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Rabies Virus Exposure of Brazilian Free-ranging Wildlife from Municipalities without Clinical Cases in Humans or in Terrestrial Wildlife

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ABSTRACT: Rabies is a zoonosis that causes thousands of animal and human deaths worldwide. Serological studies provide information concerning rabies virus circulation among animals and humans. We evaluated the circulation of the rabies virus in wildlife in nine municipalities of São Paulo State, Brazil. We took blood samples from 27 terrestrial animals of nine different mammalian species in locations without cases of rabies in human and wild terrestrial mammals. Sera were tested with the use of the rapid fluorescent focus inhibition test (RFFIT) for the detection of rabies virus–neutralizing antibodies (RVNA). The RFFIT was positive in 100% of the samples, with many (81.48%) showing protective titer levels (>0.5 IU/mL) with other samples (18.52%) showing titers representing exposure (<0.5 IU/mL). We report RVNA in novel species (e.g., *Alouatta caraya* and *Tapyrus terrestris*). Wild animals were exposed to rabies virus in municipalities without a history of human rabies cases, which demonstrated a need for research to understand the role of these animals in the circulation and transmission of the disease.

Key words: Brazil, public health, viral circulation, wildlife, zoonosis.

Rabies is a worldwide zoonosis caused by a *Lyssavirus* genus with the wildlife acting as reservoirs (Jorge et al. 2010). The sylvatic cycle is maintained in a wide variety of mammalian hosts, and the emergence of rabies from wildlife is a concern; the epidemiological chain of rabies in Brazil includes Carnivora, and Chiroptera as the principal reservoirs (Rocha et al. 2017). Rabies in wildlife has been diagnosed in 204 animals (1990–2005) in Brazil: 173 in the crab-eating fox (*Cerdocyon thous*), 25 in common marmoset (*Callithrix jacchus*), and six in crab-eating raccoon (*Procyon cancrivorous*), and 65% (82 cases, 2002–12) of human rabies was acquired from wildlife (Rocha et al. 2017).

To get information for strategies of control and prevention of rabies in wildlife and humans, our aim was to evaluate the viral circulation of rabies virus in wildlife in locations without rabies cases in human and wild terrestrial mammals. Blood samples were collected (June 2007–August 2008) from 27 free-ranging wild animals after they were hit by cars on highways in nine municipalities (São José do Rio Preto, Catanduva, Novo Horizonte, Olímpia, José Bonifácio, Promissão, Lorena, Jales e Nhandeara) of São Paulo State, Brazil. Rapid fluorescent focus inhibition test was performed. The final titer was considered as the reciprocal of the last dilution, in which 50% virus neutralization was observed (Smith et al. 1996). Titers $\geq$0.5 IU/mL were considered protective, and titers $\leq$0.5–0.1 IU/mL were considered to be due to viral exposure (WHO 2013).

All tested animals had titers of rabies virus–neutralizing antibodies (RVNA; Table 1) demonstrating the importance of wildlife as a potential source of human rabies (Rocha et al. 2017). The frequencies of titer of viral protection and exposure for each species (Table 1 and Fig. 1) demonstrated circulation of rabies virus in the state of São Paulo. Our study used two cut-off points because standard cut-off values for wildlife are often not known. Investigators assessing exposure of free-ranging wildlife to rabies virus use a cutoff of 0.50 IU/mL (Almeida et al. 2001), whereas the World Health Organization recommends this value for evaluating hu-

TABLE 1. Free-ranging animals with rabies virus–neutralizing antibodies from municipalities in São Paulo State, Brazil, with and without cases of rabies in wild terrestrial mammals and in humans. Titers ≥0.5 IU/mL were considered protective against rabies; titers <0.5 IU/mL (bold) were considered to be evidence of rabies exposure.

Species (common name)	Municipalities[a]	Anti-rabies titer (IU/mL)	Domestic animal or bat[b] rabies cases, 2007–15
Alouatta caraya (black howler)[c]	JB	1.3	Bovine (one case, 2014)
	JB	1.0	Bovine (one case, 2014)
	JB	1.3	Bovine (one case, 2014)
	SJRP	1.3	NVB (two cases, 2013 and 2014)
	NH	0.7	None
Cebus apella (tufted capuchin)[d]	NH	1.3	None
Pseudalopex vetulus (hoary fox)	JB	1.0	Bovine (one case, 2014)
	JB	0.5	Bovine (one case, 2014)
	O	**0.3**	NVB (two cases, 2011 and 2014); VB (one case, 2012)
	L	1.3	None
	L	1.3	None
	C	0.5	None
Cerdocyon thous (crab-eating fox)	N	**0.3**	None
	P	1.0	None
	SJRP	1.3	NVB (two cases, 2013 and 2014)
Chrysocyon brachiurus (maned wolf)[c]	O	1.0	NVB (two cases, 2011 and 2014); VB (one case, 2012)
	SJRP	1.3	NVB (two cases, 2013 and 2014)
	J	1.3	None
	J	0.66	None
Hydrochaeris hidrochaeris (capybara)	SJRP	1.3	NVB (two cases, 2013 and 2014)
	SJRP	1.3	NVB (two cases, 2013 and 2014)
	SJRP	1.3	NVB (two cases, 2013 and 2014)
	SJRP	1.0	NVB (two cases, 2013 and 2014)
Puma concolor (cougar)[c]	N	**0.26**	None
	N	**0.33**	None
Leopardus pardalis (ocelot)	SJRP	1.3	NVB (two cases, 2013 and 2014)
Tapyrus terrestres (South American tapir)[c]	SJRP	**0.26**	NVB (two cases, 2013 and 2014)

