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Article/Artigo

First report of rabies infection in bats, *Molossus molossus*, *Molossops neglectus* and *Myotis riparius* in the City of São Paulo, State of São Paulo, southeastern Brazil

Primeiro relato de infecção de vírus da raiva em morcegos, *Molossus molossus*, *Molossops neglectus* e *Myotis riparius* na Cidade de São Paulo, Estado de São Paulo

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ABSTRACT

Introduction: This paper presents the first report of rabies in three bat species, *Molossus molossus*, *Molossops neglectus* and *Myotis riparius* in the city of São Paulo, Brazil. **Methods:** Bats were diagnosed as positive for rabies using the fluorescent antibody test and mouse inoculation test. The isolates were characterized antigenically using a panel of eight monoclonal antibodies. The samples were also genetically analyzed by partial sequencing of the portion of nucleoprotein gene between positions 1157 and 1445nt. **Results:** Analysis of the results verified that the sample isolated from the species *M. molossus* presented antigenic variant 6, while the other two samples showed a different profile from that established in the panel, one not previously reported in the literature. The results of genetic analysis revealed that the *M. molossus* sample segregated with *Lasiurus* sp. isolates, *M. neglectus* segregated with a subgroup of *Eptesicus furinalis* isolates and the *Myotis riparius* sample segregated with *Myotis* sp. isolates. **Conclusions:** The cases reported in this paper emphasize the need for clarification of the circumstances in which cases of rabies in wildlife occur, principally in urban areas.

Keywords: Rabies. Chiroptera. Urban area. Phylogenetic analysis.

RESUMO

Introdução: Esse trabalho apresenta o primeiro registro de raiva em três espécies de morcegos: *Molossus molossus*, *Molossops neglectus* e *Myotis riparius* na Cidade de São Paulo, Brasil. **Métodos:** Os morcegos foram diagnosticados como positivos para raiva usando as técnicas padrão de imunofluorescência direta e o teste de inoculação em camundongo. Os isolados foram caracterizados antigenicamente usando um painel de oito anticorpos monoclonais (CDC/Atlanta/USA). As amostras também foram analisadas geneticamente por sequenciamento parcial do gene da nucleoproteína entre as posições 1157 e 1445nt. **Resultados:** O resultado das análises mostrou que as amostras isoladas da espécie *M. molossus* apresentou variante antigênica 6, enquanto as outras duas amostras mostraram um perfil diferente daquele estabelecido no painel e ainda não registrado em literatura. Os resultados da analise genética revelaram que a amostra de *M. molossus* segrega com isolados de *Lasiurus* sp., *M. neglectus* segrega com o isolado do subgrupo de *Eptesicus furinalis* e uma amostra de *M. riparius* segrega com isolados de *Myotis* sp. **Conclusões:** Os casos relatados neste estudo enfatizam a necessidade do esclarecimento da ocorrência de casos de raiva em morcegos, principalmente em áreas urbanas.

Palavras-chaves: Raiva. Chiroptera. Área urbana. Análise filogenética.

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INTRODUCTION

The territorial expansion of the human population, deforestation and construction of projects (roads, dams, mineral extraction and others) have contributed to the displacement of bats to the cities. The urban ecosystem provides many forms of shelter and food for the bat population. With the absence of a satisfactory number of predators, some species of bats, particularly those that are more generalist, i.e., species with less specificity regarding food preferences and habitats, have been taking advantage of these changes[1,2].

Insectivorous bats have proven to be more versatile among the different species of bats in the colonization of cities and are therefore considered highly anthropophilic[3]. Insects attracted by street lighting, widely distributed in the urban environment, are the source of food for these species of bat[2,4].

The city of São Paulo registered the presence of about 40 bats species with different feeding habits, of which 62% belong to the family Molossidae, 20% are Phyllostomidae and 8% are Vespertilionidae[5]. However, as in most Brazilian cities, about 70% of specimens forwarded for rabies diagnoses in this city have insectivorous feeding habits.

Insectivorous bats can gather in colonies of tens to hundreds of individuals in the urban area of São Paulo: roosting in roofs (86.3%); small spaces between buildings, such as expansion joints (8.7%); window-blinds (3.8%); hollow cinder blocks (0.9%) and garages (0.3%). Bats exploring refuges in houses, as reported above, increases the opportunity for contact with humans and their pets.

