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Circulation of the rabies virus in non-hematophagous bats in the City of Rio de Janeiro, Brazil, during 2001-2010

Circulação do vírus da raiva em morcegos não-hematófagos na Cidade do Rio de Janeiro no período entre 2001-2010

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ABSTRACT

Introduction: Rabies is one of the most known lethal zoonosis, responsible for 55,000 human deaths per year. It is transmitted to humans mainly by the bite of domestic or wild animals infected with the virus. This paper shows the circulation of this virus in non-hematophagous bats in the City of Rio de Janeiro, Brazil. **Methods:** A survey was performed on the number of bats that had been sent for diagnosis by the Seção de Virologia of the *Instituto Municipal de Medicina Veterinária Jorge Vaitsman* and were positive for rabies. The positive animals were identified, and the isolated viruses were sent for antigenic typification with indirect immunofluorescence. The results were compared with the antigenic panel of the Centers for Disease Control and Prevention. **Results:** During 2001-2010, the laboratory received 555 non-hematophagous bats for rabies diagnosis, with 198 (35.7%) from Rio de Janeiro City. A total of 11 (5.5%) animals were positive for this disease. Antigenic typification revealed the predominance of variant 3 in 9 (81.8%) of the isolated viruses; 1 virus was classified as variant 4 and 1 variant was identified that segregated with the viruses in insectivorous bats. **Conclusions:** The data obtained in this study showed the presence of the rabies virus in synanthropic populations of non-hematophagous bats in the City of Rio de Janeiro. The circulation of this agent in these animals represents a serious risk to human and animal health and requires attention and control measures by the authorities.

Keywords: Rabies vírus. Rio de Janeiro. Non-hematophagous bats.

RESUMO

Introdução: A raiva é uma das mais letais zoonoses conhecidas, responsável pelo óbito de 55 mil pessoas anualmente. É transmitida ao homem principalmente pela mordida de animais, domésticos ou silvestres, infectados pelo vírus. O presente estudo mostra a circulação deste agente em morcegos não-hematófagos no município do Rio de Janeiro, Brasil. **Métodos:** Na Seção de Virologia do Instituto Municipal de Medicina Veterinária Jorge Vaitsman, foi realizado um levantamento do número de morcegos recebidos positivos para o diagnóstico. Os animais positivos foram identificados, e o vírus isolado foi submetido à tipificação de variantes antigênicas pela técnica de imunofluorescência indireta, com os resultados comparados com o painel antigênico utilizado pelo Centro de Controle de Doenças (*Center of Disease Control* - CDC). **Resultados:** Entre 2001-2010, o laboratório recebeu 555 morcegos não-hematófagos para o diagnóstico da raiva, sendo 135 (35,5%) do município do Rio de Janeiro. Um total de 11 (5,5%) animais foram positivos para a doença. A tipificação antigênica revelou a predominância da variante 3, com nove (81,9%) vírus isolados, um pertencente a variante 4 e outro de uma variante que segrega com a presente em morcegos insetívoros. **Conclusões:** Os dados observados no presente estudo demonstraram a presença do vírus da raiva em populações sinantrópicas de morcegos não-hematófagos no município do Rio de Janeiro. A circulação deste agente nesses animais representa um grave risco a saúde humana e animal, e requer atenção e medidas de controle e prevenção por parte das autoridades competentes.

Palavras-chaves: Vírus da raiva. Rio de Janeiro. Morcegos não-hematófagos.

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INTRODUCTION

Among the diseases transmitted to humans by animals, rabies is known for its lethality, which is close to 100%. The disease is caused by a virus of the order Mononegavirales, family Rhabdoviridae, and genus *Lyssavirus*. The virus has a single-stranded RNA genome of negative polarity, a nucleocapsid with helical symmetry, and a bilipidic envelope. The main transmitter of this virus to humans in urban areas is the domestic dog, following inoculation of the virus by the bite of infected animals. Much feared since ancient times, rabies receives special attention from health authorities in several countries that have adopted measures to fight the illness, so that today, the urban cycle of the disease is on the verge of eradication. However, some countries still neglect the disease, such as those in Asia and Africa, where the World Health Organization estimates that there are 55,000 human deaths due to rabies every year[1].

