

Case Report

Rabies Virus Related to Vampire Bats (*Desmodus rotundus*) Isolated from a Crab-Eating Fox (*Cerdocyon thous*) in Southeast Brazil

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Abstract

In this study, rabies virus was isolated from a naturally infected crab-eating fox [*Cerdocyon thous*] in a rural area of São Paulo State, Brazil. The sample was positive for the standard techniques used for rabies diagnosis. The isolate was antigenically characterized using anti-nucleoprotein monoclonal antibodies. Studies on molecular epidemiology by patterns of nucleotide substitution in the nucleoprotein gene showed that the isolate was genetically homologous to the rabies variant maintained and transmitted by the common vampire bat *Desmodus rotundus*. This is the first report of detection of this variant from a crab-eating fox in Brazil and represents evidence of cross-species transmission. This was the unique report where this variant was isolated from a crab eating fox in Brazil.

INTRODUCTION

Rabies occurs in two main epidemiologic cycles, each involving different animal species and virus variants: a terrestrial one maintained mainly by domestic dog and several wild mammals and an aerial cycle involving different bat species [1]. The incidence of rabies transmitted by domestic animals has decreased in Brazil and in others countries from Latin America in the past years, while the importance of rabies maintained and transmitted by wild animals (bats and terrestrial mammals) has increased [2,3]. In Brazil, the rabies virus variant maintained by vampire bats is the second in importance for transmission to humans, the most important for livestock, with important economic losses and the most found in dogs and cats in areas where dog rabies has been controlled [2].

Five different rabies virus genetic lineages were identified in human cases in Brazil: two maintained by domestic dogs, one by the common vampire bat *Desmodus rotundus*, one by marmosets (*Callithrix jacchus*) and one by crab-eating foxes

(*Cerdocyon thous*) [4,5]. The last two lineages were identified in the Northeast region and restricted to this part of the country. Although there are some independent virus lineages maintained by non-hematophagous bat populations in Brazil, no human cases due to these variants were reported to date [2,5].

MATERIALS AND METHODS

A juvenile crab-eating fox was found ill in a rural area in the city of Matão (São Paulo State, Southeast region of Brazil) in 2000, without report of aggression to humans or domestic animals and died after 48 hours. All the contacts were submitted to post-exposure prophylaxis. The animal was diagnosed as positive for rabies by Fluorescent Antibody Test (FAT) and Mouse Inoculation Test (MIT).

The sample (brra5301_2000) was antigenically characterized using a panel of eight rabies virus anti-nucleoprotein monoclonal antibodies against rabies virus pre-established by the Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA) for the

