

SEROLOGICAL SURVEY FOR RABIES IN SERUM SAMPLES FROM VAMPIRE BATS (*Desmodus rotundus*) IN BOTUCATU REGION, SP, BRAZIL

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ABSTRACT: The chiropterans constitute 25% of the world's mammal fauna. Due to the destruction of their natural ecosystem, the vampire bats have moved from nature to artificial roosts closer to man and domestic animals. This phenomenon has happened particularly in rural areas. Rabies is a viral anthroozoonosis, 100% lethal, and vampire bats (*Desmodus rotundus*) represent an important role in its epidemiology. *D. rotundus* were captured at night with mesh nets in partnership with the Botucatu Defense Office and sent to the Zoonosis Diagnostic Service, at the School of Veterinary Medicine and Animal Husbandry, UNESP. Serum samples from 204 bats were analyzed by enzyme-linked immunosorbent assay (ELISA) and fluorescent antibody viral neutralization test (FAVN) for rabies antibody detection. The results showed 7.4% of sera with titers higher or equal to 0.5 U for rabies antibodies, which demonstrated viral flow circulation among the studied region. Data suggest a need for constant monitoring accomplished by epidemiological and sanitary measures.

KEY WORDS: *Desmodus rotundus*, vampire bats, rabies virus, LPC-ELISA, RFFIT.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

The chiropterans are one of the most distinguished mammals as they present special features that make them capable of true flight (19). These animals play an important role in nature, specifically, in several tropical and subtropical areas, since they are responsible for spreading seeds, flower pollination and insect population control. They constitute somewhat wide range of mammal fauna, in the number of species and population density, representing approximately a quarter of mammal fauna worldwide (23).

In spite of their ecological function, bats can be transmitters of many diseases like arbovirus, rickettsiosis, criptococosis (17), histoplasmosis, Chagas's disease, brucellosis, salmonelosis, candidiasis (8) and rabies to humans and other animals. The latter is the most severe disease that can be transmitted by bats. *D. rotundus*, the most common vampire bat of the Americas, can be found in colonies of 20 to 100 animals (16). They live in caves, tree holes, abandoned mines, home basements, drainpipes or roosts (4).

Vampire bats present some adaptations to feed on blood: their saliva enzymes inhibit blood coagulation and two ducts on each side of their tongues enable them to suck blood. Each bat ingests 15 to 25 mL of blood per night and usually feeds on the same prey and the same wound for many nights in a row; additionally, a parasitized animal can be exploited by more than one bat during the same night (18).

As they feed only on blood, they are potential transmitters of rabies, which may cause damage to equine and cattle flocks due to numerous animal deaths (14). Humans, in turn, indiscriminately kill chiropterans, vampires or not, threatening the ecological balance (18).

Attacks on humans and rabies transmission by *D. rotundus* have increased in recent decades, especially in the Amazon area, in Peru and Brazil. Some of these outbreaks have been preceded by local changes, including rapid domestic animal removal, mineral extraction or deforestation, activities that can change the natural environment where the *D. rotundus* lives (20).

An important fact regarding the epidemiological vigilance of this disease in Botucatu, and its surrounding cities, is the Castelo Branco highway that presents many embankments and, consequently, numerous areas of water drainage through large piping. These places are favorable for developing bat colonies near rural residences

and livestock. Although there are reforestation and agricultural areas in the bordering municipalities, which usually show some human activity, there are also piping, abandoned rural buildings and caves where bats can take shelter (22).

Wild animals constitute the natural reservoirs and transmit the virus to domestic animals. The most common transmission route of rabies is through infected animal saliva, especially by biting. The infection may also occur if the saliva reaches any mucous membrane or open skin wound or scratch (1). The actions necessary to hinder the disease are well known and there have been many improvements, for example, in feline and canine control in urban areas (13). On the other hand, the chiropteran population has grown in non-rural areas due to lack of metropolitan planning with respect to architectural and landscape projects. Together with the chiropteran population growth, acts of epidemiological vigilance were intensified. As a result, the number of chiropteran rabies cases increased and the virus was found even in areas where the disease had been thought to be under control (12).

Due to the disease expansion, *D. rotundus* has interfered in the aerial cycle, causing countless rabies cases in non-vampire bats, as well as in the urban cycle, being responsible by eight out of fourteen cases of the disease recorded among dogs and cats (7).

Fifteen rabies cases in humans transmitted by bats were observed in the Americas from 1995 to 2000. Bats rank second in transmission cases in Latin America. In the USA and Canada, they were the only transmitters of autochthonous human rabies (2). An outbreak has been recently detected in Pará State, Brazil, with 15 cases of rabies in humans caused by bats within a month. It has been the most serious outbreak in humans in Brazil, the largest ever registered in such short a period (3).

