NOTE *Virology*

Genetic Characterization of Rabies Viruses Isolated from Frugivorous Bat (*Artibeus* spp.) in Brazil

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(Received 6 February 2004/Accepted 13 May 2004)

ABSTRACT. In Latin America, rabies cases related to frugivorous bats have been reported since 1930's. Recently, two viruses isolated from *Artibeus lituratus* were proved to be vampire bat variants by monoclonal antibodies panels [2], but their genetic information is not well known. In this report, four rabies viruses were isolated from frugivorous bats (*Artibeus* spp.) in Brazil and their nucleoprotein gene sequences were determined. These isolates were found to be genotype 1 of lyssavirus and showed the maximum nucleotide sequence homology of 97.6–99.4% with vampire bat-related viruses in Brazil [6]. These results indicate that the Brazilian frugivorous bat rabies viruses in this study are closely related to vampire bat-related viruses that play a main role in rabies virus transmission to livestock in Brazil.

KEY WORDS: Brazil, frugivorous bat, rabies.

J. Vet. Med. Sci. 66(10): 1271–1273, 2004

Rabies in non-haematophagous bat was first definitively diagnosed in frugivorous bat (*Artibeus planirostris*) in Trinidad, 1931 [10]. After then, a few reports had described the detection of rabies virus antigen and/or rabies virus neutralizing antibody in *Artibeus* spp. in Central and South America [2, 11, 12]. In 1997, two rabies virus strains isolated from frugivorous bats (*Artibeus lituratus*) were examined with monoclonal antibodies for the first time and proved to be vampire bat variants [2].

Although the outbreaks of rabies in livestock transmitted by vampire bats have been recognized early in Latin America, the serious economic problem still remains [1]. We previously reported that major reservoirs of rabies virus in Brazil were dogs and vampire bats by analyzing 50 isolates [6]. Furthermore, the number of bat-transmitted human cases has been increasing in Americas [3, 8, 15]. However, the dynamics of the epidemiology of bat rabies virus in Brazil still remain unknown. In particular, little is known about epidemiological relationships of rabies between vampire bat and other species. In this study, we analyzed four rabies viruses isolated from frugivorous bat (*Artibeus* spp.) in São Paulo, Brazil to characterize genetic properties of these viruses and to investigate their epidemiological status in rabies virus maintenance in Brazil. This is the first available report to describe genetic characterization of rabies virus from frugivorous bat species (*Artibeus* spp.) and would give valuable information for epidemiological and public health surveillance of rabies outbreaks in South America.

Four frugivorous bats (3 *Artibeus lituratus* (AL), 1 *Artibeus planirostris* (AP)) were captured in São Paulo, Brazil in 1998. Brain materials from these bats were diagnosed as rabies-positive by means of fluorescent antibody test (FAT) using antibodies against rabies virus nucleoprotein (SANOFI DIAGNOSTIC PASTEUR, Paris, France) and passaged intracerebrally in mice followed by FAT for the verification of rabies-positive. RNAs were extracted from mouse brain suspensions with a commercial reagent (QIAamp Viral RNA Mini Kit, QIAGEN). RT-PCR and sequencing of the complete coding region of the nucleoprotein (N) gene was performed using sense primers: JW12 (5'-ATGTAACACCYCTACAAT-3', [4]), P1 (5'-CTACAATGGATGCCGACAAGA-3', [5]), BRABN-S1 (5'-GGACTAGCTATGGAATCCTG -3', [6]) and BRABN-S3 (5'-GGACTGGTGTCATTTACAGG-3', [6]), and antisense primers: N8 (5'-AGTTTCTTCAGCCATCTC-3', [7]), P2 (5'-CCCATATAACATCCAACAAAGTG-3', [5]) and BRABN-C3 (5'-TGTCCAGAGATTTTGCTCA-3'). The sequences of 1,332 bp corresponding to nucleotides 89-1,420 on the N gene determined in this study and retrieved from GenBank (Table 1) were used for phylogenetic analy-