[a] JB = José Bonifácio; SJRP = São José do Rio Preto; NH = Novo Horizonte; O = Olímpia; L = Lorena; C = Catanduva; N = Nhandeara; P = Promissão; J = Jales.

[b] NVB = nonvampire bat; VB = vampire bat (*Desmodus rotundus*).

[c] Population classified as Vulnerable (International Union for Conservation of Nature 2016).

[d] Population classified as Declining (International Union for Conservation of Nature 2016).

man/animal response to vaccination (WHO 2013). Even the presence of lower levels of detectable antibodies may represent previous natural exposure to the rabies virus and the exclusion of animals with lower titers may not be appropriate (Jorge et al. 2010).

According to the National Health System (SVS) in Brazil, rabies in wildlife must be reported to allow for the implementation of their complete prophylaxis scheme (Rocha et al. 2017). During our collection period (2007–08), there was one case of rabies among herbivores in the state of São Paulo, and other Brazilian states have had cases of rabies in humans transmitted by wildlife (SVS 2016). After our collection period in the sampled cities, rabies cases were recorded in cattle and bats (Table 1). In São Paulo State, there were no rabies cases between 2007 and 2016 in humans (SVS 2016). However, animal rabies

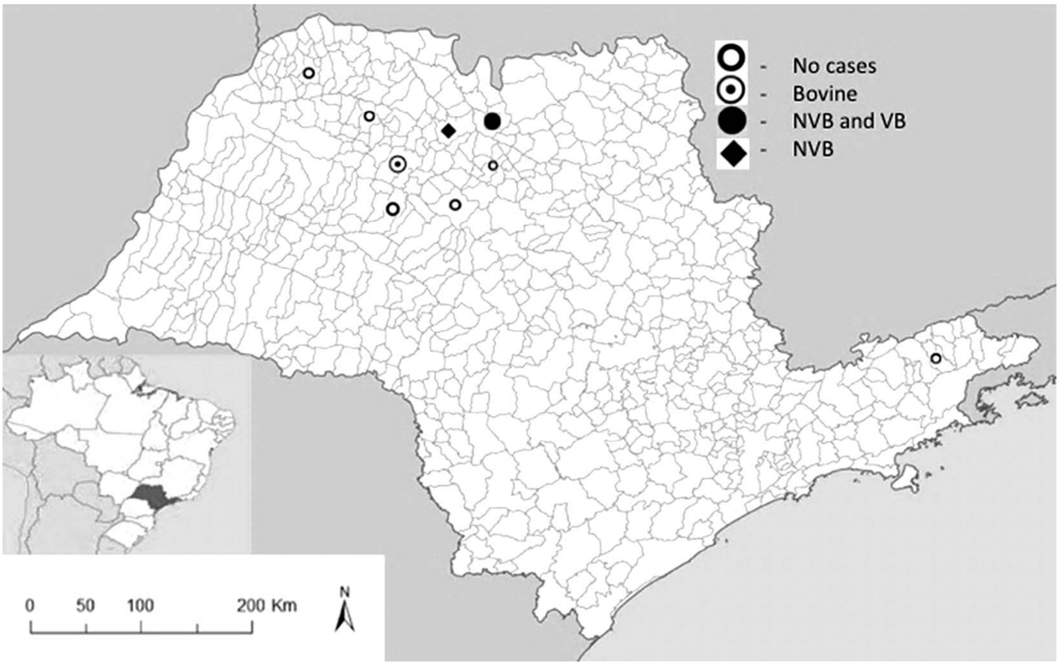

FIGURE 1. Locations of the municipalities in São Paulo State, Brazil where the collections of wild animals were made in order to test for rabies antibodies, indicating whether or not they were associated with clinical cases of rabies in animals. NVB=nonvampire bats; VB=vampire bats (*Desmodus rotundus*).

cases occurred in other municipalities that were not sampled in our study: dog and feline in 2012, 2014; dog only in 2015; and nonvampire bats in 2016 (SVS 2016). In Brazil, animal rabies is endemic, although the highest numbers of cases occur in production animals. Within São Paulo State there were no human rabies cases, only cases in domestic feline (*Felis catus*), nonvampire bats, vampire bats (*Desmodus rotundus*), wild canids, and herbivores (SVS 2016; Rocha et al. 2017). In São Paulo State, the major cases of rabies are in cattle (*Bos taurus*) and horses (*Equus caballus*) transmitted by vampire bats (Macedo et al. 2010) confirming the interaction of these species in maintaining viral circulation.