The subclinical infection may occur in bats, when viruses multiply in their fatty tissue without invading the central nervous system (CNS). This makes them efficient reservoirs that may cause infections for months or years by secretion and saliva elimination (5). The main clinical rabies signs in *D. rotundus* are: daytime eating habit, hyperexcitability, aggressiveness, incoordination, muscle spasms, paralysis and death. The fatal infection does not seem common among chiropterans. The infection cycles originate from bites (15).

The viral transmission among bats occurs through bites, breathing, scratches or nourishment and it is more likely to occur in places with diverse colonies, or due to

bat migration, which leads to the viral spreading (21). The intraspecies dissemination may cause acute disease in some individuals, while in others the abortive infection results in seroconversion. Serological tests permit the evaluation of the epidemiological situation, since the seropositivity in a colony may simply demonstrate an exposure to the virus, whereas the high prevalence associated with high antibody titers can indicate recent outbreaks (15).

Due to the importance of vampire bats in South America, this study aimed to evaluate the presence of serum antibodies in healthy free-living bats from the Botucatu region of São Paulo State.

MATERIALS AND METHODS

Two-hundred and four vampire bats were captured in the following municipalities: Bofete (36), Pardinho (10), São Manuel (45), Pratânia (24), Botucatu (16), Itatinga (28) and Anhembi (45) (Figure 1). The captures were performed during the evening, between 6 and 11 p.m., using mesh nets set up in front of roosts, caves, tree holes as well as inside piping (under highways or railways). Captured animals were sent to the Zoonosis Diagnostic Laboratory in the Veterinary Medicine and Animal Husbandry School, UNESP, Brazil, where they were properly anesthetized for blood collection via intracardiac puncture. The blood samples were centrifuged at 1600 x g for 10 minutes and the sera were frozen at -20°C. The animals were killed and, then, their brain tissues were examined for Negri bodies by direct immunofluorescence (DIF) according to Dean *et al.* (10).

Liquid-phase competitive enzyme-linked immunosorbent assays (LPC-ELISA) and rapid fluorescent focus inhibition test (RFFIT) were performed according to Cardoso *et al.* (6) with some modifications. CVS₂₃ (virus standard) propagated in the chicken embryo related (CER) cell line was inactivated and used for antigen preparation for ELISA (11) and for RFFIT (6). Both methods are recognized by the World Organization for Animal Health (OIE) as standard tests, with specificity and sensitivity higher than 80% and presenting no cross-reaction with other antigens except for rabies virus particles. A positive control serum of dog origin titrated to 132 IU/mL, obtained by the US Centers for Disease Control (CDC), stored at -20°C and diluted to 5 IU/mL was used for all assays. As negative control, another dog serum with negative results was also employed.

In order to calculate ELISA titers, the two-graph receiver operating characteristic (TG-ROC) technique was performed. Serum ranging between 0.10 and 0.20 was considered weak positive; 0.20 to 0.30, positive; and greater than 0.30, high positive. Each high positive serum was tested by RFFIT and the titers were calculated through the analysis of 20 microscopic fields in only one pool, for each serum dilution assessed in the presence of infected cells using a fluorescence microscope (Carl Zeiss Inc., USA) (160x magnification). Titers were calculated using the method of Reed and Muench in which the respective titers ≥ 0.5 IU/mL were considered positive.

