Serological Survey of Rabies Virus Infection among Bats in Brazil

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Rabies is a disease caused by a virus belonging to the family Rhabdoviridae, genus *Lyssavirus* (RABV), and is characterized as a progressive and acute encephalitis that presents high lethality. Between 2002 and 2012, 2149 wild animals were found positive for RABV in Brazil where 79% were associated with the aerial cycle of the disease. As part of the active surveillance of rabies in Brazil, the objective of this study was to investigate the presence of RABV infection in bats captured in the metropolitan region of Rio de Janeiro city. Forty-four bats were tested for RABV at Institute Jorge Vaitsman (IJV), reference laboratory for rabies in Rio de Janeiro State. Of the individuals captured and sent for the diagnosis of rabies, a total of 15 were identified as being hematophagous, all of them belonging to the species *Desmodus rotundus*. Twenty-four individuals were considered as frugivorous, four as insectivores and one individual (*Lonchophylla peracchii*) identified as having a pollen/nectar feeding habit. All specimens sent to RABV diagnosis presented negative results. Considering the active surveillance for RABV in Brazil, studies of fauna survey with serological characterization are important in the strengthening of epidemiological surveillance and protection of human life.

**Key words:** Rabies, Brazil, Bats, Serological Survey

**INTRODUCTION**

Rabies, caused by a virus belonging to the family Rhabdoviridae, genus *Lyssavirus* (RABV), is characterized as a progressive and acute encephalitis that presents high lethality (Oliveira et al, 2015). The genus *Lyssavirus* presents eight genotypes, with genotype 1 - Rabies virus (RABV), the only one present in Latin America and Brazil, and can be expressed, according to the profile, in 12 antigenic variants according to their respective natural hosts (terrestrial or aerial) (Rupprecht et al, 2002). In Brazil, seven antigenic variants of the rabies virus were found, being variants 1 and 2 isolated from dogs; variant 3, of the hematophagous bat *Desmodus rotundus*, and variants 4 and 6, of the insectivorous bats of the species *Tadarida brasiliensis* and *Lasiurus cinereus* (Brazil, 2016). Kotait et al. (2009) describes four transmission cycles for rabies virus in Brazil: urban, rural (animals and animal products), aerial wild and terrestrial wild. Between 2002 and 2012, the Ministry of Health notified 2149 wild animals positive for RABV infection where 79.2% were associated
with the aerial cycle of the disease (Rocha et al., 2015). In the same period the authors encountered 126 reported cases of human rabies, with a case-fatality rate of 99.2%.

Among the risk factors for the occurrence of rabies one might list low canine vaccination coverage, presence of stray dogs, community or with free access to the street, existence of suspected or confirmed rabies cases in dogs and cats, environmental changes and occurrence of rabies virus or the disease in hematophagous bats. Rabies surveillance in Brazil has its pillars based on (i) investigating all suspected cases of human and animal rabies, as well as determining its source of infection with active search for people under risk; (ii) determining risk areas; (iii) monitoring animal rabies in order to avoid occurrence of human cases; (iv) carrying out and evaluating focal blocks; (v) carrying out and evaluating rabies vaccination campaigns for canines and felines; evaluating prevention and control measures; and (vii) carry out educational activities on an ongoing basis (Brazil, 2016). Diagnostic laboratories of rabies virus are essential for guiding the control program, as well as for guiding epidemiological surveillance for prophylaxis in geographical areas where positive cases are reported. The agreement between the municipalities and the Ministry of Health is that biological material of 0.2% of the canine population be sent to laboratory diagnoses annually (Schneider et al., 1996; Moutinho et al., 2015).

Animal rabies is considered endemic in the State of Rio de Janeiro, where, similar to other Brazilian regions, the incidence of rabies acquired by bats is increasing in importance in the last two decades (Moutinho et al., 2015). Surveillance of this viral circulation is very important for the control of the disease, but for such action to be satisfactory and give the desired results there is a need for well-located reference laboratories, well-structured and in sufficient quantity. The difficulty of forwarding these samples is a reality in Brazil (Miranda et al., 2003). Considering the labor structure of Rio de Janeiro State for rabies diagnostics, some authors believe that it is insufficient for the processing of the expected quantity and poorly distributed from a spatial point of view, making it difficult to route the samples through the municipalities. According to the WHO, it is highly recommended that laboratory capacity allows the surveillance and detection of rabies (WHO, 2017).