In countries where urban rabies has been controlled by the authorities, great attention has been given to sylvatic rabies, where the virus has been isolated in several animal species[2-4], in addition to proven cases of human rabies by contact with these animals[5]. The presence of the virus in hematophagous and non-hematophagous bats for the maintenance of the sylvatic cycle of the disease should be highlighted. In non-hematophagous bats, the virus has been detected in several states in Brazil[6-8]. In the City of Rio de Janeiro, limited data about the virus and disease in wild animals[9] is available. This study aimed to contribute to a better understanding of the movement of the virus in Rio de Janeiro City, and to serve as a warning to local authorities.

METHODS

Data compilation

A survey was undertaken of the record book of the *Seção de Virologia* of the *Instituto Municipal de Medicina Veterinária Jorge Vaitsman* (SV-IJV), and

we analyzed the test results originating from bat samples referred for the diagnosis of rabies. We separated non-hematophagous and hematophagous bats from Rio de Janeiro City, observing the history of each animal, including species, sex, age, and origin, in order to provide a more detailed epidemiological analysis. The species were identified by morphological and morphometrical characteristics by an expert.

Antigenic typification

We carried out serological typing of the viruses isolated from positive samples passed in mice. These were sent to the national reference laboratory, the Instituto Pasteur, in the State of São Paulo, as recommended by the Brazilian Ministry of Health[10]. The brains of the bats were in the kept at the Section, preserved in glycerin, glycerol phosphate, and glycine sucrose, and stored at -20ºC.

Ethical considerations

The laboratory follows strict standards for rabies diagnosis tests, which include the use of animals, as described by Koprowski[11].

RESULTS

In the study decade, a total of 846 bats were submitted for rabies diagnosis. Of these, 555 (65.6%) were non-hematophagous species, and 198 of these (35.7%) came from Rio de Janeiro City, representing 23.4% of bats submitted for diagnosis and an average of ~20 bats per year. In total, 11 (5.6%) non-hematophagous bats from Rio de Janeiro City were positive for rabies.

Of the 11 animals diagnosed as positive, 9 (81.8%) belonged to the genus *Artibeus*, 1 belonged to *Nyctinomops laticaudatus*, and 1 belonged to *Molossus molossus*. Within the predominant genus, 5 animals belonged to *Artibeus lituratus* and 4 belonged to *Artibeus fimbriatus*. Furthermore, 5 of the 11 bats were male and 5 were female; the gender was not identified in 1 bat. From these, 8 were adult bats and 2 were young; the age was not determined in 1 bat (**Table 1**).

Viral typification of the isolates identified the virus present in the 2 species of *Artibeus* as variant 3. In the other 2 species, the isolate from *Nyctinomops laticaudatus* was variant 4, which originated from *Tadarida brasiliensis*, while the variant found in *Molossus molossus* did not belong to the antigenic typification panel used, and was considered as a specific variant of insectivorous bats, which segregates with variant 4. The antigenic profile of this isolate was AgV C4 + C10 + C12.

All positive bats were from highly populated urban areas of the city. In **Figure 1**, it is possible to observe the spatial distribution of rabies-positive bats received from 2001-2010 by the *Seção de Virologia* of the *Instituto Municipal de Medicina Veterinária Jorge Vaitsman*.

TABLE 1 - Year of receiving specimen, district where the specimen was found, species identified, and isolated variant in non-hematophagous bats from Rio de Janeiro City (2001-2010), Brazil.

Year	District	Species	Sex/age	Isolated variant
2001	Piedade	*Artibeus lituratus*	male/mature	V_3 (*Desmodus rotundus*)
2002	São Cristovão	*Artibeus lituratus*	female/NI	V_3 (*Desmodus rotundus*)
2004	Lagoa	*Artibeus lituratus*	female/mature	V_3 (*Desmodus rotundus*)
	São Cristovão	*Nyctinomops laticaudatus*	female/mature	V_4 (*Tadarida brasiliensis*)
2007	São Cristovão,	*Artibeus fimbriatus*	NI/mature	V_3 (*Desmodus rotundus*)
	Campo Grande	*Artibeus fimbriatus*	female/mature	V_3 (*Desmodus rotundus*)V_3
	Urca	*Artibeus fimbriatus*	male/mature	(*Desmodus rotundus*)
2008	Campo Grande	*Molossus molossus*	male/immature	Variant from insectivorous bat (segregates with isolates from *Nyctinomops laticaudatus*)
2009	Rocha	*Artibeus lituratus*	male/mature	V_3 (*Desmodus rotundus*)
2010	Grajaú	*Artibeus fimbriatus*	male/immature	V_3 (*Desmodus rotundus*)
	Tijuca	*Artibeus lituratus*	female/adult	V_3 (*Desmodus rotundus*)

V: variant; **NI:** not identified.

