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MICROBIOLOGY

Feline rabies caused by a viral variant of insectivorous bat

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ABSTRACT: Rabies is an invariably fatal infectious-contagious viral disease caused by viruses in the genus *Lyssavirus*, which affects the central nervous system of domestic and wild mammals. This study draw attention to the importance of insectivorous bats and domestic cats in the epidemiology of rabies. For this, two cases of rabies registered in domestic cats in the southeast region of Rio Grande do Sul were analyzed. Diagnoses were based on histological alterations and positive staining for the virus in direct immunofluorescence with confirmation by biological test, immunohistochemical evaluation and identification of the rabies virus variant by polymerase chain reaction. In Brazil, a change in the epidemiological profile of rabies has been observed, in which bats play a major role in the current chain of transmission of the disease. It is noteworthy, that the antigenic viral variant AgV-4, typical of the insectivorous bat *Tadarida brasiliensis*, was identified in the domestic cats affected in this study.
Key words: feline rabies, *Tadarida brasiliensis*, domestic cat, Agv-4 variant, insectivorous bat.

Raiva felina por variante viral de morcegos insetívoros

RESUMO: A raiva é uma enfermidade viral infectocontagiosa, invariavelmente fatal, causada por Lyssavirus, que afeta o sistema nervoso central de mamíferos domésticos e silvestres. O objetivo deste estudo foi alertar para a importância de morcegos insetívoros e dos felinos domésticos na epidemiologia da raiva. Para isso, foram analisados dois casos de raiva registrados em gatos domésticos na região sudeste do Rio Grande do Sul. Os diagnósticos basearam-se nas alterações histológicas e na marcação positiva para o vírus na imunofluorescência direta com confirmação por prova biológica, avaliação imuno-histoquímica e identificação da variante do vírus rábico por reação de polimerase em cadeia. No Brasil tem sido observado uma mudança no perfil epidemiológico da raiva, no qual os morcegos assumem papel principal na cadeia atual de transmissão da doença.Chama-se a atenção que a variante viral antigênica AgV-4, própria do morcego insetívoro *Tadarida brasiliensis*, foi identificada nos felinos domésticos afetados neste estudo.
Palavras-chave: raiva felina, *Tadarida brasiliensis*. gato doméstico, variante AgV-4, morcego insetívoro.

Rabies is an invariably fatal infectious and contagious viral disease that affects the central nervous system (CNS) of human beings and almost all species of domestic and wild mammals. The main form of transmission is through the bite of infected animals shedding the virus in their saliva (CONSALES & BOLZAN, 2007).

The genus *Lyssavirus* has currently at least 17 lyssavirus species recognized by the International Committee on Taxonomy of Viruses as well as 1-2 unclassified viruses (*Kotalahtibat lyssavirus* and *Matlobat lyssavirus*) (MARKOTTER & COERTSE, 2018), and only the classic rabies virus is reported in Brazil. This genotype is responsible for causing rabies in both air and land mammals. In addition, this virus has antigenic variants, of which four have been identified in the country: variant 2, specific for dogs; variant 3, found in the vampire bat *Desmodus rotundus*; variant 4, present in the insectivorous bat *Tadarida brasiliensis*; and variant 6, found in the insectivorous bat *Lasiurus cinereus* (ALBAS et al., 2011).

Because it is lethal and untreatable, rabies is extremely important to public health. In Brazil, according to the most recent data released by the Health and Environmental Surveillance Secretariat of the Ministry of Health, from 2010 to 2022, 45 cases of human rabies were recorded in the country, of which four were transmitted by domestic cats, nine by domestic dogs, 24 by blood-feeding bats and the rest by other wild species (SAÚDE, 2022).

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Based on the latest cases of rabies diagnosed in domestic cats infected by the AgV-4 variant of the rabies virus in Pelotas, Rio Grande do Sul - Brazil, region; this study draw attention to the importance of insectivorous bats and domestic cats in the epidemiology of rabies in the southeast of Rio Grande do Sul (RS), Brazil.

Both affected animals were male (8 moth-old – cat 1) and female (3-year-old – cat 2) domestic short hair cats. The animals lived at home but had access to the street. The cases occurred in 2014 and 2022 respectively. Cat 1 was found dead by the owners and cat 2 was euthanized after 5 days with clinical signs of great aggressiveness, photophobia, and lack of appetite. The owner reported that there was a bat in the household and the veterinarian who attended to the animal kept it in the clinic in isolation for having suspected of a neurological disease. Cats 1 and 2 were not vaccinated for rabies and owners of both animals reported that they were scratched and bitten.

