

Canine rabies in Rio Grande do Sul caused by an insectivorous bat rabies virus variant

Raiva canina no Rio Grande do Sul causada por uma variante de vírus rábico de morcego insetívoro

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ABSTRACT

Rabies is caused by rabies virus (RV), a RNA virus member of the *Lyssavirus* genus, family *Rhabdoviridae*. The aim of this study was to determine antigenic characteristics of a rabies virus isolate (RV183-07) recovered from a stray bitch that died of rabies and to infer the most likely source of contamination, since no urban rabies has been reported in the area in more than 20 years. The virus was identified by direct immunofluorescence and multiplied by one passage in mice. The antigenic profile of the isolate was determined with a panel of monoclonal antibodies to lyssavirus antigens on infected brain tissues. A fragment of the viral genome corresponding to the nucleoprotein (N) gene was submitted to reverse transcription/polymerase chain reaction and the amplicon obtained was subjected to restriction enzyme analysis. A 303 base pair fragment of the N gene was cloned, sequenced and compared to other RV sequences available at Genbank. The isolate RV183-07 displayed antigenic and genomic characteristics of rabies virus variants whose natural reservoir is the non-hematophagous bat *Tadarida brasiliensis*. Therefore, the most likely source of contamination of the bitch was an incidental contact with an infected bat of that species, common inhabitants of the area. In view of that, the status of “urban rabies-free” of the area was not compromised.

Keywords: rabies, variants, canine rabies, urban rabies, insectivorous bat rabies, diagnosis.

RESUMO

A raiva é causada pelo vírus da raiva (VR), um RNA vírus membro do gênero *Lyssavirus*, família *Rhabdoviridae*. O objetivo deste trabalho foi determinar as características de uma amostra (RV183-07) isolada de uma cadela que morreu em uma área livre de raiva urbana, onde não ocorriam casos da infecção em cães há mais de 20 anos, buscando inferir a fonte mais provável de contaminação da mesma. O vírus foi identificado por imunofluorescência direta, multiplicado em camundongos e analisado antígenicamente frente a um painel de anticorpos monoclonais antilissavírus. Um fragmento do genoma viral correspondente ao gene da nucleoproteína (N) foi submetido à transcrição reversa seguida da reação em cadeia da polimerase e o amplicom obtido foi submetido à análise com enzimas de restrição. Um fragmento de 303 pares de bases do gene N foi clonado, sequenciado e comparado com outras sequências do vírus da raiva disponíveis no Genbank. O isolado RV183-07 demonstrou características antigênicas e genômicas de vírus que têm os morcegos não hematófagos da espécie *Tadarida brasiliensis* como hospedeiro natural. A fonte de contaminação mais provável da cadela foi um contato acidental com um morcego não hematófago da espécie *Tadarida brasiliensis*, habitante comum daquela área. Em vista disso, o status de “livre de raiva urbana” da área não foi comprometido.

Descritores: raiva, variantes, raiva canina, raiva urbana, raiva em morcego insetívoro, diagnóstico.

INTRODUCTION

Rabies is a widespread zoonotic disease caused by rabies virus (RV), a member of the *Lyssavirus* genus, family *Rhabdoviridae* [1]. Epidemiologically, RV is maintained in distinct biological cycles, with distinct natural reservoir species. In the urban cycle, the domestic dog is the main RV reservoir [1]. In the sylvatic cycle, in Latin America, the main natural reservoir is the vampire bat *Desmodus rotundus*. However, RV variants adapted to different bat species, including insectivorous and frugivorous bats, have been frequently reported. Such variants are antigenically and genetically distinct to variants whose natural reservoirs are haematophagous (vampire) bats [4]. Antigenic differentiation can be achieved by monoclonal antibody analyses [4]. Genomic comparisons can be accomplished by a number of molecular methods. Restriction enzyme analysis (REA) of genome fragments amplified by reverse transcription/polymerase chain reaction (RT-PCR) have often been employed, usually complemented by nucleotide sequencing [4].