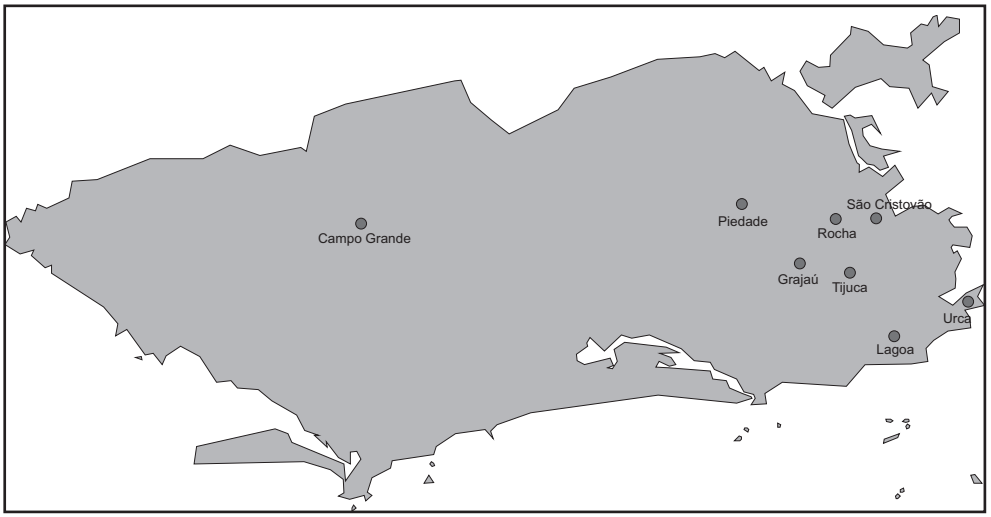

FIGURE 1 - Districts of Rio de Janeiro City, Brazil, where the rabies-positive bats were found.

DISCUSSION

The first definitive diagnosis of rabies in non-hematophagous bats was made for an *Artibeus planirostris* individual in the City of Port of Spain, Trinidad, in 1931[12]; however, it was only in 1953 when attention was given to those new reservoirs, due to an accident involving a 7-year-old boy with a female yellow bat, *Dasypterus floridanus*, which bit him on the chest. The bat was diagnosed as positive for rabies on the basis of the discovery of Negri bodies in its brain by using direct immunofluorescence, with confirmation by a biological test. In this context, reports began to emerge of people who had died from rabies transmitted by non-hematophagous bats, with these reports still being published until today[13].

In this decade, a total of 815 positive cases of rabies in non-hematophagous bats were identified in Brazil, with Rio de Janeiro being responsible for 1.4% of the reported cases[14]. In the national literature, several studies conducted in the State of São Paulo report higher numbers of animals sent for diagnosis. During 1997-2003, Albas et al.[15] reported the analysis of 3,552 bats from the west region of São Paulo, with 1.6% of the bats being positive, although they did not distinguish between hematophagous and non-hematophagous bats. In 2006, Cunha et al.[16] analyzed the presence of rabies virus in 7,393 animals, with 1.3% positivity in non-hematophagous bats. In the following year, Scheffer et al.[2] published the diagnosis results of 4,393 specimens received by the Instituto Pasteur, with 1.9% positivity in non-hematophagous bats. It is possible to observe that, despite the difference in sample size, the positivity of non-hematophagous bats in our study was similar to that in the above mentioned studies. In the City of Rio de Janeiro, a larger study with more samples is required in order to reveal the true prevalence of rabies in non-hematophagous bats. Except for the report of Silva et al.[9], similar studies are lacking in the city and even in the State of Rio de Janeiro. Thus, this paper seeks to contribute to a better understanding of this disease in the city, and to encourage the implementation of health education programs and investment in monitoring systems of these reservoirs in order to increase the number of animals sent for diagnosis. Such measures would be very useful for a better understanding of the disease state and for the implementation of prophylaxis and control.