During necropsies, fragments of organs and the brain of both cats were collected and fixed in 10% buffered formalin. Samples of the telencephalon and cerebellum of both cats were refrigerated and sent for fluorescent antibody (FA) and DNA extraction and purification for molecular diagnosis in Regional Diagnostic Laboratory (LRD) – Federal University of Pelotas (UFPel) and Desidério Finamor Veterinary Research Institute-IPVDF. The partial sequencing of the N gene and the G gene of the lyssavirus was carried out, according to the protocol established by CARNIELI et al. (2008), using the sequences of primers described by ORCIARI et al. (2001) for N gene and by SATO et al. (2004) for G gene. Sequencing of amplicons were performed using the Sanger sequencing methodology. The generated sequences were then analyzed using the online tool "Basic Local Alignment Search Tool (BLAST)".

Formalin-fixed samples were cleaved, embedded in paraffin, cut into 3µ thick sections, and stained using the hematoxylin and eosin technique. After the histological evaluation, selected sections of the telencephalon and cerebellum were sent for the immunohistochemistry (IHC) technique, performed according to the technique described by PEDROSO et al. (2008).

To perform the immunohistochemistry technique for rabies, histological sections were made 5mm thick and applied to positive slides (ImmunoSlide-EasyPath), dried vertically at room temperature, before heating them in an oven at 60º C for 3-4 hours. After the sections were dewaxed in xylene and rehydrated in decreasing levels of alcohol until distilled water. Endogenous peroxidase was blocked by incubating the slides in a 3% hydrogen peroxide solution in distilled water for 15 minutes at room temperature, and then washing them in distilled water three times for two minutes. For antigen retrieval, Citrate buffer was used (2.1g of citric acid in 1 liter of distilled water, adjusting the pH to 6.0 with 0.5% NaOH). The slides were then placed in polypropylene staining jars for 15 minutes in a water bath in a commercial stainless steel pan with dimensions of 24x20x20cm (height x width x length) with a capacity of two liters, previously heated to a temperature of 100 ºC. Afterwards, the slides were cooled for 5 minutes at room temperature. To reduce nonspecific binding ("background"), the sections were treated with 5% skimmed milk (Molico) diluted in distilled water for 15 minutes. The sections were covered with a solution containing the primary antibody. A 1:500 dilution in PBS was used for the polyclonal antibody (anti-rabies polyclonal Chemicon #5199) recommended for direct immunofluorescence. The sections were incubated in a humid chamber at 37 ºC for 60 minutes. Subsequently, they were washed in distilled water and treated with biotinylated secondary antibody (DAKO LSAB 2 kit, DAKO Corp., Carpinteria, CA) for 20 minutes in a humid chamber at room temperature. Soon after, they were washed in distilled water and treated with the Streptavidin-peroxidase conjugate (DAKO Corp., Carpinteria, CA) for another 20 minutes each in a humid chamber at room temperature, being washed again in distilled water and subjected to development with the chromogen DAB. for 5 minutes. The sections were washed in distilled water and counterstained with Harris hematoxylin for 1 minute, then washed in running water for 1-2 minutes and dehydrated in alcohol grades, clarified in xylene and mounted with Entellan (Merck, Darmstadt, Germany Sigma Chemical Co., Saint Louis, USA). The slides were then evaluated under an optical microscope.

In the histological evaluation of the brain, eosinophilic round to oval intracytoplasmic inclusion bodies were identified in the cytoplasm of neurons. In one of the cats, there was also non-suppurative encephalitis, characterized by perivascular cuffs of lymphocytes, plasma cells, and macrophages (Figure 1A).

In both cases, the FA results were positive for rabies virus in the samples by the biological test in suckling mice. In the IHC evaluations, positive immunomarking for the rabies virus were observed in the cytoplasm of the telencephalon and Purkinje neurons and their dendrites in the molecular layer of the cerebellum (Figure 1B). The RT-PCR results