In the state of Rio Grande do Sul (RS), southern Brazil, urban rabies has not been detected since 1988 [3]. Nevertheless, rabies in vampire bats remains endemic [1]. However, on a growing number of occasions, non-haematophagous bats have been found infected with RV in urban areas [1]. In this study, a case is reported where rabies virus was isolated from a stray bitch which, as deduced from the antigenic and genomic characteristics of the isolate, was probably infected by a RV variant whose natural reservoirs are insectivorous bats of the species *Tadarida brasiliensis*.

MATERIALS AND METHODS

Virus isolation

In January 2007, a stray bitch in the city of Tapes (eastern RS, Brazil) was found dead by local residents. The bitch apparently did not develop any signs of disease. However, as it was occasionally fed by locals, citizens notified public health authorities. The public health service collected the dead animal, which was submitted to the laboratory for rabies diagnosis (sample number 183-07).

Routine identification of rabies virus antigens was performed by the direct fluorescent antibody test (DFAT) following standard diagnostic procedures [1]. A polyclonal anti-rabies nucleocapsid fluorescein conjugate (Pasteur Institute, São Paulo, Brazil) was used. Virus

identification was confirmed by mouse intracerebral inoculation followed by DFAT on brain tissues of infected mice [1].

Antigenic characterization

The antigenic profile of isolate RV183-07 was determined by indirect immunofluorescence (IIF) with a panel of eight monoclonal antibodies (Mabs) raised to lyssavirus antigens [4]. Three other RV isolates, named 1024 (from an insectivorous bat), 576/RS (from a haematophagous bat) and 585/BA (from a case of urban dog rabies) were included in the tests for comparisons.

Genomic characterization

Total RNA was extracted with Trizol (Invitrogen, Carlsbad, CA, USA) and submitted to reverse transcription-polymerase chain reaction (RT-PCR) with primers targeting the N gene as described by Tordo [5].

The amplicon obtained was subjected to restriction enzyme analysis (REA) with *Bgl*II and *Pvu*II endonucleases. Cleavage was performed at 37°C for 1 hour. The products were submitted to electrophoresis in 0,7% agarose, stained with ethidium bromide by and visualized on a UV transilluminator [2].

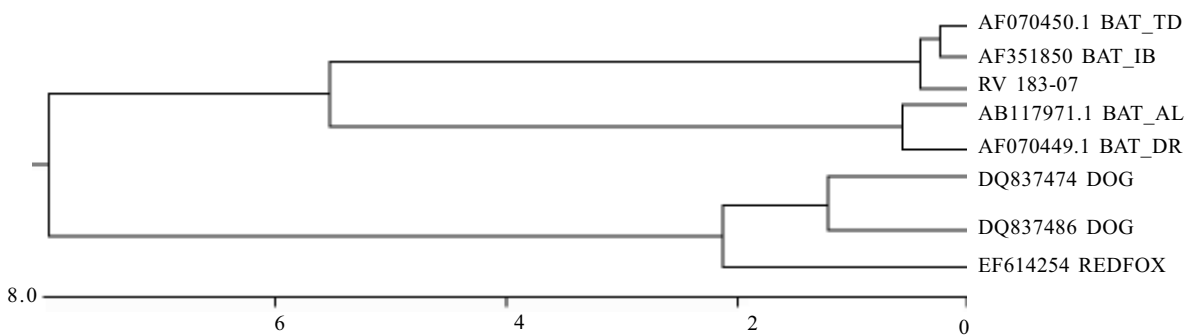
Nucleotide sequence analysis

A fragment of the N gene was targeted for sequence amplification and comparative analyses. Primers corresponding to positions 66-82 (sense primer: 5'-CTACAATGGATGCCGAC-3') and 385-365 (anti-sense primer 5'-TGGGGTGATCTTRTCTCCTTT -3') were used to amplify the isolate RV183-07 by RT-PCR as described above. The amplicon obtained was cloned into plasmid pCR2.1 (Invitrogen) and sequenced three times in both senses, from three different clones. Sequencing was carried out with the DYEnamic ET terminators sequencing kit (GE Healthcare, Giles, United Kingdom) following the manufacturer's protocol, in a MegaBACE 500 automatic sequencer (GE). Assembled sequences with high quality were aligned using Clustal V method (weighted), gap penalty 10 and gap length penalty 10. Homology analyses were performed with basis on RV sequences available at the GenBank database.