Bats have been studied as a possible natural reservoir and/or for transmitting pathogens, including the rabies virus[6,7]. In the northern region of Brazil, hematophagous bats are the main reservoirs

of rabies virus in the wild cycle. In urban areas, this virus has been isolated mainly from insectivorous and frugivorous bats[8-12]. However, up to now, no human rabies cases have been registered in Brazil resulting from aggression by these bats involving viral variants related to these species.

In the city of São Paulo, rabies has been controlled. The last case of rabies in humans occurred in 1981 and in pets in 1983[13]. However, the rabies virus is still circulating in the bat populations, with an average of two to four bats diagnosed as infected with the virus per year.

During the period from 1988 to 2009, 46 cases of rabies were reported involving 12 species of bats in the city. Almost all of these cases involved insectivorous bats[14], 23 belonging to the Molossidae family, 21 to Vespertilionidae and two to the Phyllostomidae family; one frugivorous (*Artibeus lituratus*) and one nectarivorous (*Glossophaga soricina*)[14].

Studies of Brazilian samples of rabies virus isolated from different bat species, characterized by the technique of monoclonal antibodies using a panel from the CDC (Centers for Disease Control and Prevention, Atlanta, GA, USA) and gene sequencing, have proven the existence of numerous species involved in the maintenance and transmission of rabies virus in nature, serving as reservoirs and transmitters[7,12,15].

This study reported the first occurrence of rabies in three bat species, two belonging to the Molossidae family (*Molossus molossus* and *Molossops neglectus*) and one to the Vespertilionidae family (*Myotis riparius*). Although *M. molossus* has already been diagnosed as positive for rabies in several other cities and states in the country, this is the first report in the city of São Paulo. For the other two species, this work presents the first reported occurrence of rabies in Brazil.

METHODS

Samples of the rabies virus characterized in this study were obtained from bats captured on three occasions by the Center for Zoonosis Control of the city of São Paulo (*Centro de Controle de Zoonoses*, CCZ-SP) in response to complaints from the population.

In all the events, the bats were diagnosed as positive for rabies using the fluorescent antibody test[16] and the mouse inoculation test[17].

In order to characterize the isolates, these were submitted to antigenic typing using a panel of eight monoclonal antibodies provided by the CDC, Atlanta, USA, for the characterization of rabies virus isolated in Latin American samples[18].

The samples were also genetically analyzed by partial sequencing (289nt) of the portion of the carboxyl-terminal of the nucleoprotein gene between positions 1157 and 1445nt. A phylogenetic tree was constructed using the Neighbor-Joining method (NJ) with Kimura MEGA, version 4. A comparison of samples with the same sequence of proteins was conducted based on the database available in GenBank. The bootstrap values were calculated for 1,000 replicas.

Ethical considerations

The primary reference regarding euthanasia of animals is the AVMA Guidelines on Euthanasia, June 2007[19].

RESULTS

First event

In April 2005, a bat was found hanging on the wall of a residence in the western zone of the city. Two teenagers decided to raise it as a pet. In addition to the teenagers, three adult people came into contact with the bat, though none of them were bitten. After two days with no success in feeding the bat, the CCZ-SP was notified to rescue it. It was an adult female of the *Molossops neglectus* species. As required in the Rabies Control Program Protocol, all the people involved received antirabies treatment.

Second event

In October 2005, the CCZ-SP was called to capture a bat fallen in the yard of a residential home in the northwestern zone of the city. The bat, an adult male of *Myotis riparius*, was still alive. No known contact with people or other animals occurred.

Third event

In March 2008, a bat that entered a 2nd floor apartment located in the northwestern zone of the city was removed by CCZ-SP. The bat, an adult male of *Molossus molossus*, was caught by the family's dog, a female who was nursing and had been previously vaccinated. The same was taken to the veterinarian and received a booster vaccination against rabies.

Antigenic characterization: the reaction patterns observed in bats samples with the 8 pre-established reactivity patterns that characterize rabies virus variants in animals involved with rabies virus maintenance and transmission in Latin America are reported in **Table 1**.

Genetic characterization: the results of the genetic analysis revealed that the isolates segregated with other isolates from insectivorous bats in Brazil and other countries from the Americas, resulting in independent clades with high bootstrap values (**Figure 1**).

TABLE 1 - Reaction patterns detected in isolates of bats from the city of São Paulo, SP, Brazil.