Bats have attracted the attention of the scientific community for being important hosts to emerging human diseases. Recognized for hosting and transmitting various infectious agents of public health importance, such as Rabies Virus, Coronavirus, Histoplasma, among others, in the last decades studies have pointed out the Chiroptera also as potential hosts of proteobacteria belonging to the genus Rickettsia, Bartonella and Coxiella with the participation of different species of arthropod vectors and amplified by vertebrates in complex zoonotic cycles in which humans may be included accidentally (Bai et al., 2011; Ferreira et al., 2015).

Potential source of many zoonotic infections, bats differ from other wild reservoirs by their often gregarious social organization, their ability to fly, long life expectancy and by acting as binding hosts, transmitting the infectious agent, most often indirectly to human beings, characteristics that, associated with their increasing contact with man, increase the risk of transmission of infectious agents (Bennett, 2006; Hayman et al., 2013; Shi, 2013). The occurrence of rabies transmitted by bats has been increasing considerably in Brazil. There are several studies in different regions pointing to this growth, besides the change in the epidemiological profile of the disease (Queiroz et al., 2009; Moutinho et al., 2015), mainly in the rural area (Wada et al., 2011). Until 2009, 41 species of bats had already been identified in Brazil with the rabies virus (Sodré et al., 2010). In the State of Rio de Janeiro this epidemiological profile change has already been identified in several regions (Moutinho et al., 2015). In Brazil, Desmodus rotundus appears as the main species involved in bovines rabies (Kotait et al., 2009; Favoretto et al., 2013). This species has also been involved in human cases. Many authors also focus on the importance of herbivorous bats in the indirect transmission of rabies, with many species adapted to life in urban areas (Pacheco et al., 2010; Sodré et al., 2010). As part of the active surveillance of rabies in Brazil, the objective of this study is to investigate the presence of rabies virus infection in bats captured in Rio de Janeiro State.

**METHODS**

This is a sectional descriptive epidemiological study that aims to verify the presence of rabies virus in bats captured by sampling of convenience during expeditions conducted in the Atlantic Rainforest in the Southeastern of Brazil. The present study is inserted in the project “Bats (Mammalia, Chiroptera) as indicators of biotic integrity in an area of anthropic tension in the Maciço da Pedra Branca” with the license (No 19037-1) granted by the Brazilian Institute of the Environment and Renewable Natural
Bat specimens were collected in Metropolitan Region of Rio de Janeiro, in the neighborhood of Jacarepaguá, (22°56'22"S, 43°24'12"W). All the sampling sites comprise rainforest areas formed by the Atlantic Forest biome. Although the region of the city of Rio de Janeiro included in the study is the most anthropized because it is surrounded by a large urban center, the other areas are associated with ecological tourism.

**Field methodology and sampling**

In order to capture the bats, mist nets were used for six hours each night, on forest edges or on pre-existing tracks. Once captured, the animals were searched for ectoparasites. The captured animals were processed in a field laboratory and the samples were collected according to strict biosafety standards, with all the researchers equipped with biosafety level 3 equipment (motorized filters, positive pressure masks and other accessories) that guarantee the safety of the members of the team against aerosolized agents. In the field laboratory base the animals were anesthetized, following recommended procedures (Mares-Guia, 2014), for collecting bionomic data (body weight and body and tail measurements) and those related to sex and reproductive activity. All specimens were submitted to blood collection by cardiac puncture using 1-3 ml syringes according to animal size. After euthanasia, samples of organs and viscera of the specimens (kidney, liver, spleen, lung and heart) were extubated (total bleeding) and were conditioned in liquid nitrogen. In addition to the taxonomic identification based on external

![Figure](representative_map_of_the_three_regions_of_capture_of_bats.png)
morphology, cranial morphometry and karyotype analysis, the identification of the animals was also performed from bone marrow samples for cytogenetic studies associated with karyotyping. Liver samples were fixed and preserved in ethanol for molecular phylogenetic studies of bats. All animals collected were taxidermized and their skeletons prepared to be deposited as testimonial material in scientific collections of universities in each study region.