RESULTS

Antigenic characterization

Mab analysis revealed that isolate RV183-07 displayed an antigenic pattern usually found in RV variants whose natural reservoirs are bats, indicating the likely origin of the recovered virus.



*Hosts of origin of the rabies virus isolates whose sequences were used in the present study (source: Genbank):

- AF070450.1 BAT_TD: *Tadarida brasiliensis* species.
- AF351850 BAT_IB: Insectivorous bat of undetermined species.
- AB117971.1 BAT_AL: *Artibeus lituratus*.
- AF070449.1 BAT_DR: *Desmodus rotundus*.
- DQ837474 DOG and DQ837486 DOG: Domestic dogs.
- EF614254 REDFOX: *Vulpes vulpes*.

Figure 1. Comparison between the first 281 nucleotides of rabies virus (RV) nucleoprotein (N) gene of isolate RV183-07 and seven other RV sequences available at Genbank (Phylogenetic tree based on using Clustal V method (weighted); gap penalty 10, gap length remover vírgula daqui penalty 10)

Genomic characterization

REA of the DNA fragment amplified from RV183-07 gave rise to two *Bgl*II fragments of 1000 and 530 bp, and two *Pvu*II fragments of 1100 and 430 bp. The REA cleavage pattern obtained was similar to that observed with other bat RV variants, and unlike the pattern usually displayed by RV variants found in dogs [4].

Nucleotide sequence analysis

By aligning the sequenced fragment of RV183-07 with equivalent RV sequences available at Genbank, high homology was found between RV183-07 and RV variants whose natural reservoirs are the insectivorous bats of the Brazilian free-tailed bat species *Tadarida brasiliensis* (Figure 1).

DISCUSSION

The present study reports an investigation on the origin of a rabies virus variant isolated from a bitch in a canine rabies-free area. Antigenic and REA suggested that the virus was a RV variant whose natural reservoirs are bats [1,3,4]. Phylogenetic analysis defined that the recovered virus had characteristics of RV variants recovered from insectivorous bats of the Brazilian free-tailed bat species *Tadarida brasiliensis* [4], fairly abundant in the area of occurrence of the case. Thus, isolate RV183-07 is a variant adapted to *Tadarida brasiliensis* and its detection in a canine was more likely an occasional spillover from a bat of that species.

The determination of the species of origin of the case was important to allow appropriate management of sanitary vigilance resources; as the isolate RV183-07 was originated from an episode of incidental contamination of a bitch, the status of “canine rabies free” of the area was not compromised. Strict surveillance on rabies infections in bats must be maintained. In addition, these findings highlight the need of a better understanding of the biology of rabies in non-haematophagous bat species and its role in rabies epidemiology.

CONCLUSIONS

The RV isolate examined in this study (RV183-07), recovered from a bitch that died of rabies in the city of Tapes, RS, revealed antigenic and genomic characteristics of RV variants which have the “free tailed” bat *Tadarida brasiliensis* as natural host. Therefore, the more likely source of infection for the bitch was an incidental contact with an infected bat of that species. In view of these findings, the status of “urban rabies-free” of the area should not be compromised.

Acknowledgements. All procedures involving animal care, handling and experimentations were performed under veterinary supervision and according to the recommendations of the Brazilian Committee on Animal Experimentation (COBEA; law no. 6.638 of May 8th, 1979). Work funded by FEPAGRO, CNPq and FAPERGS. H.B.C.R.B. is a doctorate fellow at the Programa de Pós-graduação em Ciências Veterinárias (PPGCV), UFRGS. P.M.Roehe is a CNPq researcher 1C.

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