; Hoskins, 1998; Woods, 2001). The presence of these viruses is noteworthy because co-infection with one of these agents and CDV or CPV may cause increased morbidity and mortality and may prolong viral shedding in infected canids (Evermann et al., 1980; Pratelli et al., 1999, 2001).

The results of the feline virus survey were more encouraging, although our sample size was small. The lower prevalence of pathogen exposure in cats may be a result of their apparently low density in this region, or their less social nature. Antibodies to three viruses were not found at all, including feline immunodeficiency virus and feline coronavirus, two of the more worrisome pathogens for wild feline populations. We cannot conclude that these viruses are not present in the domestic cat population, but even if every family owned one cat—highly unlikely given the unpopularity of cats in the region—we can be 95% confident that these viruses are present in less than 20% of the population (Cannon & Roe, 1982). In addition, the single positive result for FeLV antigen was considered a weak positive, indicating the need for a retest in 3–4 weeks to confirm infection. Because a retest was not possible, we cannot confirm the presence of this virus in this animal. Antibodies to feline panleukopaenia were found in all 14 cats (Table 6). This virus is of conservation concern because it can cause clinical disease in felines, procyonids and mustelids and it persists in the environment (Greene, 1998a; Stein et al., 2000: Table 1). Infection with feline calicivirus, a pathogen known to cause disease in wild felids (Sabine & Hyne, 1970; Kodai et al., 1997), was also widespread in the cats we sampled. In most cases, this virus causes high morbidity but low mortality in domestic cats (Gaskel & Dawb, 1998); however, more virulent strains that cause high mortality do exist (Pedersen et al., 2000).

A striking result was the high seroprevalence of *T. gondii* exposure in dogs and the large number of dogs with titres suggestive of active infection (Fig. 2). Although most cats had antibodies (Table 6), their titres were generally quite low (Fig. 3). This is not surprising, since cats are the definitive host for this parasite. They are probably infected as kittens and may have either partial or complete immunity to re-infection. Both dogs and cats are most probably infected via the ingestion of raw meat, including rodents and other wild mammals. Humans are also commonly exposed by this route, but children may be more at risk for infection by ingestion of contaminated soil (Tenter, Heiroth & Weiss, 2000). In these semi-rural areas of Bolivia, cats are allowed to roam freely and indoor litter boxes are not provided. Cats are therefore defaecating in soil where children can be readily exposed.

Leptospirosis is a zoonotic bacterial disease considered by some to be a re-emerging infectious disease in the tropics (Branding-Bennett & Pinheiro, 1996). Many serovars exist and serological testing often reveals some degree of cross-reactivity among the different serovars. Only the serovars *hardjo* and *canicola* were recognised in our samples (Table 5). Dogs are the primary host of *canicola*, so this serovar is probably endemic in this area (Greene, Miller & Brown, 1998). The *serovar hardjo* is most often found in cows and other hoofed animals, including wild bovids. Interestingly, most positive dogs were found in Ixiamas, which has a large dairy farm (Tables 4 & 5). However, the serovar *canicola* was found in four out of the five Ixiamas dogs with titres, so the presence of cattle may be coincidental. Two dogs from Ixiamas had titres ≥1600, which is considered suggestive of active or recent infection. The few dogs with positive titres from San Buenaventura and Tunupasa had titres of 100, all with serovar *hardjo*. Unfortunately, our cross-sectional data are insufficient to document active infection; paired titres separated by 2–3 weeks would be necessary. The low seroprevalence of *Leptospira* found in domestic cats is probably related to their smaller ranging patterns and their natural dislike of standing water. Data concerning human *Leptospira* infection from the region at this time would be helpful in determining if dogs could serve as sentinels for human and wildlife infections.

Heartworm disease caused by *Dirofilaria immitis* is found worldwide in tropical, subtropical and temperate climates, although it has a higher prevalence in warmer regions (Anderson, 2001). In general, wherever heartworm disease occurs in domestic dogs, it also occurs in domestic cats, but at a lower prevalence (Ralston, Stemme & Guerrero, 1998). It has been diagnosed in many wild carnivore species, including canids, felids, mustelids and pinnipeds (Anderson, 2001). In the present study, we found a significantly higher prevalence of this disease in dogs from San Buenaventura, which is the only community we sampled that is located near a large body of water (Table 4). Foci of heartworm disease around rivers have been previously reported (Okoniewski & Stone, 1983) and are probably related to proximity to mosquito breeding areas. Our results show, however, that even in Tunupasa and Ixiamas, which are more distant from a major river, the disease is present.