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Table 1. Viruses used in this study

Name	Country of isolation	Year of isolation	Host	Variant host	Accession no.
BR-AL1	Brazil (São Paulo)	1998	*Artibeus lituratus*[c]		AB117969[a]
BR-AL2	Brazil (São Paulo)	1998	*Artibeus lituratus*[c]		AB117970[a]
BR-AL3	Brazil (São Paulo)	1998	*Artibeus lituratus*[c]		AB117971[a]
BR-AP1	Brazil (São Paulo)	1998	*Artibeus planirostris*[c]		AB117972[a]
BRdg2	Brazil (Goias)	1999	Dog		AB083792[b]
BRdg15	Brazil (Goias)	1999	Dog		AB083798[b]
BRhr31	Brazil (Goias)	1998	Horse	*Desmodus rotundus*[c]	AB083804[b]
BRbv32	Brazil (São Paulo)	1994	Cow	*Desmodus rotundus*[c]	AB083805[b]
BRbv39	Brazil (Tocantins)	1999	Cow	*Desmodus rotundus*[c]	AB083811[b]
BRbv45	Brazil (Mat Grosso)	1999	Cow	*Desmodus rotundus*[c]	AB083814[b]
BRbv49	Brazil (São Paulo)	1989	Cow	*Desmodus rotundus*[c]	AB083817[b]
BRbv50	Brazil (Goias)	1999	Cow	*Desmodus rotundus*[c]	AB083818[b]
BRA-VB	Brazil	1985	Vampire bat		U22479
TRI-DR	Trinidad	1995	Cow	*Desmodus rotundus*[c]	AF351852
PER-HM	Peru	1996	Human	*Desmodus rotundus*[c]	AF045166
USA-TB	USA(FL)	1988	*Tadarida brasiliensis*[c]		AF394876
USA-EF	USA(AR)	1999	*Eptesicus fuscus*[c]		AY170397
USA-MC	USA(CA)	1987	*Myotis californicus*[c]		AF394871
CAN-LN	Canada	1980	*Lasionycteris noctivagans*[c]		AF351841
CHI-IB	Chile	1988	Insectivorous bat		AF351850
CHI-TB	Chile	1987	*Tadarida brasiliensis*[c]		AF070450
CAN-RC	Canada	?	Raccoon		U27220
USA-RC	USA	?	Raccoon		U27218
MEX-DG	Mexico	1991	Dog		U22477
FRA-FX	France	1991	Red fox		U22474
YUG-FX	Yugoslavia	1972	Red fox		U22839
NAM-JK	Namibia	1992	Jackal		U22649
NIG-HM	Nigeria	1983	Human	Dog	U22488
SRI-BV	Sri Lanka	2001	Cow		AY138550
THA-HM	Thailand	1983	Human	Dog	U22653
Mokola	?	?	?		Y09762
ABL	Australia	1998	Human		AF418014

a) Isolates from fruit bat (*Artibeus* spp.) in this study.
b) Described in the work of Ito *et al.* [5].
c) Scientific names are used only within the bat origins.

sis by the neighbor-joining methods using the Clustal X program [13] and the bootstrap probabilities of each node were calculated using 1,000 replicates. The TREEVIEW program [9] was used to obtain the graphic output.