The most representative group of wildlife with rabies in Brazil are bats. In addition, wild canids, the crab-eating fox and the hoary fox (*Pseudalopex vetulus*), and marmosets are regularly found to have rabies (Rocha et al. 2017). Among the six species of wild canids in Brazil, the crab-eating fox is the primary transmitter of rabies in the northeast (Carnieli et al. 2008). In our study, in São Paulo state (southeast region), 59% of the rabies-positive wild animals were carnivores showing the importance of these animals in the terrestrial rabies cycle (Rocha et al. 2017).

From 211 free-ranging wild carnivores tested from two Brazilian biomes, 26 individuals (12.3%) had RVNA $\geq$0.10 IU/mL; all locations had antibody-positive animals, suggesting circulation of virus, and the risk posed by rabies for conservation of Brazilian carnivores (Jorge et al. 2010). This was evidenced by mortality caused by rabies in the Ethiopian wolf (*Canis simensis*; Sillero-Zubiri et al. 1996). In both in the study by Jorge et al. (2010) and our own study, the wild canids: maned wolves (*Chrysocyon brachyurus*), crab-eating foxes, hoary foxes, cougars (*Puma concolor*), and ocelots (*Leopardus pardalis*) were rabies positive, suggesting their potential role as reservoirs. However, it is difficult to draw conclusions about the wild cats. For example, serum from jaguars (*Panthera onca*) had 0.10 IU/mL of RVNA, suggesting exposure (Furtado et al. 2013). In our study, felines had RVNA levels indicative of exposure and protection (Table 1). The transmission of rabies via predation seems a more likely route in this study to explain the exposure, however, we cannot infer the precise source of infection or the mode of transmission. The protective level titers (>0.5 IU/mL) could be due to a second exposure through oral transmission, because this type of transmission results in a long-lasting specific humoral response that increases immunity (Zhang et al. 2008). Regarding the conservation of species, although some carnivore populations are not considered to be threatened (Table 1), the transmission potential of these animals should be considered.

Monkeys are fourth in order of importance in transmitting rabies to humans; the marmosets (*Callithrix* spp.) and capuchins (*Cebus* spp.) are most commonly involved in transmission (Rocha et al. 2017). We reported the same species, the tufted capuchin (*Cebus apella*), with protective RVNA and found a new species of nonhuman primate, the black howler monkey (*Alouatta caraya*) as serologically positive, which may suggest a new participant in the rabies cycle. Circulation of rabies virus in capuchin monkeys seems to be important in São Paulo State (Machado et al. 2012) and the marmosets maintain a unique variant of rabies virus in northeastern Brazil (Rocha et al. 2017), although without viral isolation or sequences, it is not possible to determine the virus source responsible for the immune response in capuchin monkeys.

Environmental areas have been reduced and fragmented in Brazil, where capuchin monkeys have been living, and where this species has been exposed to rabies virus (Machado et al. 2012). To date, there is no report of human rabies transmitted by capuchin monkeys. Kobayashi et al. (2013), determined the chiroptera-related rabies virus is the source of rabies infection in the capuchin monkeys, and there is a risk that the capuchin acts as a rabies reservoir. Nonhuman primates have transmission potential in Northeast Brazil; their role in São Paulo State is as yet unknown. The monkeys sampled in our study are vulnerable to extinction (Table 1), which is of concern, as all the animals had RVNA.

Capybaras (*Hydrochaeris hydrochaeris*) have been reported with RVNA (Almeida et al. 2001), and we report the first RVNA of exposure in tapirs (*Tapirus terrestris*). Although we do not know the potential for rabies transmission in these species, a human injury caused by a capybara bite was classified as a risk for rabies transmission (Oliveira Vieira et al. 2015). Although rabies is not reported in tapirs, the disease should be kept in mind, as the tapir is a large terrestrial mammal at some risk of extinction (Table 1).

In our study, because of the new species reported with RVNA and bearing in mind the small number of samples collected from some species, it is difficult to draw conclusions about the importance of these species for rabies. There is a high likelihood that exposure originated from other wild animals, and that there is a risk of transmission to humans or animals. Viral circulation in species bearing a possibility of extinction is worrying, because diseases can cause a significant decline in wildlife populations and may represent a threat to biodiversity with implications to the conservation of species (Table 1; International Union for Conservation of Nature 2016). Our sample sizes varied among locations, but our results indicate that rabies circulated in all the regions (Fig. 1), reinforcing the importance of serology and of testing the brains of road-killed animals as an adjunct surveillance tool. Standardization of cut-off values of RVNA for specific animal species should be the object of future discussions.

The presence of exposure or protective levels of antibodies indicates that the animals had contact with rabies virus, but the ways that animals can contact the virus in nature are unclear. The rabies virus is circulating among wildlife in municipalities in São Paulo State, Brazil that have lacked clinical cases of rabies in humans or wild terrestrial mammals. Our results in apparently healthy animals suggest rabies virus contact, and the human exposure to animals serologically positive for rabies is a significant problem for public health and in the conservation of these species.

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