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Detection of Rabies Virus Antibodies in Brazilian Free-Ranging Wild Carnivores

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ABSTRACT: *Rabies virus* is a pathogen of major concern in free-ranging wild carnivores in several regions of the world, but little is known about its circulation in Brazilian wild carnivores. Sera from 211 free-ranging wild carnivores, captured from 2000 to 2006 in four locations of two Brazilian biomes (Pantanal and Cerrado), were tested for rabies antibodies. Twenty-six individuals (12.3%) had neutralizing antibody titers ≥0.10 IU/ml. The four sampled locations had antibody-positive animals, suggesting that *Rabies virus* circulates in all of these regions. Results underscore the risk posed by rabies for conservation of Brazilian carnivores and the possibility of the animals acting as reservoirs for the *Rabies virus*.

Key words: Brazil, conservation, free-ranging wild carnivores, public health, rabies, serology.

Domestic dogs (*Canis familiaris*) have historically been considered the main reservoir species of rabies throughout the world. However, with successful management programs focused on controlling the *Rabies virus* in dogs, wildlife have overtaken domestic animals as the most important *Rabies virus* reservoirs in Europe and North America (Smith and Baer, 1988; Wandeler, 1988). Similarly, in Latin America, where efforts to control rabies in humans have been focused on vaccinating dogs and cats (*Felis catus*), the sylvatic cycle, maintained in a wide variety of mammal hosts, is emerging as a new issue for public health (Bernardi et al., 2005).

Free-ranging wild canids have been diagnosed as rabid in the northeastern Brazilian states (Bernardi et al., 2005), and a cycle in these animals that is independent of the cycle in domestic dogs has been reported (Carnieli et al., 2008). In that study, the crab-eating fox (*Cerdocyon thous*) was genetically identified as the main reservoir of *Rabies virus* among wild canid species in northeastern Brazil.

In addition to its relevance for public health, rabies represents a potential threat to the conservation of free-ranging wild carnivores. This has been demonstrated by the extensive mortality caused by rabies outbreaks in isolated populations of the most endangered wild canid, the Ethiopian wolf (*Canis simensis*; Sillero-Zubiri et al., 1996). The purpose of this study was to assess the exposure to *Rabies virus* of free-ranging wild carnivores in four locations of two Brazilian biomes, Pantanal and Cerrado.

Serum samples were obtained from the Biological Samples Bank of the National Research Center for the Conservation of Natural Predators (CENAP) of the Chico Mendes Institute for the Conservation of Biodiversity (ICMBio), a Brazilian Governmental agency. Samples were collected from free-ranging wild carnivores captured as part of ecologic studies in four locations in Pantanal and Cerrado (Fig. 1), Brazil, from 2000 to 2006 and stored at −70 C. Numbers of individuals of each species captured and locations of capture are specified in Table 1.

During immobilization, the physical condition and any clinical signs of rabies

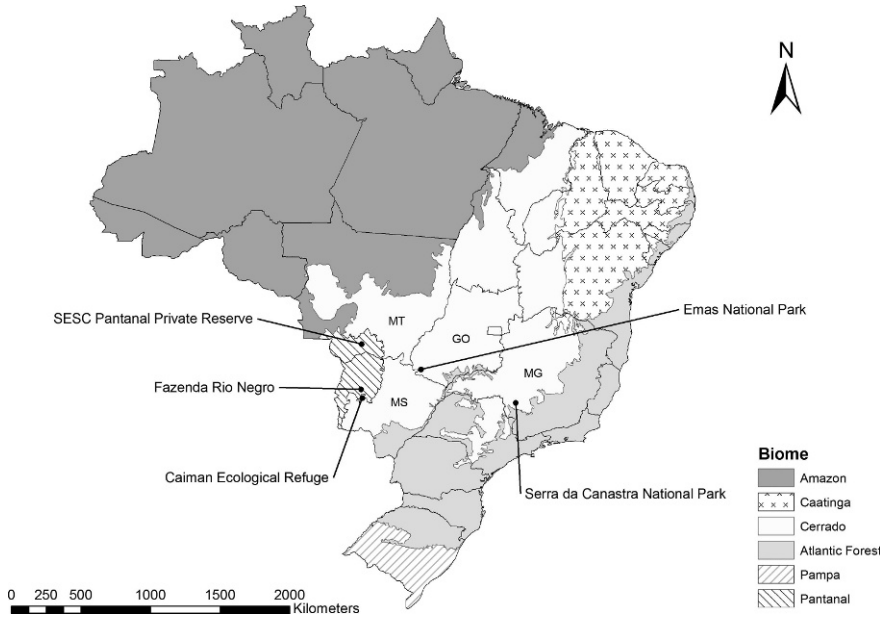

FIGURE 1. Map of Brazil and its biomes. The named locations are sites where free-ranging wild carnivores were captured and tested for antibody to *Rabies virus*. MG=Minas Gerais; GO=Goias; MT=Mato Grosso; MS=Mato Grosso do Sul.

were assessed by clinical examination. Seventy-three individuals from Emas National Park (ENP) and 24 from Serra da Canastra National Park (SCNP) were fitted with radio collars and monitored using radiotelemetry. Monitoring of individuals was related to the objectives of the ecologic studies and not to objectives of our study. Samples were tested by simplified fluorescent inhibition microtest (Favoretto et al., 1993) at Instituto Pasteur (São Paulo, São Paulo, Brazil). Animals with neutralizing antibody titers ≥0.10 IU/ml were considered positive. Serology was performed on all samples in 2007. Because results were only known after capture and sampling were complete, it was not possible to resample individuals based on serologic results.