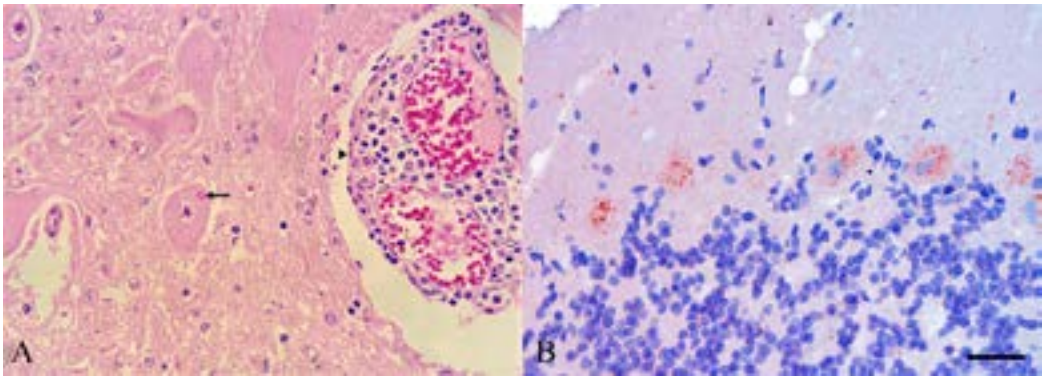

Figure 1 - Feline rabies caused by a viral variant of insectivorous bat. Cat n. 1. (A) Negri's corpuscle in the cytoplasm of temporal cortex neurons (arrow), and perivascular cuffs of lymphocytes, plasma cells, and macrophages (arrowhead). Obj 40x. HE.Cat n. 2. (B) Positive immunostaining for rabies in the cytoplasm of Purkinje neurons in the cerebellum. Obj 40x. Bar: 200µm.

(GenBank accession OR634834) were positive in both animals, and the genetic sequencing showed that both *Lyssavirus* sequences had more than 99% similarity with *Lyssavirus* sequences from bats species *Tadarida brasiliensis* (99.85% identity for the N gene and 99.67% identity for the G gene), the antigenic variant (AgV-4) of the rabies virus.

In the current study, the diagnosis of rabies in cats was based on epidemiological data, clinical signs, histopathological findings, and positive results for rabies in tests such as FA, RT-PCR, and IHC. Furthermore, Negri bodies were observed on microscopic examination, indicating rabies; histological techniques have the advantage of being fast, practical, and inexpensive (INSTITUTO PASTEUR, 2009).

Notably, in one of the cases, the characteristic rabies encephalitis was not observed. This can be explained by the fact that the animal was euthanized. Tissue damages are more easily observed when the brain is examined after the disease runs its natural course, giving time for the lesions to develop fully (BECK et al., 2017). If the animal is euthanized in the early stages of the disease, lesions and Negri bodies may not be evident. In addition, in some rabies cases, the lesions can be very discreet or absent, demonstrating the importance of always performing other techniques, such as IHC, to assist in making the diagnosis (MARCOLONGO-PEREIRA et al., 2011). However, immunohistochemistry does not differentiate the genetic variant of the rabies virus.

RT-PCR is important due to the genetic characterization and typing of Lyssavirus, contributing to epidemiological studies (KIMURA et al., 2006; WADHWA et al., 2017; GIGANTE et al., 2018). In the present study, the rabies virus variant identified in the RT-PCR of feline samples was the antigenic variant (AgV-4), which, in Brazil, is specific to the insectivorous bat *Tadarida brasiliensis* (ALBAS et al., 2011). There are approximately 170 species of bats identified in the country, and rabies virus has already been isolated from 36 individuals of this species. In urban centers, species of bats of the genera *Artibeus*, *Eptesicus*, *Molossus*, *Myotis*, *Nyctinomops*, and *Tadarida* are predominantly found. The presence of these infected animals in urban areas demonstrates the high importance of the aerial cycle in maintaining the rabies virus in the environment, representing risks to public health since many have synanthropic habits (INSTITUTO PASTEUR, 2009).

Although, dogs continue to be the main species targeted by anti-rabies immunization programs, the importance of domestic cats in the epidemiology of the disease is becoming increasingly evident. In Brazil, from 2010 to 2014, eight cases of human rabies transmitted by dogs were recorded. From then on, from 2015 to 2022, four of the five cases of human rabies transmitted by domestic species had the cat as the aggressor animal, while the dog was responsible for only one case (SAÚDE, 2022), demonstrating the greater participation of domestic cats as transmitters from the rabies virus to man. A similar situation has been observed in the Pelotas region, which has had no record of dog rabies since 2000, with the only cases observed in the urban area occurring in domestic cats. Thus, feline rabies

cannot be neglected, especially in endemic regions, being influential in differential diagnoses.

Attention is drawn to the importance of the domestic cat in the epidemiology of rabies due to the exponential growth of this species in urban areas and the high numbers of their colonies of insectivorous bats in urban areas of the south of RS, favoring viral circulation and transmission.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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