Rabies diagnosis

All bats were tested for RABV at Institute of Diagnosis, Surveillance, Sanitary Surveillance and Veterinary Medicine Jorge Vaitsman (IJV), the reference laboratory for rabies in Rio de Janeiro State. The institute offers several services including actions to control rabies in the city, such as vaccination, diagnosis, education, and the observation of suspected/aggressive animals. Brain tissue fragment and parotid salivar gland were collected for the direct immunoflorescence (DIF) test as described by Dean and Abelseth (1996). Up to eight prints were made on each laminae distributed in two rows, each corresponding to one animal. Positive and negative control slides were prepared. The laminae were observed with an immunofluorescence microscope with a 40x objective, making a complete scan of the whole field. The exam searched for characteristic fluorescent spotlights, fluorescences of varying size, well defined contours, characteristic morphology.

Analysis

The results were quantified for bat species, RABV positivity, tissues analyzed, functional group regarding feeding habits, separation between males and females, and the sampling sites of the species. In addition, data comprising the number of reports of rabies in Brazil in the last four years were obtained from the Ministry of Health's Health Surveillance Secretariat (Brazil, 2017).

RESULTS

A total of 44 bats were captured during a active surveillance of rabies in Jacarepaguá/RJ. The bats belonged to the families Vespertilionidae and Phyllostomidae, divided in 9 genera and 13 species (Table 1). Bats captured in Jacarepaguá in December 2013, March, May and September 2014 were sent to diagnostic analysis for rabies at the Jorge Vaitsman Institute, all of which presented negative results (Table 1). The same number of males and females were submitted to the diagnosis of rabies, being 22 individuals of each sex.

Of the individuals captured and sent for the diagnosis of rabies, a total of 15 were identified as being hematophagous, all of them belonging to the species Desmodus rotundus. Twenty-four individuals were considered as frugivorous, four as insectivores and one individual (Lonchophylla peracchii) identified as having a pollen/nectar feeding habit.

Brazilian surveillance system has reported an important amount of rabies cases in the last years, including human confirmation in 2013, 2015 and 2016 (Table 2). Bats are an important aspect in this scenario with several cases of rabies recorded by the surveillance system. It is worth mentioning the high number of reports of rabies cases in non-hematophagous bats, and it is quite possible that these numbers demonstrate underreporting, however, the data show a reduction in these reports with values dropping from 139 positive bats in 2013 to 71 in 2016. For hematophagous bats, the number has remained relatively stable in the last four years with 18 positives in 2013, falling to 11 and 6 and an increase in 2016 to 19. Bovine and equine animals presented high values for the rabies notification with cattle being the group with the highest number of notifications among all evaluated with 778 cases in 2013 and a reduction to 320 in 2016.

DISCUSSION

Considering the increasing importance of Chiroptera and its relationship with several zoonotic agents that cause human diseases, studies of fauna survey with serological characterization for rabies virus antigen and other agents of diseases of importance in public health are important in the strengthening of epidemiological surveillance and protection of human life. In the present study, developed with samples of bats captured in three Brazilian states, no positive DIF was found for rabies virus antigen. These data do not necessarily indicate the lack of circulation of rabies virus in the cities surveyed, instead the quantitative sampled may not have been sufficient to reach the prevalence of positivity in the Chiroptera fauna of these localities. Albas et al. (2005), in a survey in the Brazilian municipality of Presidente Prudente, in cattle, of 205 samples analyzed, 7.8% were positive, and in the
chiroptera, of 3,552 samples, 1.6% was positive. This result, as well as those presented here, show a necessity of a greater collection effort to achieve positivity. In Rio de Janeiro city, Souza et al (2014) found a single positive bat for rabies in a residence in the neighborhood of Barros Filho, in an urbanized area. The specimen was identified as *A. lituratus*, a species of common frugivorous bat in the city. Samples for laboratory diagnosis of rabies from bats collected in the Jacarepaguá neighborhood, the same as in this study, also presented negative results.

