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Short Communication

Rabies virus monitoring in bat populations in Rondônia state, Brazil

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Abstract

Introduction: The Jirau hydroelectric power plant built in Rondônia state has environmental impacts that could be relevant to rabies outbreaks. **Methods:** Bat populations were monitored for rabies by fluorescent antibody testing and simplified fluorescent inhibition microtesting between 2010 and 2015. **Results:** All 1,183 bats tested negative for rabies. The prevalence of rabies antibodies was 17.5% in 1,049 bats. **Conclusions:** The rabies antibody dosage was not reactive in samples collected before the environmental changes, and there was a progressive increase in subsequent collections that could indicate an increase in rabies virus circulation among bats and risk of a rabies outbreak.

Keywords: Rabies. Rabies antibodies. Chiroptera.

Bats are one of the most important reservoirs and vectors of the rabies virus worldwide. In Brazil, a marked decrease in rabies among humans and domestic animals was observed after the 1990s; since then, geographically isolated human rabies outbreaks transmitted by the hematophagous bats *Desmodus rotundus* have been documented. Rabid bats have been reported in Brazilian states and the virus has been isolated from 43 of the 172 bat species present in the country[1,2].

In the Rondônia state, the Jirau hydroelectric power plant was built on the Madeira River. The implementation of huge enterprises demands a series of alterations to the landscape, with anthropic activities of high environmental impact that can destroy artificial and natural shelters used by bats.

The aim of this study was to monitor the rabies virus in bat populations in the area surrounding the Jirau hydroelectric power plant in the Rondônia state in the North Region of Brazil, between 2010 and 2015.

Jirau's bat populations were monitored between 2010 and 2015 in three districts (Caiçara, Abunã, and Mutum) in the municipality of Porto Velho, Rondônia. Samples collected in the districts were classified as "anthropic". Samples collected in areas of primary forest with low disturbance pressures were classified as "forest", and samples collected from rocky outcrops on the Madeira River bank were classified as "stones." Collections were made twice a year, in the dry and rainy seasons.

Before collecting the bats, the local population was interviewed to elicit their knowledge about rabies and bats.

To capture the bats, mist nets and harp traps were used, and shelters were searched. Bat collection was authorized by the Instituto Brasileiro do Meio Ambiente (IBAMA) under the licenses 260/2010 and 190/2012. Specimens were deposited at the Zoology Museum of São Paulo University, São Paulo state, Brazil.

Rabies diagnosis was made using a fluorescent antibody test FAT[3] and a simplified fluorescent inhibition microtest SFIMT[4]. The cutoff value was 0.5 IU/mL. Blood samples were collected by cardiac puncture after sedation with ketamine hydrochloride. The rabies tests were performed at the Rabies Laboratory of the Zoonosis Control Center in the city of São Paulo, Brazil.

The statistical analysis was performed using Chi square, Kruskal-Wallis, and Dunn tests with the software BioEstat 5.3[5].

One hundred fifty-eight people were interviewed in the three districts, all adults between 20 to 60 years old. Among those interviewed who had a formal education (85%), on average, 77% studied for four years, 20% studied for eight years, and 3% had

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gone to college. A majority of the people interviewed (93.5%) declared knowing about rabies; however, they associated rabies with dogs. Only 52.5% knew that bats can transmit rabies, and 53.9% knew that medical care was necessary in the case of a bat bite. Although 91.5% of the respondents declared knowing that domestic animals should receive an annual rabies vaccination, only 59.7% of the animals were regularly vaccinated.

In surveys made in the Mutum district, 93.1% of houses were vulnerable to bats, and the insectivorous bat *Molossus molossus* was roosting in 70% of these dwellings. This species was also observed in attics in houses in the Abunã district living in cohabitation with the insectivorous bat *Eumops perotis.*

In total, 1,183 bats were analyzed, belonging to 59 species and 7 families. Phyllostomidae (79.9%) and Molossidae (15.5%) families were predominant. Of these, 836 were captured in anthropic areas, 316 in the forest, and 31 in rocky outcrops. All bat brains tested negative for rabies.

Sera of 1,047 bats were analyzed (754 bats from anthropic areas, 262 from the forest, and 31 from "stones"). The prevalence of rabies antibodies was 17.5%. The families Phyllostomidae and Molossidae had a seroprevalence rate of 18.9% and 7.7%, respectively. Rabies antibodies were detected in 31 of the 59 species tested (**Table 1**).