The 1,411-nt sequences (corresponding to nucleotides 71–1,482 of PV sequence, [14]) of the viruses isolated from Brazilian frugivorous bats (BR-FBs: BR-AL1, BR-AL2, BR-AL3 and BR-AP1) were determined by direct sequencing. The nucleotide homology within BR-FBs showed greater than 99.0% and the amino acids homology showed 100%. Fifteen nucleotide substitutions existed in the 1,411-nt sequences in BR-FBs and all of them were synonymous substitutions (data not shown). The nucleotide and deduced amino acid sequence analysis and phylogenetic analysis were carried out on 1,332 nucleotide of BR-FBs and 26 representative rabies viruses (genotype (GT) 1), Mokola virus (GT 3) and Australian bat lyssavirus (ABLV) (GT 7). The nucleotide sequence identity of BR-FBs with isolates of GT 1, Mokola virus and ABLV was 83.8–99.4%, 72.2–72.5 % and 77.9–78.1 %, respectively, showing that BR-FBs belong to GT 1 of lyssaviruses. Among the isolates of GT 1, BRbv50 and PER-HM showed the greatest nucleotide identity with BR-FBs (98.9–99.4 %), and 100% amino acid identity. And the other bat-related isolates of GT 1 showed 88.6–97.9% nucleotide and 96.8–99.6% amino acid identity with BR-FBs, respectively. On the other hand, the nucleotide and amino acid sequence homology between BR-FBs and carnivoran-related isolates was 84.0–85.7% and 95.5–97.2%.

Phylogenetic analysis was carried out to figure out the genetic relationship between BR-FBs and the other worldwide isolates by using the Mokola virus as an outgroup (Fig. 1). The GT 1 viruses, analyzed in this study, were divided into two groups. One group was made up of carnivora-related isolates and the other was consisted of bat-related isolates including BR-FBs and isolates from raccoon in North America. BR-FBs formed a cluster with vampire bat-related isolates in Brazil [6], Trinidad and Peru. Furthermore, all BR-FBs consisted one sub-group with PER-HM, BRbv32, BRbv49 and BRbv50 with high bootstrap value 96.8%.

These data suggested that BR-FBs are genetically homo-

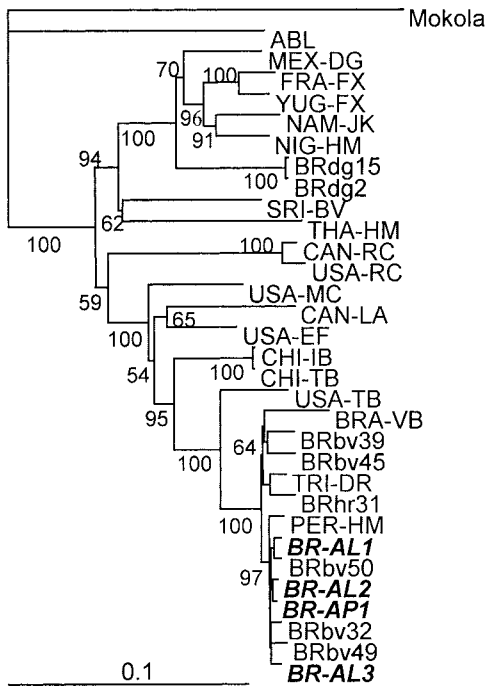

Fig. 1. Neighbor-joining tree is described in the figure based on 1,332 nucleotide sequences of the N gene of 32 isolates shown in Table 1. The four isolates determined in this study are shown in bold italics. Percentage bootstrap values out of 1,000 replicates are indicated at each corresponding nodes.

geneous and had a close relationship to vampire bat-related rabies viruses isolated in South America. In 1997, Delpietro *et al*. reported that isolates from *Artibeus lituratus* were antigenically same variants as vampire bat-related viruses [2].

In this work, we have determined N gene nucleotide sequences of rabies viruses isolated from frugivorous bats (*Artibeus* spp.) for the first time. The phylogenetic analysis reveals that these viruses are closely related to vampire bat-related rabies viruses that are important in rabies virus transmission to livestock in Brazil. Further study should be carried out to investigate relationships between vampire bat and this genus (*Artibeus*) in the context of rabies epidemiology in Brazil

ACKNOWLEDGEMENT. We are grateful to Marli F. C. SANTOS for providing some isolates used for phylogenetic analysis in this study. This work was financially supported in part by a Grant-in-Aid for Scientific Research (No. 14405032) from the Japan Society for the Promotion of Science.

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