Over half of the dogs tested were positive for *Sarcoptes scabiei* antibodies (Table 4). This is a zoonotic parasite, which causes clinical disease in free-ranging Bolivian carnivores (Deem et al., 2002; C. V. Fiorello, unpublished results) and disease epidemics resulting in population declines in other carnivore species (Lindstr"{o}m et al., 1994; Pence & Windberg, 1994; Shibata & Kawamichi, 1999). Antibodies to this parasite wane and are not detectable by about 2 months after resolution of infection (Arlian et al., 1996; Lower et al., 2001). The presence of antibodies therefore indicates a current or very recent infection, unlike antibodies to most viral pathogens, which persist for years (Greene, 1998b). In Tunupasa, all but one of
the dogs tested were positive, an alarming statistic. Why 
the prevalence of mange was higher in Tumupasa is not 
clear. The overall health of the dogs from Tumupasa did 
not seem to differ from that of the other two towns and no 
other disease agents were found disproportionately more 
in Tumupasa.

The use of serology, which documents infection but not 
disease, may be viewed as a limitation or an advantage of 
this study. The stratified prevalence survey employed here 
is commonly used to estimate the frequency of infection in 
a population (Greiner & Gardner, 2000a). Disadvantages 
to serology include the possibility of cross-reaction of 
reagents to antibodies against other agents (false positives) 
as well as the (less likely) possibility for false negatives. 

The presence of antibodies is dependent on numerous 
factors, including the immune status of the host, the 
timing of the initial infection and the outcome of the 
infection (Greiner & Gardner, 2000a). The detection of 
antibodies is further dependent on the accuracy of the 
test, the presence of cross-reacting substances in the host, 
and proper test execution (Barr, 1996; Greiner & Gardner, 2000a). Even if the test is carried out 
correctly, determining what constitutes a positive result 
may be somewhat arbitrary, since cut-off values often 

varies as well. For example, antibodies to feline immunodeficiency virus found 
in the serum of an adult cat using a Western blot nearly always 
indicates that the cat is currently infected with the virus 
(Barr, 1996). However, antibodies to feline coronavirus found 
in the serum of a cat by any method indicates that 
was probably infected by a feline coronavirus, or 
related coronavirus, at some point in the past (Barr, 
1996). Neither positive result tells you with certainty if 
the cat in question is, or will become, clinically ill, nor 
does it tell you which viral strain infected the cat or if 
that strain was particularly virulent. Paired titres from 
the same individual at different times would have been useful 
to determine the presence and timing of infection, but 
this was not possible for this study. While serology has 
its drawbacks, it provides information on the exposure 
history of a given individual, so that it is not necessary 
to sample an animal during the short period of clinical 
disease to document infection.

At least five of the dogs we sampled were purchased 
or bred specifically for hunting and these dogs go into 
the forest regularly. Even those dogs that were not 
considered to be hunting dogs by their owners sometimes 
accompanied them hunting and all dogs were unconfined 
and free to roam at will. Direct contact with wildlife did 
occur; owners occasionally pointed out scars or healing 
wounds on their dogs that they attributed to encounters 
with peccaries (although when questioned, all of the 
owners admitted that they had not actually witnessed 
the encounter). Even if hunting dogs infrequently have 
direct contact with wild carnivores, they are urinating 
and defaecating in the forest where wildlife may be exposed. 

Because many carnivore species use urine and faeces to 
mark their territories, they are likely to investigate any dog 
urine and faeces they encounter in the forest. Given that 
dog and cat pathogens can and do cross taxonomic lines, 
this situation allows for disease transmission. Dogs from 

a large population in which many diseases are endemic 

forest and may contact susceptible wildlife species, 
which may have little or no immunity to these pathogens. 

In the dense rainforest of the Amazon basin, outbreaks 
of infectious diseases in carnivores may go unnoticed 
or unrecognised, leading to population declines of these 

already threatened species.

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