Sera samples from the first collection were not reactive. The prevalence showed a progressive increase in subsequent collections (**Table 2**). The Kruskal-Wallis statistical analysis showed that the differences observed in the serology of the twelve collections were significant (H = 232.7; g.l. = 11; $p = 0.0001$). The Dunn test showed that the results of the first collection were different from those of the eleven subsequent collections ($p < 0.05$).

Seroprevalence was higher in bats collected from the forest (24.8%) compared to that in bats collected from anthropic areas (15.2%) for all individual collections. The statistical analysis

TABLE 1: Rabies antibodies prevalence in bats according species, Jirau-RO, 2010-2015.

Taxon	Serum samples (n)	Reagent (n)	Prevalence (%)*
Family Emballonuridae			
Subfamily Emballonuridae (4 genera, 6 species)			
Cormura brevirostris	2	0	
Peropteryx leucoptera	1	0	
Rhynchonycteris naso	3	0	
Saccopteryx leptura	4	0	
Saccopteryx bilineata	2	0	
Saccopteryx gymnura	1	0	
Family Phyllostomidae (25 genera, 42 species)			
Subfamily Micronycterinae			
Micronycteris minuta	2	0	
Subfamily Desmodontinae			
Desmodus rotundus	3	2	
Subfamily Lonchorhininae			
Lonchorhina aurita	1	0	
Subfamily Phyllostominae			
Chrotopterus auritus	4	1	
Lophostoma brasiliensis	1	1	
Lophostoma carrikeri	1	0	
Lophostoma silvicola	18	8	44.4
Macrophyllum macrophyllum	9	2	
Mimon crenulatum	7	0	
Phylloderma stenops	3	0	
Phyllostomus discolor	5	2	
Phyllostomus elongatus	10	1	10
Phyllostomus hastatus	4	0	
Tonatia saurophila	4	0	
Trachops cirrhosus	45	16	35.5
Subfamily Glossophaginae			
Glossophaga commissarisi	1	0	
Glossophaga soricina	44	14	31.9
Glossophaga sp	1	0	

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Subfamily Lonchophyllinae			
Lonchophylla thomasi	2	1	
Subfamily Carolliinae			
Carollia benkeithi	1	1	
Carollia brevicauda	3	1	
Carollia perspicillata	510	77	15.1
Carollia sp.	10	0	
Subfamily Glyphonycterinae			
Trinycteris nicefori	3	0	
Subfamily Rhinophyllinae			
Rhinophylla fischerae	4	0	
Rhinophylla pumilio	20	4	20
Subfamily Stenodermatinae			
Artibeus concolor	2	0	
Artibeus fimbriatus	2	2	
Artibeus lituratus	23	2	8.7
Artibeus planirostris	22	6	27.3
Artibeus obscurus	30	9	31.0
Artibeus sp.	2	1	
Chiroderma villosum	1	0	
Dermanura cinerea	3	1	
Dermanura glauca	1	1	
Dermanura gnoma	9	3	
Dermanura sp	3	0	
Mesophylla macconnelli	4	1	
Platyrrhinus brachycephalus	1	0	
Platyrrhinus incarum	4	0	
Sphaeronycteris toxophyllum	1	0	
Sturnira lilium	3	1	
Sturnira tildae	9	0	
Uroderma bilobatum	4	0	
Uroderma magnirostrum	1	1	
Uroderma sp.	1	0	
Vampyressa bidens	1	0	
Family Mormoopidae (1 genus, 3 species)			
Pteronotus gymnonotus	1	0	
Pteronotus parnellii	12	8	66.7
Pteronotus personatus	1	1	
Family Noctilionidae (1 genus, 2 species)			
Noctilio albiventris	1	1	
Noctilio leporinus	1	0	
Family Thyropteridae (1 genus, 1 species)			
Thyroptera tricolor	2	0	
Family Molossidae (3 genera, 3 species)			
Molossus molossus	98	6	6.1
Eumops perotis	33	3	9.1
Nyctinomops laticaudatus	38	4	10.5
Family Vespertilionidae (1 genus, 1 species)			
Myotis riparius	3	1	
Myotis nigricans	1	0	
Total	**1047**	**183**	**17.5**

*Prevalence was calculated in species with n > 10 specimens only.

