Received: January 23, 2004
Accepted: May 11, 2004
Published online: February 11, 2005.

J. Venom. Anim. Toxins incl. Trop. Dis.
V.11, n.1, p.84-87, 2005.
Short communication - ISSN 1678-9199.

RABIES IN THE BIG FRUIT-EATING BAT *Artibeus lituratus* FROM BOTUCATU, SOUTHEASTERN BRAZIL

LANGONI H. (1), LIMA K. (1), MENOZZI B. D. (1), SILVA R. C. (1)

(1) Zoonoses Searches Nucleus (NUPEZO) – School of Veterinary Medicine and Animal Husbandry (FMVZ), UNESP, Botucatu, São Paulo, Brazil.

ABSTRACT: Rabies is a viral disease of mammals transmitted through the bite of a rabid animal. A frugivorous adult male bat, *Artibeus lituratus*, family Phyllostomidae, was diagnosed as positive to rabies by direct immunofluorescence (DIF) and mouse inoculation test (MIT) of the bat's brain, both performed at the School of Veterinary Medicine and Animal Husbandry - FMVZ, UNESP, Botucatu, São Paulo State, Brazil. The animal collided with the window of a commercial establishment in the urban area during the day. With regard to DIF, a high amount of Negri bodies of several sizes was observed in the brain. The spleen and right kidney presented some Negri bodies too. In relation to MIT, the mice presented paralysis in the 7th day, and died in the day after with several characteristic small bodies. The reverse transcriptase-polymerase chain reaction (RT-PCR), followed by *hemi-nested* RT-PCR (hnRT-PCR) resulted in an amplification of fragments from the bat's brain viral RNA, 432bp in RT-PCR, and 274bp in hnRT-PCR, confirming the diagnosis. Therefore, the hnRT-PCR and DIF have good sensitivity and specificity, providing and confirming the diagnosis of the clinical samples in a short period of time.

KEY WORDS: southeastern Brazil, rabies, Phyllostomidae, frugivorous bat.

CORRESPONDENCE TO:
H. LANGONI, Núcleo de Pesquisas em Zoonoses, Faculdade de Medicina Veterinária e Zootecnia, UNESP, Distrito de Rubião Jr, s/n, Caixa Postal 560, 18.618-000, Botucatu, SP, Brasil. Phone: 55 14 3811-6270. Fax: 55 14 3811-6075. Email: hlangoni@fmvz.unesp.br

INTRODUCTION

Rabies is a preventable viral disease of mammals, often transmitted through the bite of a rabid animal. Rabies viruses are highly neurotropic, infecting the central nervous system, and causing encephalopathy and ultimately death in all warm-blooded species. The principal rabies hosts today are wild carnivores and bats. These viruses replicate in muscle cells before invading the peripheral and central nervous system (5,6).

A research on the rabies virus isolation from bats in Botucatu, São Paulo, from 1992 to 1993, identified two positive frugivorous bats, both *Carolia perspicillata*, representing 0.92% of the total bats (2/216) and 1.43% of the non-hematophagous bats (2/140) (8).

In the period from 1992 to 2000, in Botucatu, we verified that 3/1480 (0.2%) bats examined showed low positivity to rabies virus isolation.

One of these, a hematophagous bat, *Desmodus rotundus*, was captured in Anhembi (60 km distant from Botucatu). The other two were insectivorous bats: a *Tadarida brasiliensis*, found at UNESP, Botucatu, during the day, in 1996; and a *Molossus molossus*, captured in the rural area (25 km distant from Botucatu) at night, in a colony of 12 animals (7).

After any human exposure, the animal's brain should be rapidly examined in order to detect lyssavirus infection. Antigen detection by direct immunofluorescence (DIF) is the most widespread screening method. Results are usually confirmed by mouse inoculation test (MIT) or viral isolation on murine neuroblastoma cells. More recently, RNA detection by reverse transcription-PCR (RT-PCR) has been proposed as a rapid and sensitive alternative. However, as RT-PCR is not as faster as DIF, and rabid animals' brains usually contain high amounts of virus, very few laboratories have adopted this technique. On the other hand, direct sequencing of RT-PCR products is the most common technique for virus identification and molecular epidemiology studies (2,3).

Located in the south-central region of São Paulo State, at an altitude of 700-900m in the top of the Cuesta and 400-500m in its front, Botucatu (22º52'20"S, 48º26'37"W) has an area of 1496 km^2 at the Superior Median Tiete Hydrographic Basin, which drains into the Tiete River Basin to the north and the Pardo River Basin to the south (4).