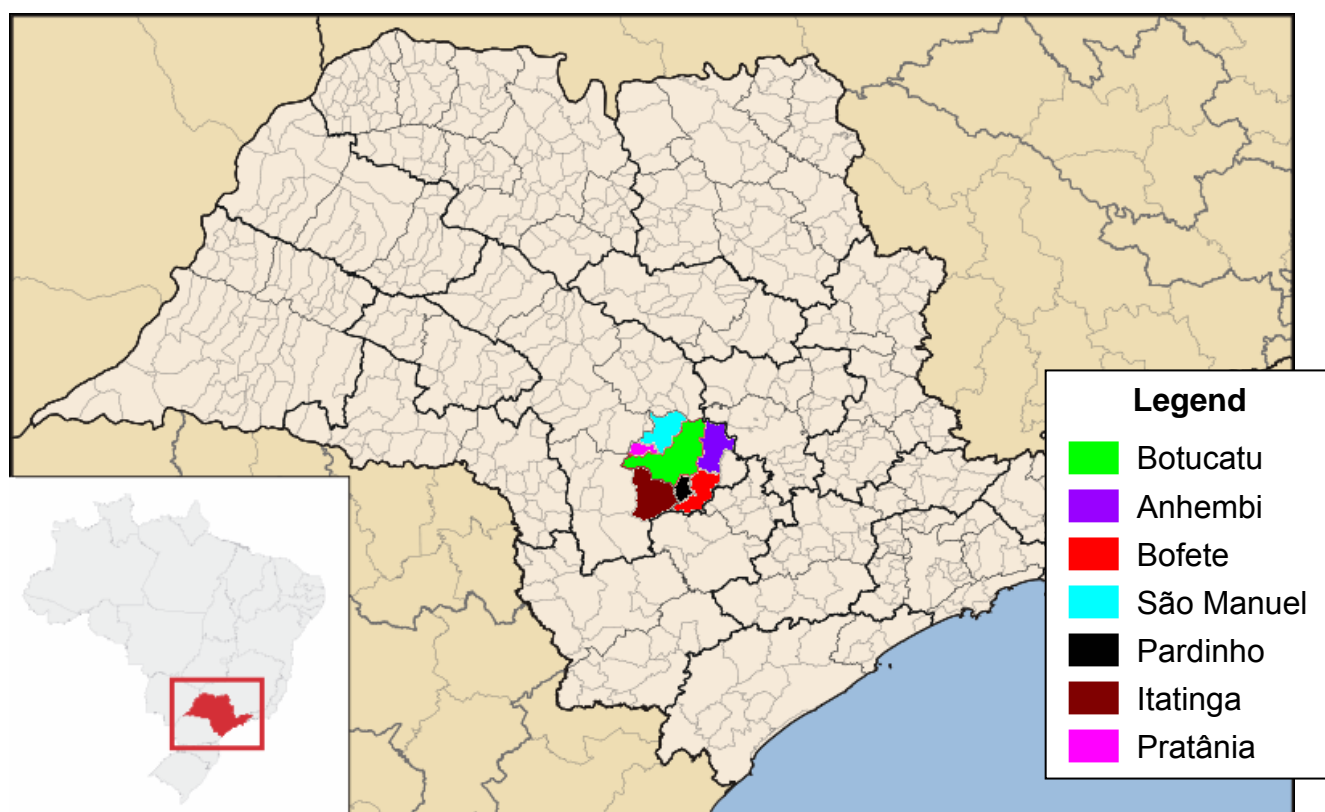


Figure 1. Cities comprising the Botucatu region – in São Paulo State, Brazil –where the vampire bats were captured in 2008.

Approximate scale: 1:600,000. Adapted from Wikimedia Commons, available from: <http://commons.wikimedia.org>.

RESULTS AND DISCUSSION

All animals were negative for Negri bodies by DIF.

In the LPC-ELISA, from 204 serum samples analyzed, 92 (45.10%) reacted weakly (titer from 0.10 to 0.20); 19 (9.31%) presented average reactions (titer between 0.20 and 0.3); 22 (10.78%) reacted strongly (titer greater than 0.30); and 71 samples did not react. Every serum with titer higher than 0.3 was examined by RFFIT; among this group, 15 samples (7.35%) were positive, presenting titers greater than 0.5 IU/mL (Table 1).

In another study performed in Rio Preto, Minas Gerais State, Brazil, by Piccinini *et al.* (19), 59 vampire bats – 15 (60%) out of 25 males and 17 (50%) out of 34 females – had presented the virus in brain and/or in gland tissues, which revealed rabies infection. These results show a significant difference when compared to the 7.35% positivity of the current report, which presumably indicates a higher viral circulation in both distinct regions.

Souza *et al.* (22) found, in the same Botucatu region, only 0.1% positivity among 895 studied bats by employing the immunofluorescence technique for brain tissue. On the other hand, Cortês *et al.* (9), in another study in the same area, found 0.9% positivity. The results of the present study may indicate subclinical disease, without Negri bodies detected by the immunofluorescence technique. The animals presenting subclinical infections constitute efficient reservoirs because the virus can multiply in their fatty tissues without affecting the central nervous system. In reservoir animals, the rabies virus can endure and is eliminated through both respiratory secretion and saliva, so that they are capable of infecting other bats, humans as well as domestic and wild animals.

The epidemiological characteristics of the disease are still poorly understood. Consequently, more research and implementation of an epidemiologic vigilance system are necessary (14). *D. rotundus* plays an important role in rabies circulation in the Botucatu region, being capable of infecting a great variety of animals.

Table 1. Numbers and percentages of *D. rotundus* bats infected by rabies (RFFIT serology), in Botucatu region, 2008

Origin	Number of captured animals	Number of positive animals	Positivity (%)
Bofete	36	2	5.56
Pardinho	10	1	10.00
São Manuel	45	3	6.67
Pratânia	24	0	0
Botucatu	16	0	0
Itatinga	28	4	14.29
Anhembi	45	2	4.44
Total	204	15	7.35

CONCLUSIONS

The studied region presents viral flow circulation through bats, thus a constant monitoring accomplished by epidemiological and sanitary measures is necessary to maintain the control of rabies.

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