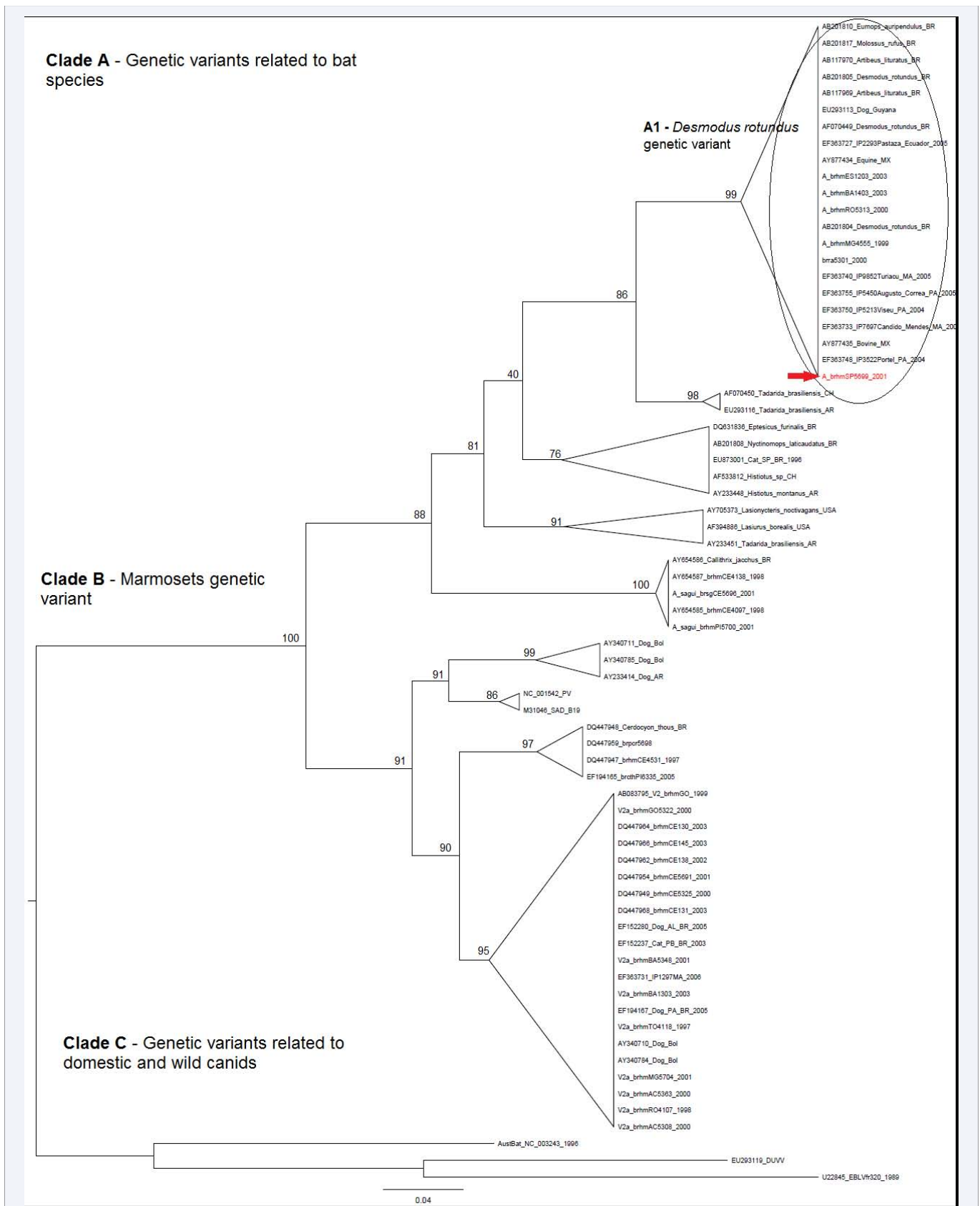


Figure 1 Phylogenetic tree of nucleotides from the carboxi-terminal region of the N-gene of the rabies virus sequence obtained from the isolate of a *Cercopithecus thous* in Brazil and representative sequences from Latin America. The tree was obtained with the GARLi program, using the maximum likelihood method. The verification of the continuity of the topology was made using bootstrap analysis of 10000 models.

antigenic characterization of rabies isolates in epidemiologic for studies in Latin America, was used [6,7].

The genetic characterization was performed using Reverse Transcriptase PCR (RT-PCR) as previously described [8,9] using the primers 21g and 304 with a 1478bp amplicon. The RT-PCR products were sequenced using the Sanger methodology. The obtained sequence was edited using the Bio-Edit program. The genetic comparison were carried out using a 320 base pairs, between the 1157 and 1476 positions, corresponding to the amino terminal portion of the nucleoprotein gene. The phylogenetic tree was reconstructed using the maximum-likelihood (ML) method with Kimura-2 evolutionary distance correction statistics. The branching pattern was statistically evaluated by bootstrap analysis of 10000 replicates with the GARLi program to determine the confidence value of each node. Trees were converted to a graphic format using the FigTree program [http://tree.bio.ed.ac.uk]. The sequence from this study was compared with homologous sequences available on GenBank and related those circulating in the main rabies reservoirs in Latin America.

RESULTS AND DISCUSSION

The antigenic pattern observed was identical to the antigenic variant 5 (AgV-5), one of the profiles related to vampire bats and described in the panel used. In Brazil, the more common antigenic variants in this panel, especially those responsible for human cases, are the AgV-2, related to domestic and wild canids and the AgV-3, related to *Desmodus rotundus* bats [6,7].

The genetic sequence obtained from sample brra5301_2000, GenBank accession number KX443700, showed 99% identity [e-value: 1e-159, BLASTN search] with rabies viruses belonging to the *Desmodus rotundus* genetic lineage isolated in Brazil.

The obtained phylogenetic tree obtained Figure (1) with the selected sequences presented three different clades: clade A, formed by lineages maintained and transmitted by hematophagous and non-hematophagous bat populations; clade B, representing a genetic lineage related to marmosets and clade C, composed by lineages maintained and transmitted by domestic and wild canids populations. Sample brra5301_2000 segregated in Clade A and in the genetic subgroup maintained by *Desmodus rotundus* (A1), and this relationship was statistically supported by high bootstrap values (99% for group A1). In Brazil, the genetic lineage associated with *Desmodus rotundus* is usually antigenically characterized as AgV-3, but AgV-5, the antigenic variant identified in this study, is also related to vampire bats.

The crab-eating fox is a relatively common wild canid found throughout Latin America where it occupies diverse habitats and adapts well to deforestation, agricultural and horticultural development [10].

In the Northeast region of Brazil, the rabies virus maintained and transmitted by the crab-eating fox is well characterized and studied [11,12]. This wild species is responsible for the maintenance of an independent genetic lineage, a spillover from the lineage maintained by domestic dogs, and this variant is also antigenically characterized as AgV-2 (the same of domestic dogs in Brazil). The lineage is restricted to the Northeast region, transmitting to humans and other wild and domestic

animals species [2,12]. The crab-eating fox is responsible for a considerable annual number of aggressions [bites and a smaller percentage of scratches] and post-exposure treatments in humans and in diagnosed rabies cases reported in these animals [13].

The genetic lineage identified in the sample under study is that maintained by *Desmodus rotundus*. This is the first and only occurrence in which this variant has been isolated from a *Cerdocyon thous* in Brazil. The isolation of the vampire bat lineage from this species was previously reported in Argentina [14]. Only one possible case of the vampire bat lineage of rabies was previously notified in a wild terrestrial mammal in Brazil: in a human that died with the disease and had report of a bite by a red brocket deer [*Mazama americana*] in 2012 [15].

The rabies virus variant maintained by populations of *Desmodus rotundus* was previously described in different species of animals including dogs, cats, livestock, different species of non-haematophagous bats and humans [6]. The possibility of inter species transmission of the vampire bat variant is an important issue, especially if we consider the importance of this bat species for rabies epidemiology in Brazil and the rest of Latin America. This is the first report of a rabies virus transmission between a common vampire bat and a crab-eating fox in Brazil. It exemplifies the complex rabies virus epidemiology and this virus intricate pathway of interspecies transmission and highlights the importance of constant rabies surveillance in these and other wildlife species to monitor the emergence of new transmission cycles essential for rabies prevention and control.

CONCLUSION

The study of rabies virus isolated from different host species obtained from different geographical areas facilitates the understanding of rabies epidemiology and prevention. It can be used to explain the transmission from reservoir species to non-reservoir animals and humans, and to improve the surveillance of the emergence of specific strains of the virus into new species.

These results demonstrate the possibility of the occurrence of another case of spillover, the possibility of direct transmission from foxes to humans and one more time disclose the emergence of new epidemiological cycles of wildlife rabies in Latin American, indicating the need to develop surveillance programs for rabies control in wildlife.

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