Bats species	C1*	C4*	C9*	C10*	C12*	C15*	C18*	C19*	AgV
Myotis riparius	-	-	+	+	+	-	+	-	NC
Molossus molossus	+/-	+	+	+	+	-	-	-	6
Molossops neglectus	-	+	-	+	+	-	-	-	NC

C1: 62-3-1, C4: 62-15-2, C9: 62-52-1, C10: 62-52-2, C12: 62-62-4, C15: 62-97-3, C18: 62-143-2, C19: 62-146-3, AgV: Antigenic Variant, NC: Not compatible.

*Monoclonal antibodies against the rabies virus nucleoprotein selected by the CDC (Centers for Disease Control and Prevention), Atlanta, USA and PAHO (Pan-American Health Organization) to identify variants of viruses existing in Latin America.

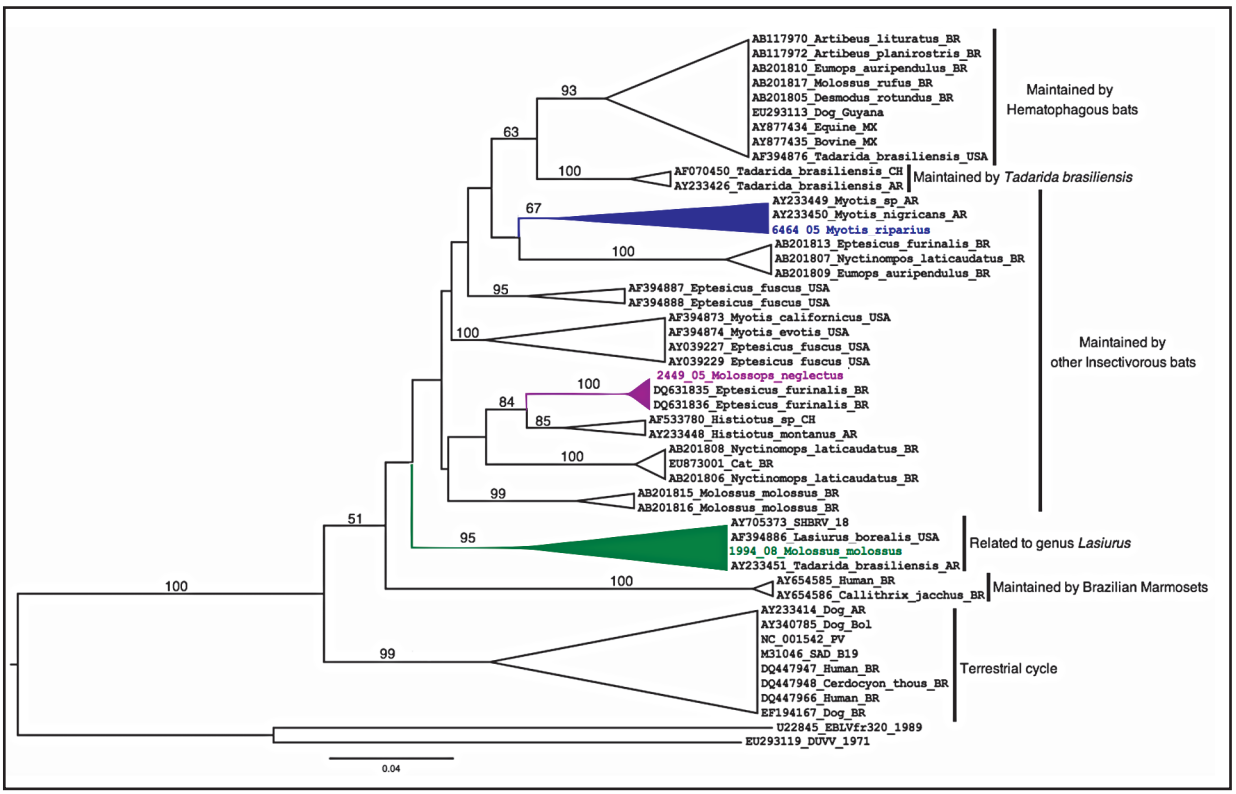

FIGURE 1 - Phylogenetic tree based on partial sequencing (289nt) of the carboxyl-terminal portion of the nucleoprotein gene between positions 1157 to 1445nt, constructed using the Neighbor-Joining method (NJ) with Kimura MEGA. The bootstrap values were calculated for 1,000 replicas.