TABLE 2: Rabies antibodies prevalence in bats according area of collection, Jirau-RO, 2010–2015.

Collection	samples	Prevalence (%)			
	n	Total	Forest	Anthropic	Stones
C1	70	0	-	0	-
C2	72	2.8	2.3	0	5.3
C3	62	6.4	20.0	3.8	-
C4	99	20.2	28.6	17.0	16.7
C5	81	14.8	26.9	9.1	-
C6	102	36.3	44.4	31.8	-
C7	73	21.9	-	21.9	-
C8	119	24.4	28.0	23.4	-
C9	83	36.1	52.4	30.6	-
C10	104	8.6	16.7	7.0	-
C11	90	8.9	17.9	4.8	-
C12	92	17.4	18.5	16.9	-
Total	**1047**	**17.5**	**24.8**	**15.2**	**9.7**

showed that the habitat where the bats were collected was associated with antibody titers ($x^2 = 12.2$, df = 1, p = 0.0005). Seroprevalence was 9.7% in bats collected from rocky outcrops (**Table 2**).

A higher proportion of bats were captured during the dry season (56.2%). However, the seroprevalence was higher among bats captured during the rainy season (19.2%) in relation to the dry season (15.2%). This difference was not significant ($x^2 = 2.81$, df = 1, p = 0.09).

The proportion of males was higher than that of females (53.1% and 46.9%, respectively); however, rabies seroprevalence was higher in females (18.9%) than males (16.5%). This difference was not significant ($x^2 = 1.03$; df = 1; p = 0.34).

The proportion of adults was higher than that of juveniles (91.7%). Rabies seroprevalence was higher in juveniles (20%) in relation to adults (17.4%). This difference was not significant ($x^2 = 0.35$; df = 1; p = 0.55).

From the interviews, the relationship observed between the human population and the bats was based on prejudice, fear, and erroneous concepts. The role of these animals in the control of insects, as pollinators, and as seed dispersal agents was unknown. It is necessary to provide more information to the citizens about the ecological role of bats, their role in the rabies cycle, and the importance of the vaccination of domestic animals.

The implementation of huge enterprises, such as the Jirau hydroelectric power plant, demands making a series of alterations to the landscape, such as vegetation suppression, movement of large areas of land, demolition of houses, and relocation of resident human populations, among other activities of high environmental impact. During this process, artificial and natural shelters used by bats are destroyed.

Factors observed in the study area that are associated with the occurrence of human rabies outbreaks in Brazil included small human population groups in a remote rural area, change in the type of human productive process, deforestation, and communities living under poor conditions, including vulnerable housing and relatively limited access to health services[6]. The monitoring of bat populations is a way to control possible rabies outbreaks.

The dosage of rabies antibodies was not reactive in bats from the first collection and showed a progressive increase for every subsequent collection. The increase in the levels of antibodies indicates an increase in the circulation of the rabies virus in bat populations in the area. The highest seroprevalence was in collection 6 (36.3%), and the highest average of the titers was in collection 9 (0.54 UI/mL). High seroprevalence of the rabies virus in many bat species in the same area suggests that frequent survival after exposure may be a general feature of bat rabies.

A cutoff of 0.5 IU/mL was established by the World Health Organization[7] for sera of vaccinated humans and animals; however, antibodies in any titer are indicative of contact between the animal and the rabies virus. In bats from Jirau, the antibodies were acquired naturally, and the expressive levels indicate that the rabies virus circulates actively in these bats. According to Klimpel[8], virus contact can stimulate B lymphocytes to produce antibodies specific for viral antigens. The antibodies can neutralize the virus by blocking virus-host cell interactions or recognizing viral antigens on virus-infected cells, which can lead to antibody-dependent cytotoxic cells or complement-mediated lysis. Neutralization by antibodies is most effective when the virus is present in fluids (e.g., serum). The simplified fluorescent inhibition microtest (SFIMT) was used in our bat samples to detect neutralizing antibodies.

A seroprevalence rate higher in bats of the forest areas compared to that in anthropic areas was also observed by Salas-Rojas et al.[9] and Thoisy et al.[10]. The diversity of species increases the circulation of the virus due to more frequent interspecies interactions in areas with higher species richness. In this study, a higher diversity of species (26) collected from the forest areas in relation to species collected from anthropic areas (14) was observed.