*Carollia perspicillata, D. rotundus* and *A. lituratus* were the most frequent species collected in this study. *Desmodus rotundus* is one of the principal hematophagous species found in Brazil (Corrêa et al, 2013). Casagrande et al (2014) after a survey of bats in Pernambuco State, found 88.2% of non-hematophagous species (with a 2% of positivity for rabies virus) and 11.8% of *D. rotundus* from which all brains were negative for the anti-rabies virus antibodies. Some studies have reported the difficulty of finding hematophagous bats with RABV brain infections or even with least clinical signs of rabies, including Sugay and Nilsson (1966) with 3.8% of positivity and Souza et al (1997) with 5.1% of samples from hematophagous bats positive for anti-RABV antibodies.

*Desmodus rotundus* is considered the most important reservoir and vector of rabies virus in Brazil (Greenhall et al 1983, Schneider et al, 2009; Correa et al, 2013). The main source of infection of rabies virus in cattle is probably the

| Species                  | Diet                      | Tissue*       | # Males | # Females | Result | Place** |
|--------------------------|---------------------------|---------------|---------|-----------|--------|--------|
| *Glossophaga soricina*   | Nectarivorous/ Frugivorous| NT / SG       | 0       | 2         | Negative | RJ     |
| *Desmodus rotundus*      | Hematophagus              | NT / SG       | 9       | 6         | Negative | RJ     |
| *Carollia perspicillata* | Frugivorous               | NT / SG       | 2       | 2         | Negative | RJ, BA, SC |
| *Artibeus lituratus*     | Frugivorous               | NT / SG       | 2       | 2         | Negative | RJ, BA, SC |
| *Artibeus fimbriatus*    | Frugivorous               | NT / SG       | 1       | 2         | Negative | RJ, SC |
| *Artibeus obscurus*      | Frugivorous               | NT / SG       | 1       | 0         | Negative | RJ, SC |
| *Sturnira lilium*        | Frugivorous               | NT / SG       | 4       | 2         | Negative | RJ, SC |
| *Sturnira tiliae*        | Frugivorous               | NT / SG       | 2       | 0         | Negative | RJ     |
| *Vampyressa pressil*     | Frugivorous               | NT / SG       | 0       | 2         | Negative | RJ     |
| *Myotis nigricans*       | Insectivorous             | NT / SG       | 1       | 1         | Negative | RJ     |
| *Micronycteris sp.*      | Insectivorous             | NT / SG       | 0       | 1         | Negative | RJ     |
| *Micronycteris minutata* | Insectivorous             | NT / SG       | 0       | 1         | Negative | RJ     |
| *Lonchophylla peracchii* | Pollen/ Nectar            | NT / SG       | 0       | 1         | Negative | RJ     |

Table 2. Number of reported cases of rabies in Brazil from 2013 to 2016.

|                     | 2013 | 2014 | 2015 | 2016 |
|---------------------|------|------|------|------|
| Human               | 5    | 0    | 2    | 2    |
| Canine              | 32   | 15   | 85   | 9    |
| Feline              | 4    | 5    | 8    | 6    |
| Hematophagous bats  | 18   | 11   | 6    | 19   |
| Non-hematophagous bats | 139 | 136 | 77 | 77 |
| Wild dogs           | 18   | 6    | 17   | 14   |
| Bovine              | 778  | 730  | 320  | 320  |
| Equines             | 105  | 111  | 48   | 48   |
| Other herbivores    | 8    | 17   | 3    | 3    |
| Production animals  | 10   | 8    | 4    | 6    |
| Nonhuman primates   | 7    | -    | 4    | 0    |
hematophagous bats, because in Brazil the virus variant isolated in cattle is that same isolated from *D. rotundus* (Heinemann et al, 2002). The increase in numbers of rabies cases in hematophagous and non-hematophagous bats has been observed, as well as the number of bats that have been diagnosed in every country (Teixeira et al, 2015), possibly as a result of increased knowledge about the role of bats in cycle of rabies transmission and the greater opportunity of perception of the presence of the species in the environment altered by man.