TABLE 1. Number of carnivores sampled and tested for the rabies-virus antibody by species and site in Brazil.[a]

Species	ENP	SESC Pantanal	SCNP	Southern Pantanal	Total
Cerdocyon thous	26	41	1	—	68
Chrysocyon brachyurus	59	8	24	—	91
Speothos venaticus	—	1	—	—	1
Lycalopex vetulus	1	—	—	—	1
Procyon cancrivorus	—	13	—	—	13
Panthera onca	2	—	—	12	14
Leopardus pardalis	6	4	—	—	10
Puma concolor	1	7	—	—	8
Leopardus colocolo	5	—	—	—	5
Total	100	74	25	12	211

[a] ENP=Emas National Park; SESC=Serviço Social do Comércio; SCNP=Serra da Canastra National Park.

TABLE 2. Carnivores considered positive for exposure to *Rabies virus* at four locations in Brazil, their date and location of capture, and their neutralizing-antibody titer.

Species	Date of capture	Location[a]	Titer (IU/ml)
Chrysocyon brachyurus	15 May 2005	ENP	0.10[b]
Chrysocyon brachyurus	19 August 2004	ENP	0.27[b]
Chrysocyon brachyurus	27 September 2004	ENP	0.10[b]
Chrysocyon brachyurus	13 June 2004	ENP	0.10[b]
Chrysocyon brachyurus	25 March 2004	ENP	0.20[b]
Chrysocyon brachyurus	31 July 2003	ENP	0.10[b]
Chrysocyon brachyurus	18 May 2003	ENP	0.10[b]
Chrysocyon brachyurus	4 May 2003	ENP	0.10[b]
Chrysocyon brachyurus	1 November 2002	ENP	0.13[b]
Chrysocyon brachyurus	23 September 2002	ENP	0.27[b]
Chrysocyon brachyurus	7 July 2001	ENP	0.13[b]
Chrysocyon brachyurus	24 July 2004	SCNP	0.27[b]
Chrysocyon brachyurus	22 November 2005	SCNP	0.13[b]
Cerdocyon thous	25 November 2005	ENP	0.27
Cerdocyon thous	16 July 2006	ENP	0.10
Cerdocyon thous	11 August 2003	Southern Pantanal	0.10
Cerdocyon thous	6 November 2004	SESC Pantanal	0.20
Speothos venaticus	2 August 2006	SESC Pantanal	1.60
Panthera onca	3 December 2005	Southern Pantanal	0.13
Panthera onca	26 February 2006	Southern Pantanal	0.10
Panthera onca	5 June 2006	Southern Pantanal	0.10
Leopardus pardalis	17 April 2002	ENP	0.13[b]
Leopardus pardalis	10 July 2005	ENP	0.10[b]
Puma concolor	20 March 2003	SESC Pantanal	0.10
Leopardus colocolo	3 May 2005	ENP	0.13
Procyon cancrivorus	22 September 2005	SESC Pantanal	0.27

[a] ENP=Emas National Park; SESC=Serviço Social do Comércio; SCNP=Serra da Canastra National Park.

[b] Monitored using radiotelemetry.

Among the 211 wild carnivores tested, 26 had titers of rabies-neutralizing antibodies ≥0.10 IU/ml for a frequency of 12.3%. Titers and the locations of capture for positive animals are shown in Table 2. The frequencies of exposed specimens for each region were 16% (16/100) for ENP, 5.4% (4/74) for the Serviço Social do Comércio (SESC) Pantanal, 8% (2/25) for SCNP, and 33.3% (4/12) for Southern Pantanal, Brazil. Specimen frequencies for each exposed species were 14.3% (13/91) for maned wolves (*Chrysocyon brachyurus*), 5.8% (4/69) for crab-eating foxes, 100% (1/1) for bush dogs (*Speothos venaticus*), 0% (0/1) for hoary foxes (*Lycalopex vetulus*), 23.1% (3/13) for jaguars (*Panthera onca*), 20% (2/10) for ocelots (*Leopardus pardalis*), 12.5% (1/8) for pumas (*Puma concolor*), 20% (1/5) for pampas cats (*Leopardus colocolo*), and 7.7% (1/13) for crab-eating raccoons (*Procyon cancrivorus*). To our knowledge, this is the first report of exposure of free-ranging maned wolves, bush dogs, jaguars, ocelots, pumas, and pampas cats to *Rabies virus*.