PV and SAD_B19: vaccine strains, DUVV: Duvenhage virus, EBLVfr: European Bat Lyssavirus from France, SHBRV: Silver Haired Bat Rabies Virus, BR: Brazil, AR: Argentine, CH: Chile, MX: Mexico, USA: United States of America. Codes AB117970, AB117972, AB201805-AB201810, AB201813, AB201815-AB201817, AF070450, AF394873, AF394874, AF394876, AF394886-AF394888, AF533780, AY039227, AY039229, AY233414, AY233426, AY233448-AY233451, AY340785, AY654585, AY654586, AY705373, AY877434, AY877435, DQ447947, DQ447948, DQ447966, DQ631835, DQ631836, EF194167, EU293113, EU293119, EU873001, M31046, NC_001542, U22845: GenBank Accession Number.

DISCUSSION

Of the bats diagnosed as positive for rabies in the city of São Paulo from 1988 to 2009, 38 (82.6%) were found or captured in atypical circumstances and at unusual times, such as on the ground, inside residences and hanging in buildings, including the three positive cases reported in this paper and 8 (17.4%) were captured while roosting in roofs.

Bats of the Molossidae family are the most predominant in urban areas in Brazil and a number of its species are those most frequently reported inside houses or having invading homes.

Although *M. molossus* is one of the most abundant bats in the city of São Paulo, representing 40% of the bats collected from 1988 to 2009[14], only in March 2008 was the first occurrence of the disease registered in this species. This fact demonstrates that rabies virus circulates at a low level among this species, which has a positivity of approximately 0.06% (1711/1) in this city.

For *Molossops neglectus* (Molossidae) and *Myotis riparius* (Vespertilionidae), species that have low population densities and few registered captures in the State or city of São Paulo, this is the first occurrence of a positive diagnosis for rabies in Brazil.

In urban areas, bats use shelters in numerous buildings, indicating high human synanthropy. Although the possibility of accidents involving bats, humans, dogs and cats exists, the risk can be considered low, since positivity for rabies in bats is less than 1% in the city of São Paulo[14].

Of the 46 bats diagnosed as positive in this city over the past 21 years, 23.9% came into direct contact with people and 13.0% with domestic animals. In these circumstances, all the individuals were instructed to seek immediate medical attention and the animals were forwarded for veterinary medical consultation. Moreover, the actions and guidelines recommended in the State Program of Rabies Control for the State of São Paulo were followed.

Given the events outlined here, the facts emphasize the importance of health agencies in orientating the public not to directly handle bats, especially when found in atypical situations, and highlight the importance of maintaining the vaccination of domestic animals up to date.

Regarding antigenic characterization, analysis of the results verified that the sample isolated from the species *M. molossus* presented antigenic variant 6, associated with the insectivorous bat *Lasiurus cinereus*. The other two samples showed a different profile from those established in the panel, one not previously reported in the literature (**Table 1**).

The sample from *M. molossus* segregated with *Lasiurus* sp. isolates, bats that are also insectivorous. Although these two species have the same feeding habits, they roost in different shelters in the city of São Paulo. *M. molossus* roosts in the roofs of buildings and forms medium to large colonies, unlike *Lasiurus* sp., which roosts alone or forms small colonies, usually in palm trees. The *M. neglectus* sample segregated with a subgroup of *Eptesicus furinalis* isolates. This group (*E. furinalis*) consisted of five specimens from the same

