PHYLOGENY OF A RABIES VIRUS IDENTIFIED IN A CAT CLOSELY RELATED TO VAMPIRE BAT RABIES BASED ON THE NUCLEOPROTEIN GENE

ABSTRACT

Rabies-infected bats are preyed upon by dogs and cats and the transmission of virus from bats to domestic animals has been reported. It is expected that bat-related virus variants might be found with higher frequency in dogs and cats living in urban areas where the terrestrial cycle has been controlled. This article reports the genetic characterization of one sample of rabies virus from a cat that had contact with a bat on the border of São Paulo city. The sample was genetically typed as variant 3, associated with Desmodus rotundus, suggesting that bats and their rabies virus variants must from now on be considered in the epidemiology of rabies of urban domestic animals and in public health policies.

INTRODUCTION

Carnivores remain the main cosmopolitan transmitters of rabies and, among these, dogs are the most important in developing countries, but a surprising rise in the number of cases of human rabies transmitted by bats is occurring in such countries. In developed countries in North America and Europe, rabies still persists in the wild cycle, mainly among bats, foxes, coyotes, raccoons and skunks (WHO 2000).

In Latin America, vampire bats are the main reservoirs of rabies in rural areas, but non-hematophagous bats also play a role in rabies transmission. While terrestrial carnivores are responsible for a terrestrial cycle, bats make aerial one. Vampire bats are responsible for almost all cases of rabies in herbivores such as bovine and horses (PAHO 1997). Recently, in Portel and Viseu municipalities, Pará State, Northern Brazil, vampire-bat transmitted rabies caused 21 human deaths (Travassos da Rosa et al. 2004).

In the last two decades, rabies control programs carried out in Brazil, based on animal population control, yearly massive vaccination of dogs and cats, epidemiological surveillance (laboratory-based diagnosis), pre and post-exposure prophylaxis of humans and health education, have led to a decrease in the number of cases in the urban cycle, with a fall in the incidence of human rabies (PAHO 1997).
As a result, antigenic studies revealed that variants of rabies virus classically associated with dog rabies have not been detected in diagnostic laboratories in São Paulo State since March 1998 (Carrié et al. 2000, Carrié et al. 2001).

Consequently, bat rabies in urban centers started to attract greater interest, given the rise in the number of bats infected by rabies virus, mainly those from the genus *Artibeus*, often found in the urban areas as a result of their synanthropism. These animals carry a specific rabies virus variant (AgV 3), classically associated to *Desmodus rotundus*. (Carrié et al. 2001, Brandão et al. 2004).

Rabies-infected bats are easily preyed on by dogs and cats and the transmission of rabies virus from bats to domestic animals has already been reported (Del Pietro et al. 1987). Thus, it is expected that bat-related virus variants might be found with a higher frequency in dogs and cats living in urban areas, where the terrestrial cycle has been controlled.

Molecular epidemiology of rabies is an invaluable tool to track back the origin of a given virus variant and to study its diversity and dynamic in an ecosystem, to assign a virus variant to a specific host and to design vaccine studies (Bourhy et al. 1993, Smith et al. 1993).

The nucleoprotein (N), a 450-amino acid-long protein, is the most conserved of rabies virus structural proteins due to functional constraints such as its role in the protection of the genome against cellular nucleases, its importance on the nucleocapsid assembly and regulation of transcription and replication (Wunner 2002).

Thus, analysis of the N gene, be it based on partial (200-300nt) or full-length (1350nt) sequencing, allows one to draw more significative clusterings regarding each rabies virus variant or each host-specific variant when compared to the other rabies virus genes, and thus is the most suitable approach to the molecular epidemiology of rabies; distance-based methods of phylogenetic analysis, such as neighbor-joining, are computationally fast methods that in this case can resolve clade associations as efficiently as maximum parsimony and maximum likelihood (Smith 2002).

This study aimed to characterize a rabies virus variant detected in a bat-contacted cat in the Municipality of Cotia, located at the Great São Paulo City, by partial sequencing of the N gene.

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**MATERIALS AND METHODS**

**Sample.**

The biological sample used in this study was a central nervous system (CNS) sample originated from Cotia (São Paulo State), from a 3-month old mixed breed stray female cat (register number 1099M) positive to rabies by direct fluorescent antibody test (DFA) as described by Dean et al. (1996). According to the sample submission file, the cat was reported as possibly having had contact with bats.