None of the positive animals presented any clinical signs of rabies. Thirteen positive maned wolves from ENP and two from SCNP were monitored continuously for periods of 6–51 mo. During that time, no abnormal behaviors, lack of muscular coordination, or any typical signs of rabies were noted. The two positive ocelots were monitored for 15 and 24 mo, respectively, without any clinical signs of rabies. Similarly, none of the 80 radio-collared animals that were negative for serology and were monitored for variable

periods presented any notable clinical signs of rabies.

Several investigators that have assessed exposure of free-ranging wildlife to *Rabies virus* used a cutoff of 0.50 UI/ml (Almeida et al., 2001; Deem et al., 2004). The World Health Organization (WHO, 1992) recommends that cutoff for evaluating human and animal response to vaccination, not for natural exposure to the virus. Even the presence of lower levels of detectable antibodies may represent previous natural exposure to *Rabies virus* (Rosatte and Gunson, 1984). However, we cannot discard the possibility that the lower levels of antibody are the result of nonspecific inhibition.

In this study, the bush dog had the highest titer of rabies-neutralizing antibodies among the tested carnivores. However, because only one individual was sampled, it is difficult to draw conclusions about the importance of bush dogs for rabies maintenance in the sylvatic cycle. That individual was captured in a rural community. Thus, there is a high likelihood that exposure originated from domestic animals, and there is a risk of transmission from the bush dog to humans or other domestic animals. Because bush dogs are vulnerable to extinction (IUCN, 2004), these results have implications for the conservation of the species.

Larger numbers of maned wolves and crab-eating foxes were sampled, and these species had a relatively high frequency of exposure to the virus, suggesting their potential role as reservoirs for the *Rabies virus*. Fewer jaguars, pumas, ocelots, pampas cats, crab-eating raccoons, and hoary foxes were sampled, making it difficult to make inferences about their role in *Rabies virus* transmission. However, apart from the hoary fox, all tested species had rabies antibody-positive individuals, suggesting that they had been exposed to the virus. Although wild canids and procyonids are known to serve as *Rabies virus* reservoirs elsewhere in the world, no wild felids are known to be reservoirs. Hence, it is difficult to draw conclusions about the importance of these wild cats in the circulation of *Rabies virus*. This subject deserves more focused study in the future. Nevertheless, our results, along with the high rabies antibody titer found in an oncilla (*Leopardus tigrinus*) in Bolivia (Deem et al., 2004), show that rabies may be a threat to the conservation of these felids, as well as to other exposed South American carnivores, especially endangered species.

The most common transmission route for *Rabies virus* is through contaminated saliva inoculated through the bite of an infected animal during the phase of viral excretion. Oral infection of carnivores via ingestion of contaminated carcasses has also been demonstrated (Ramsden and Johnston, 1975), but the importance of this route in free-ranging wildlife has seldom been explored. Although ingestion of contaminated carcasses may lead to lethal infection, it is possible that some animals respond only with the production of neutralizing antibodies (Ramsden and Johnston, 1975).

A *Rabies virus* strain adapted to a specific species may be less pathogenic and more immunogenic to phylogenetically distant species, resulting in a higher proportion of animals of that species with rabies-neutralizing antibodies in the regions where these strains occur (Rosatte and Gunson, 1984). In Brazil, *Rabies virus* isolates associated with vampire bats (*Desmodus rotundus*) have been reported in several areas, including the states of Mato Grosso, Mato Grosso do Sul, Goias, and Minas Gerais (Ito et al., 2001; Bernardi et al., 2005), where our samples were obtained. Therefore, there is considerable chance that some of the antibody-positive animals in this study were exposed to a vampire bat–related strain of *Rabies virus* or were exposed via the oral route, with a low viral load.

The Southern Pantanal had the highest proportion of exposed animals (33.3%), followed by ENP (16%), SCNP (8%), and

the SESC Pantanal (5.3%). Although these results may be a consequence of higher rates of exposure in the Southern Pantanal and ENP regions, they also may reflect the occurrence of *Rabies virus* strains for which wild carnivores are not natural reservoirs, or they may reflect alternative routes of transmission. Sample-size variation among locations makes comparison among the proportions difficult. Nevertheless, our results indicate that rabies circulates in all of these regions.

The circulation of *Rabies virus* in free-ranging wild carnivores has received attention from the Brazilian government and researchers in the past few years. Instances of aggression toward humans by rabid wild canids in northeastern Brazil justify that focus. Our results show that wild carnivores in the states of Mato Grosso, Mato Grosso do Sul, Goias (representing the central-western region), and Minas Gerais (southeastern region) are also exposed to *Rabies virus*, reinforcing the importance of serology as an adjunct surveillance tool for rabies activity in wild animals (Almeida et al., 2001). A surveillance plan should be developed for these regions and should include the capability to identify the strains circulating among the wild carnivores. Testing the brains of road-killed animals collected in a structured and systematic manner could be useful. Surveillance results can inform control efforts for *Rabies virus* circulation among wild animals in these regions.

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