colony collected in the city of Jundiaí, about 60km from the origin of the sample (DQ631835 and DQ6318356). The two species that form this clade are insectivorous bats. The sample of *Myotis riparius* segregated with samples of *Myotis* sp., isolated in Argentina (**Figure 1**). The cases reported in this paper emphasize the need for clarification of the circumstances in which cases of rabies in wildlife occur, principally in urban areas. This is important to continue to expand current knowledge concerning the rabies potential of bats in the epidemiological rabies cycle and, consequently, to establish appropriate measures for the management and control of this population, including considering their behavior and biology. Moreover, it is important to respond to the population's general lack of knowledge by providing guidance concerning prevention, health promotion for humans and domestic animals and inform the public regarding the real benefits and risks involving bats.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Fenton MB. Bats. New York: Facts on File Incorporation; 1992.
2. Bredt A, Silva DM. Morcegos em áreas urbanas e rurais: manual de manejo e controle. Brasília: Fundação Nacional de Saúde. Ministério da Saúde; 1996.
3. Taddei VA, Vizotto LD, Sazima I. Uma nova espécie de *Lonchophylla* do Brasil e chave para identificação das espécies do gênero (Chiroptera, Phyllostomidae). Cienc Cult 1983; 35:625-629.
4. Rydell J, Racey PA. Street lamps and the feeding ecology of insectivorous bats. Paper presented at: Symposium the Zoological Society of London; 1995; London, England.
5. Martorelli LFA, Almeida MF, Kataoka APAG, Gama AR, Sodré MM, Trezza-Neto J, et al. Full-length sequence of rabies virus from insectivorous bats, São Paulo City, Brazil. Paper presented at: XX International Conference on Rabies in the Americas. Abstract Book; 2009; Quebec, Canada.
6. Brandão PE, Scheffer K, Villarreal LY, Achkar S, Oliveira RN, Fahl WO, et al. Coronavirus detected in the vampire bat *Desmodus rotundus*. Braz J Infect Dis 2008; 12:466-468.
7. Favoretto SR, Carrieri ML, Cunha EMS, Aguiar EAC, Silva LHQ, Sodré MM, et al. Antigenic typing of Brazilian rabies virus samples isolated from animals and humans, 1989-2000. Rev Inst Med Trop Sao Paulo 2002; 44:91-95.
8. Langoni H, Lima K, Menozzi BD, Silva RC. Rabies in the big fruit-eating bat *Artibeus lituratus* from Botucatu, Southeastern Brazil. J Venom Anim Toxins Incl Trop Dis 2005; 11:84-87.
9. Cunha EMS, Silva LHQ, Lara MCCSH, Nassas AFCN, Albas A, Sodré MM, et al. Bat rabies in the north-northwestern regions of the state of São Paulo, Brazil: 1997-2002. Rev Saude Publica 2006; 40:1082-1086.
10. Scheffer KC, Carrieri ML, Albas A, Santos HCP, Kotait I, Ito FH. Rabies virus in naturally infected bats in the state of São Paulo, Southeastern Brazil. Rev Saude Publica 2007; 41:389-395.
11. Silva MV, Xavier SM, Moreira WC, Santos BCP, Esberard CEL. Vírus rábico em morcego *Nyctinomops laticaudatus* na cidade do Rio de Janeiro, RJ: isolamento, titulação e epidemiologia. Rev Soc Bras Med Trop 2007; 40:479-481.
12. Castilho JG, Canello FM, Scheffer KC, Achkar SM, Carrieri ML, Kotait I. Antigenic and genetic characterization of the first rabies virus isolated from the bat *Eumops perotis* in Brazil. Rev Inst Med Trop Sao Paulo 2008; 50:95-99.
13. Ramos PM, Ramos OS. Acidentes humanos com macacos em relação a tratamentos profiláticos para a raiva, no município de São Paulo, Brasil. Rev Soc Bras Med Trop 2002; 35:575-577.
14. Kataoka APAG, Martorelli LFA, Almeida MF, Gama AR, Sodré MM, Amatuzi E, et al. Bats rabies virus in São Paulo City, Brazil, from 1988 to 2009. Paper presented at: XX International Conference on Rabies in the Americas; 2009; Quebec, Canada.
15. Albas A, Souza EAN, Lourenço RA, Favoretto SR, Sodré MM. Antigen profile of rabies virus isolated from different species of non-hematophagous bats in the region of Presidente Prudente, State of São Paulo. Rev Soc Bras Med Trop 2009; 42:15-17.
16. Dean DJ, Abelseth MK, Atanasiu P. The fluorescent antibody test. *In:* Meslin FX, Kaplan MM, Koprowisk H, editors. Laboratory techniques in rabies. 4th ed. Genebra: World Health Organization; 1996.
17. Koprowisk H. The mouse inoculation test. *In:* Meslin FX, Kaplan MM, Koprowisk H, editors. Laboratory techniques in rabies. 4th ed. Genebra: World Health Organization; 1996.
18. Pan-American Health Organization. Los Anticuerpos Monoclonales en la Caracterización y Vigilancia de los Virus de la Rabia en América Latina y el Caribe. Pan Am J Public Health 2000; 8:214-217.
19. American Veterinary Medical Association. AVMA Guidelines on Euthanasia. Formerly Report of the AVMA Panel on Euthanasia. Washington: American Veterinary Medical Association; 2007. Available from: http://www.avma.org/issues/animal_welfare/euthanasia.pdf/.