On August 20, 2003, a frugivorous adult male bat, *Artibeus lituratus*, family Phyllostomidae, was diagnosed as positive to rabies by the Zoonoses Diagnostic Service (SDZ) of the Veterinary Hygiene and Public Health Department (DHVSP), located at the School of Veterinary Medicine and Animal Husbandry (FMVZ), UNESP, Botucatu, São Paulo. It collided with the window of an urban commercial establishment during daytime, probably due to behavior alteration. The animal was taken to SDZ, where DIF and MIT of the brain were performed. With regard to DIF, a high amount of very characteristic Negri bodies of several sizes was observed. We also found few small fluorescent Negri bodies in the spleen and right kidney. The inoculated mice that had positive brain suspension developed paralysis since the 7th day; one of them was found dead in the 8th day; and the others died in this same day, presenting great amounts of characteristic small bodies, what confirmed the diagnosis.

In the reverse transcriptase-polymerase chain reaction (RT-PCR) of the bat's brain viral RNA, the external primers 505 (5'ATAGAGCAGATTTTCGAGACAGC3') and 937 (5'CCTCAAAGTTCTTGTGGAAGA3') were used in the synthesis of the complementary DNA (cDNA) by reverse transcription reaction and amplification of the cDNA obtained in this reaction, producing a fragment of 432 bp. The samples were submitted to an initial extension of 94ºC for 3 minutes; 35 cycles of 94ºC for 45 seconds, 55ºC for 1 minute, and 72ºC for 10 minutes; and final extension of 72ºC for 10 minutes.

This product was reamplified by *hemi-nested* RT-PCR (hnRT-PCR), using the internal primer 779 (5'CCCATATAACATCCAACAAAGTG3'), and generating a fragment of 274 bp. The hnRT-PCR was submitted to the same protocol of RT-PCR amplification. Both RT-PCR and hnRT-PCR were performed as described by Luciane M. Alves, 2001 (1).

Despite this isolation of the rabies virus be in a single specimen, the objective of this report is to emphasize the importance and the role of this frugivorous bat species in the aerial cycle of rabies, and the possible transmission to other species of bats and also to man. The virus isolation from bats and its characterization are important activities of epidemiological surveillance for the rabies control.

REFERENCES

1 ALVES LM. *Patogenia do vírus rábico; amostras ERA e PV administradas por via oral em hamster (M. auratus)*. São Paulo: Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, 2001. 44p. [Dissertação – Mestrado].

2 BOURHY H., KISSI B., AUDRY L., SMRECZAK M., SADKOWSKA-TODYS M., KULONEN K., TORDO N., ZMUDZINSKI JF., HOLMES EC. Ecology and evolution of rabies virus in Europe. *J. Gen. Virol.*, 1999, 80, 2545-57.

3 HEATON PR., McELHINNEY LM., LOWNINGS JP. Detection and verification of rabies and rabies related viruses using rapid cycle PCR. *J. Virol. Methods*, 1999, 81, 63-9.

4 MARTINS D. *Classificação climática de Botucatu e São Manuel*. Botucatu: Departamento de Ciências Ambientais – Faculdade de Ciências Agronômicas/UNESP, 1996. 2p.

5 MURPHY FA., BAUER SP. Early street rabies virus infection in striated muscle and later progression to the central nervous system. *Intervirology*, 1974, 3, 256-68.

6 RAY NB., EWALT LC., LODMELL DL. Rabies virus replication in primary murine bone marrow macrophages and in human and murine macrophage-like cell lines: implications for viral persistence. *J. Virol.*, 1995, 69, 764-72.

7 SOUZA LC. *Vigilância epidemiológica no município de Botucatu : significado dos quirópteros na manutenção do vírus rábico*. Botucatu: Universidade Estadual Paulista, Faculdade de Medicina Veterinária e Zootecnia, 2001. 60p. [Tese – Livre-Docência].

8 SOUZA LC., LANGONI H., CAMARGO FM., PEREIRA VG., CAMARGO MJB. *Quirópteros como reservatório de raiva no município de Botucatu*. In: *VIROLÓGICA*, 93, Porto Alegre, 1993. Resumos... Porto Alegre, Sociedade Brasileira de Virologia, 1993. 275.