The low number of animals sent for rabies diagnosis in this study is probably caused by the misinformation of the population in respect to the risk that the bats represent. The media, an important vehicle for disseminating information to the population, does not give due prominence to this fact, which must be stimulated by the relevant authorities. Its performance in this context would be important, mainly in the event of human cases, which would raise the attention of the population and could lead to a rise in the number of bats sent for diagnosis[17]. Such a situation occurred in Brazil for a case that occurred and was confirmed in July 2001, in Dracena City, State of São Paulo, in which a woman died from rabies following infection with a variant 3 virus. The reporting of this case by the media led to a rise in the number of bats sent for diagnosis[15]. The large number of Chiropteran bats has more chances to be detected in densely populated locations, which increases the chances of encountering rabid or even dead bats, such as in Rio de Janeiro, the most populous City of the state, with an estimated population of over 6 million inhabitants[18]. Nevertheless, this increase in the detection of rabid bats will only occur if the population is aware of the risk that animals with abnormal behavior pose, and if they are well informed of the correct steps to be taken, such as to never touch these animals and to call local authorities at the regional *Centro de Controle de Zoonoses*.

A total of 41 species of bats have been diagnosed as being infected with the rabies virus in Brazil[19]. In this study, there was a predominance of species from the *Artibeus* genus, which is to be expected because this genus contains the most species that have adapted for living in cities, and is in agreement with the national literature, whose authors report the presence of non-hematophagous bats belonging to the *Artibeus* genus carrying the virus in different cities and states among Brazil[20].

Regarding the sex and age of the animals, we identified 5 male and 5 female bats; the sex of 1 bat was unidentified. From those bats, 8 were mature bats, 2 were immature bats, and the age of 1 bat could not be determined. This pattern has been identified by other authors, with no difference in the sex distribution and the presence of more mature than immature animals infected[17,19]. According to Sodré et al.[19], few studies include the age of the animals in their reports, and this information could be useful in determining some epidemiologically important means of rabies transmission between bats, such as transmission via milk or the transplacental route.

The presence of variant 3, revealed by viral typification of the isolates in all species of *Artibeus*, is in agreement with other studies, indicating a strong relationship of this variant with bats from this genus[21,22]. However, although it is the same variant, phylogenetic differences between viruses isolated from *Artibeus* and *Desmodus rotundus* have already been demonstrated[22], showing the great capacity of the virus to adapt to different hosts.

The isolation of a variant that does not fit any reaction profile is not uncommon nowadays. Sodré et al.[19] presented the antigenic profile of diverse isolates from Brazil, and it is possible to see that most isolates are not compatible with the standard variant profiles. Nevertheless, the reaction profile of our isolate from *Molossus molossus* was the same as that reported in previous studies[19], but we were unable to further characterize this isolate. More studies are required for a better characterization and comprehension of this isolate.

The circulation of variant 3 in non-hematophagous species complicates issues for health professionals with respect to the diagnosis of human rabies. Although not fully proven, infection by this variant has been associated with the paralytic form of the disease, which is less common and more difficult to diagnose[23]. This fact includes rabies as a differential diagnosis of acute flaccid paralysis, although the disease does not appear in the epidemiological measures according to the Health Ministry. The diagnosis between the different causes of this syndrome is complex, but it is of tremendous importance for the prognosis and treatment of the patient, who may die in the absence of the correct diagnosis[24].

All of the locations from which the positive bats came from are actually densely populated by humans, considering that Rio de Janeiro City is considered a completely urban area. Human encroachment and destruction of the bat's habitat caused a new rearrangement in rabies epidemiology because it has imposed on wild animals, which are potential reservoirs of the virus, the need to adapt to new conditions for their survival. Thus, they started to use human buildings as shelters, or near structures and vegetation, which increased the chances of contact between sick animals, humans, and pets[25].

Excluding Campo Grande, all areas are located close to the geographical distribution of rabies-positive bats, suggesting a concentration in the bat population. Considering that in other locations in Brazil, there is knowledge about endemic areas, and even with the low number of animals sent for rabies diagnosis, the presence of the virus was detected, it may be speculated that an endemic area in the southern zone to the north of Rio de Janeiro may exist. However, the low number of animals assessed in the present study does not allow us to make a more definite conclusion since bats are highly adaptable and could be scattered throughout the city. It is important to consider the presence of stray animals in urban environments, which can become infected and transmit the disease to humans. A large number of positive bats and also rabid stray dogs and cats are found in many places around the City, and could be unnoticed because of underreporting. These observations are in agreement with those of Silva et al.[9]. Despite this not being the focus of this study, the necessity for the effective control of stray animals in the urban environment is evident.

This study makes clear the need for further measures for the effective control of urban rabies. Campaigns for the mass vaccination of dogs and cats, largely responsible for the sharp decline in the incidence of rabies in these animals, must be supplemented with measures concerning synanthropic wild animals, especially non-hematophagous bats, by informing the population about the risks they represent if handled.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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