Understanding the epidemiological situation of rabies in the study area is essential to analyze the rate of rabies seroprevalence. Almeida et al.[11] analyzed the sera of 1,618 bats collected in urban shelters in São Paulo state, where rabies is under control, and observed a prevalence of 5.9%. Costa et al.[12] and Oliveira et al.[13] analyzed the sera of 307 and 441 bats collected in rural and urban areas of Pará state, where rabies is endemic, and the prevalence was 50.8% and 50.3%, respectively.

More bats were captured during the dry season (56.2%), but the seroprevalence was higher among bats captured in the rainy season. Costa et al.[12] observed a higher proportion of seropositive bats in the rainy season, and this may be because bats spend more time in their colonies during this season, which may result in higher rates of social encounters, such as grooming or agonistic behavior resulting in bites and scratches that would increase the chance of passing rabies to a much larger number of individuals.

No significant differences were recorded between sex and age of bats in accordance with Thoisy et al.[9], Almeida et al.[11], and Costa et al.[12]. However, Oliveira et al.[13] found significantly higher proportions of males and adults that were seropositive for rabies.

The most frequently collected bat species during the study was *Carollia perspicillata* (48.7%), a frugivore with synanthropic habits that used culverts as shelters in Jirau. The neotropical genus *Carollia* includes some of the most abundant species of mammals. Rabies-seropositive individuals of this species have been frequently reported[10,12,13].

Another very common species in urban areas is the nectarivorous bat *Glossophaga soricina*. This species was collected in culverts, abandoned buildings, and nets while flying at night. A high percentage of seropositive bats was observed in this species (31.9%), which is in concordance with Oliveira et al.[13].

No study about rabies serology was found in the literature for the bats *Trachops cirrhosus* and *Pteronotus parnellii.* A high proportion of seropositive bats was observed (35.5% and 66.7%, respectively). Cohabitation of *T. cirrhosus* with *C. perspicillata* and *G. soricina* was observed in culverts.

Rabies antibodies prevalence in *M. molossus* and *E. perotis* was 6.1% and 9.1%, respectively. Almeida et al.[11] found a seroprevalence of 3.2% and 9.1% in these species.

The bat *Nyctinomops laticaudatus* is a colonial species that in Jirau uses large rocky outcrops as shelters where there is intense body contact among individuals, and the temperature is around 40°C. These facts could be associated with rabies virus transmission and the presence of antibodies. The prevalence of 10.5% was similar to that observed by Almeida et al.[11] (12.5%).

A seroprevalence of 24.7% was observed in the genus *Artibeus*. The bats *Artibeus lituratus* and *A. planirostris* are colonial frugivores with synanthropic habits that exhibit aggressive behavior and are frequently observed with visible scars on their wings that are compatible with bites. These bats are among the most common species with confirmed rabies in Brazil[11,14,15].

D. rotundus is the main bat species that carries and transmits the rabies virus in Brazil. Expressive prevalence of rabies antibodies has been observed in this species [10,11,12,15]. In this study, although intensive surveys were conducted in locations normally used by this species as shelters, only 11 specimens were collected over 6 years, all in a forest area with mist nets. This species probably lives in low densities in the Jirau area. In Rondônia state, the last human rabies case was reported in 2004, which was transmitted by a dog. The last case reported for a domestic animal (a dog) was in 2006. However, the rabies virus has remained in circulation among herbivores and swine (181 cases between 2008 and 2016). Although there is a high density of cattle in Rondônia, the rabies virus circulates at a low rate compared to other states in Brazil.

A study about the influence of stress, as measured by the maintenance of the rabies virus in bat colonies of *Desmodus rotundus*, investigated its effects by analyzing its influence on population densities and immunological profiles of the colonies. The conclusion was that an accidental stressful event can be easily overcome by the population, while a persistent vertical immunodepression due to stress factors can have a devastating impact on the colony, even if it occurs at relatively low rates[16].

Since the first collection was made before the environmental changes, the results for the dosage of rabies antibodies in subsequent collections could be a reflection of the perturbation the bat populations experienced, which was caused by the stress of forced migration to search for new shelters and new foraging areas; the competition for space, food, and females with bat populations already established in the shelters; and the establishment of a new social hierarchical status in the colonies.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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