Non-hematophagous bats have shown to be important in epidemiology of rabies and are the most frequent in surveys and serological studies. The genus *Artibeus* was frequent in the samplings of the present study. These bats are part of the family Phyllostomidae, subfamily Stenodermatinae. They are predominantly frugivorous animals, but may eventually present a more general diet, feeding on insects, pollen and nectar (Reis et al, 2013). Characteristics of this species, such as the formation of colonies that reach more than 20 individuals in the reproductive age (Exberard et al, 1998) may contribute to a rapid and widespread transmission of RABV among these animals. In addition, *A. lituratus* and *A. fimbriatus* specimens can fly long distances in one night. Costa et al. (2006) reported the displacement of a specimen of *A. fimbriatus* in the state of Rio de Janeiro, which was recaptured 20 days after the first capture, at a distance of 21.7 km. In 2012, in the study by Reis et al (2012), two specimens of *A. lituratus* were recorded about 20 km from the marking site, 24 months after the first capture. The dispersion capacity of these Chiroptera might considerably increase the endemic area of RABV. Luis et al (2013) compared bats with rodents as reservoirs of zoonotic viruses. In this study it was demonstrated that bats harbor a much larger number of zoonotic viruses when compared to rodents and the level of interspecific contact frequently observed in shelters formed by a diverse set of bat species is one of the justifications.

Casagrande et al (2014) also reported 88.5% of the total positive cases among non-hematophagous bats, with positive individuals from species *A. lituratus* and *Myotis nigricans*, also collected in the present study. The laboratory Jorge Vaitsman, a reference for the diagnosis of rabies in Rio de Janeiro, received, between 2001 and 2010, samples of 135 non-hematophagous bats, where 11 were detected positive for rabies (Souza et al., 2012). The authors report that the main factors that contribute to the spread of rabies in domestic herbivores are the increase in the food supply represented by the significant herd increase, the environmental modifications caused by deforestation and disordered occupations, and the provision of artificial shelters for these bats like tunnels, cisterns, and abandoned buildings. Herbivorous bats are accidental hosts of the rabies virus, despite participating in the epidemiological chain of rabies in the rural environment; only contribute as sentinels to the existence of the virus (Souza et al, 2014).

The sampling sites in this study comprised urban and sylvatic areas with the aim of finding positive bats for RABV. Considering the urban environment, RABV has been identified in several species of bats, hematophagous and non-hematophagous, representing a risk to public health, since many have synanthropic habits. Hematophagous bat *D. rotundus* is considered the main host of the rabies virus being responsible for the direct infection of domestic animals and possibly humans (Batista et al, 2007). In addition, hematophagous bats have interfered in the air cycle of transmission of the disease, with innumerable cases in non-hematophagous species of the urban cycle, transmitting rabies to dogs and cats. With rapid urban growth, bats have colonized new ecological niches and left their natural night shelters to occupy man-made environments. The alteration of nature with the consequent reduction of natural resources has led to hematophagous bats searching for new food source alternatives, such as humans making them alternative food options for bats and being searched for as food sources only In the absence of cattle. Urbanization has been providing a variety of resources to bats, especially non-hematophagous, which have been found positive for rabies, such as plants used for afforestation by day and night shelters and other plants as food (Uieda et al. 1996). Also, nocturnal public lighting of cities attract insects that are habitually consumed by several species of insectivorous bats (Teixeira et al, 2015).