**Genetic typing.**

Sample 11099M was tested by reverse transcriptase polymerase chain reaction (RT-PCR) targeted to a 1350-base pair fragment of the nucleoprotein (N) gene with primers (sense primers N1 5'ATGGATGCGACAGATT 3' and anti-sense primer N2 5' TTATGACGACTCGAATA 3') described by Carniel (1999), using Superscript II Reverse Transcriptase (Invitrogen™) and Taq DNA Polymerase (Invitrogen™) according to the manufacturer's instructions.

The PCR product was excised from agarose gel and purified with QIAquick gel extraction kit (Qiagen™), submitted to sequencing reaction with anti-sense primer and DYEnamic ET Dye Terminator (Amersham Biosciences™) according to the manufacturer's instructions in four replicates and the sequences resolved in a MegaBACE DNA sequencer (Amersham Biosciences™).

The chromatograms where manually compared with Chromas 2.23 software (© 1998-2002 Technelysium Pty Ltd) and the reverse-complement consensus sequence to the four replicates was obtained with Bioedit (Hall 1999).

For the phylogenetic analysis, the final sequence of the strain 11099M was aligned by the CLUSTAL method with Bioedit (Hall, 1999) with homologous sequences derived from the GenBank (accession numbers shown in figure 1) or available at Pasteur Institute (cat strain 10566, human strains 2810, 7263 e 2788, fox strain 5109 and the bat strain 3979M obtained from an *Artibeus sp*), the alignment used to build a Neighbor-Joining distance tree with Kimura-2 parameters model and 1000 bootstrap replicates with Mega 2.1 (Kumar et al. 2001).

**RESULTS**

The sequencing of the cDNA corresponding to strain 11099M resulted in a 481-nucleotide long
sequence corresponding to the 5' end of N gene. The sequence has been assigned the GenBank accession number AY563517.

The phylogenetic tree has shown that strain 11099M segregated in the polytomic cluster corresponding to variant 3 (Desmodus rotundus) of rabies virus supported by a bootstrap value as high as 100, while strains corresponding to variant 2, classically associated with the urban cycle of rabies, were found in clearly distinct groups (Figure 1).

DISCUSSION

Control measures against rabies in São Paulo State have been successful for canine and feline rabies control. However, rabies epidemiology is highly influenced by ecological forces and human activity, which allows multiple antigenic and genetic variants to be found, despite failures to find non-2 variants in dogs and cats (Ito et al. 2003, Kasenpimolporn et al. 2004).

The predatory behavior of dogs and cats against bats makes possible the transmission of rabies virus from bats to these animals (Del Pietro et al.1987, Del Pietro et al.1994, Favi et al. 2002).

In fact, several studies have reported this kind of transmission in many States of Brazil and in other countries, such as Chile, Venezuela and Mexico (Carrieri et al. 2001, Kotait et al. 2001, Favi 2001, Schaefer et al. 2002, Velasco-Vila et al. 2002, Vulkelic 2001).

Evidence of bat-related rabies transmitted to humans via cats has been reported on two occasions. The first case occurred in Dracena, São Paulo State, Brazil (Kotait et al. 2001) and the second one in Costa Rica (Badilla et al. 2003), resulting in the death of two persons.

Taking into account that routine vaccination of cats is not a common practice in Latin America, the identification of new rabies virus variants in felines, thought to be restricted to wild animals, signals a change in rabies epidemiology in urban domestic animals, posing a risk to public health.

It is noteworthy that the genetic rabies virus variant here reported has been isolated in Cotia city, near São Paulo city where no canine or feline rabies has been reported since 1983, according to Coordenação Estadual do Programa de Controle da Raiva, São Paulo, Instituto Pasteur.
The sample 11099M was genetically typed as variant 3, from *Desmodus rotundus*. Thus, one could argue that the cat had contact with an infected bat, most probably a *Desmodus rotundus* or an *Antibeus* sp., this last often found carrying the *D. rotundus* variant (Carrieri et al. 2001, Brandão et al. 2004).

This probably represents a recent pattern in the rabies cycle. Data from samples of infected cats and dogs from 1987 and 1999, despite the higher prevalence of variant 2, depict an increasing trend in the frequency of variant 3 in these animals (Favoretto et al. 2002).

Taken together, these findings suggest that the role of bats and its rabies virus variants must from now on be considered more seriously in rabies of urban domestic animals and public health policies. Continuous surveillance needs to be carried out to broaden knowledge of the distribution of this new epidemiological feature. One might also suggest the need for a review of the definitions of controlled and non-controlled areas as established by international organizations such as OPAS (Organização Panamericana de Saúde) and OIE (World Organization for Animal Health).

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REFERENCES