The Institute of Diagnosis, Surveillance, Sanitary Surveillance and Veterinary Medicine Jorge Vaitsman, also known as Instituto Jorge Vaitsman (IJV), founded in 1917, was created to carry out the control of tuberculosis in cattle producing milk. The institute had the objective of providing veterinary medical assistance to donkeys used in public services, such as garbage collection. Today, it offers a variety of services and treatments, including actions to control rabies in the city, such as vaccination, diagnosis, education, and the observation of suspect/aggressive animals. Since then, the institute has been
acting as a branch of rabies surveillance for the state of Rio de Janeiro, in addition to performing diagnoses of leishmaniasis, leptospirosis, sporotrichosis, toxoplasmosis and the performance of human serology tests for rabies. The State Rabies Prevention Program in Rio de Janeiro started the animal vaccination campaign in 1983, resulting in a significant reduction in rabies cases in dogs and cats and, as a consequence, a reduction in human rabies. After 21 years without occurrence of human rabies in the State of Rio de Janeiro, a case was confirmed in December 2006 in the municipality of São José do Vale do Rio Preto due to a bat accident (Menegheti, 2012). The control actions represented by vaccination coverage, reaching levels higher than 90% of the estimated canine and feline population between 1990 and 2009 contributed to the achievement of satisfactory results. In recent years, the occurrence of rabies in the state of Rio de Janeiro is restricted to animals from the rural area: cattle, horses and bats. Dogs and cats have not been detected positive since 2002. In Rio de Janeiro State, between 2007 and 2010, 170 cases of positive rabies cases were confirmed in cattle, 40 in horses, 14 in non-hematophagous bats, and 03 in hematophagous bats. Of the 98 cases confirmed in the State in 2011, 13% occurred in the city of Rio de Janeiro and 12% in Valença (Menegheti, 2012). In 2016, the IJV detected a positive bat in the Grajaú neighborhood.

Table 2 indicates the presence of rabies-positive monkeys in Brazil. The non-human primates are reservoirs of various zoonoses including rabies, and the involvement of monkeys in human accidents shows that Cebus (monkey-nail) and Callithrix (sagui) are among the most frequent species (Ramos et al, 2002). The population in Brazil is aware that dogs and cats can transmit rabies, but many do not know that bats, monkeys and other wild animals are also transmitters of the disease, so when they are bitten by these animals, they do not seek medical care and are at serious risk of being victims of the disease (Aguiar et al, 2011). It is important to note that laboratory surveillance of Yellow Fever in Brazil should take into account the diagnosis of rabies primarily in those primates that are sent for the confirmation of yellow fever in episodes of epizootics and/or outbreaks including human cases. In 2017, several Brazilian states reported hundreds of epizootic events in primates and human cases of yellow fever, mainly in the Southeast region, and the diagnosis of rabies should be placed in this context as a protection for laboratory professionals who manipulate dead animals for the removal of viscera, extraction of genetic material and proceedings of diagnostic.

None of the 44 bat samples tested in this study was positive for rabies antigen, which in no way indicates the reduction of RABV virus circulation in these localities, nor should a relaxation be observed in the surveillance actions, since the bats are one of the main maintainers of the virus in the wild chain of the cycle. These conclusions may differ from that found by other authors in other countries where the lack of findings suggests the absence of an active rabies infection (Mani et al, 2017). Thereby, active bat surveillance for RABV in other countries has also reported a very low prevalence of viral antigen or nucleic acid in healthy bat populations (Picard-Meyer et al, 2011; Nokireki et al, 2013; Ellison et, 2014; Schatz et al, 2014)

The lack of a sampling with longer duration and/or with greater expansion of the search area could be a limiting factor of this study for the lack of positive results. Also as a limitation of the study, a larger number of specimens could have been sent for diagnosis, which would increase the chance of detection. From a qualitative point of view, however, the samplings were able to collect several species of bats, with haematophagus bats and frugivores or insectivores being collected in the sampling sites. In addition, the species captured in this study have reported positivity for RABV in several other studies (Uieda et al., 1995; Martorelli et al., 1996; Albas et al, 2005; Rocha et al, 2015). We can suggest that the detection of seronegative bats might indicate the presence of animals with antibody levels below the threshold of detection using current tests and that actions aimed at the surveillance of rabies in Brazil needs be prioritized. Considering that the aerial cycle of the disease is showing great growth in the country, the risk of virus transmission by bats is always high, regardless of species and severity of the injury, so all bat aggression is considered severe (Moutinho et al, 2015). In addition, the environmental changes that have been occurring in rural areas, the lack of urban planning and architectural and landscape projects have interfered in the spatio-temporal distribution of various zoonotic diseases and contribute to the great population growth of bats, hematophagous or not, in urban areas.
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