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DETECTION OF RABIES VIRUS IN ORGANS OF BATS OF GENUS ARTIBEUS BY MEANS OF HEMI-NESTED RT-PCR AND REAL TIME RTPCR TECHNIQUES

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Molecular techniques have been used increasingly as tools for the diagnosis by detecting the rabies virus genome. This study aimed to detect the presence of rabies virus in the wash of skull and in different organs of the genus *Artibeus* bats using the hemi-nested RT-PCR (hnRT-PCR) and Real Time RT-PCR molecular techniques. From approximately 4,000 specimens of bats received at the Institute Pasteur for rabies diagnosis, 30 bats of the genus *Artibeus* were selected, with records of positive results for rabies by the traditional techniques of direct fluorescent antibody test (FAT) and inoculation of murine neuroblastoma cell line (N2A). Salivary glands, urinary bladders, kidneys, lungs, and also the washes of the skullcaps of the specimens were collected. The scraping of the skull was performed with the aid of sterile pipette tips and then washed with 1,000µL diluent composed of 0.85% saline solution, supplemented with 2% Bovine Fetal Serum, free of rabies virus specific antibodies and containing 0.1% Gentamicin Sulfate. The urinary bladders were diluted using the same diluent mentioned above, to 1:20 (w/v) and other organs were diluted 1:10 (w/v). The extraction of total RNA was carried out using the TRIzol® and the reverse transcription was followed by PCR and hnRT-PCR using primers specific for the gene encoding the N protein. From the product derived by the reverse transcription, the Real Time RTPCR technique was run by using primers and probes specific for the antigenic variant 3 of rabies virus. When evaluated the total samples analyzed, the overall results of the sensitivity for both the hnRT-PCR and Real Time RT-PCR techniques was 86%. A comparison between the hnRT-PCR and Real Time RT-PCR techniques performed by Fisher's exact test has revealed that the proportion of positives detected for the washing of the skull was similar to that of the organs examinations (P> 0.05). In relation to the positive results found in hnRT-PCR and Real Time RT-PCR techniques were 100% in brain washes, 90% and 93.33% in the salivary glands, 83.33% and 90% in urinary bladders, 80% and 93.33% in kidneys, and 76.67% and 50% in lungs. These results suggest that both the hnRT-PCR and the Real Time RT-PCR techniques can be used as complementary methods for the rabies diagnosis and the techniques are sensitive enough for use in studies of pathogenesis. The Real Time RT-PCR technique performed in this study proved effective in detecting the rabies virus in different organs and extra neural tissues with the advantage of being a faster and more sensitive procedure.

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IDENTIFICATION OF THE SPECIES OF RESERVOIRS AND HOSTS OF THE RABIES VIRUS AND OTHER PATHOGENS BY SEQUENCING OF THE CYTOCHROME-B MITOCHONDRIAL DNA GENE

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The identification of animal species that transmit pathogens such as the rabies virus is of the utmost importance for public health and the natural history of infectious and contagious diseases. Diagnostic laboratories very often receive mauled or decomposing animal carcasses, particularly of bats, rendering morphometric identification unviable. The existence of different regional names for the same animal, morphological variability and the lack of staff trained in zoological identification constitute a serious problem for epidemiological surveillance. Molecular techniques are used routinely and effectively in systematics, evolution and ecology to identify species and can even be used to identify hybrids that originated from genetically close animals, in which the differences very often go undetected by morphometry. Some mitochondrial DNA (mt- DNA) genetic markers, such as control region sequences and the genes encoding cytochromes b and c, are frequently used in the genetic identification of species. Many of the genetic sequences for these genes are stored in public-domain websites such as GenBank, allowing new sequences to be compared with existing ones in databases. The objective of this study is to build a database with genetic sequences from the cytochrome b gene of rabies reservoir species for use in the identification of these species. mt-DNA fragments were amplified and sequenced as described previously by Carnieli et al. (2008), using the primers 5'- CGACTAATGACATGAAAAATCACCGTTG-3' (sense) and 5'- TATTCCCTTTGCCGGTTTACAAGACC-3' (antisense) described by Martins et al. (2007). Sixty-six mt-DNA samples from different species of wingless Brazilian mammals and fifty-four samples from different species of chiropterans were sequenced. Analysis of the genetic sequences from these wingless mammals highlighted the problem of genetic identification of species as only a few sequences of mt-DNA from wingless mammals of Brazil were found in GenBank. For example, there are seven species of marmosets (genus *Callithrix*) but mt-DNA sequences for only some of them are deposited in GenBank. However, the cytochrome b gene sequences obtained from bats in this study, together with morphometric identification carried out in parallel, allowed us to name the species with certainty. From the fifty-four mt-DNA samples from chiropterans, nineteen species from eight genera and four different families were identified. Thus, the method described here is efficient in the identification of animal species and the search for samples of mt-DNA in Natural History Museums and Zoos may complement and certify unequivocally the sequences in the database under construction. Financial Support: Instituto Pasteur, Brazil

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PHYLOGENETIC ANALYSIS OF RABIES VIRUS IN THE STATE OF RIO GRANDE DO SUL, SOUTHERN BRAZIL

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Rabies is a worldwide zoonosis caused by rabies virus (RABV), a member of the *Lyssavirus* genus, family *Rhabdoviridae*. In nature, RABV is maintained in cycles with distinct natural reservoirs. In the urban cycle, the main reservoir for the virus is the domestic dog, on the other hand, in the sylvatic cycle different species can be the reservoir. In Latin America, the main natural RABV reservoir is the haematophagous bat *Desmodus rotundus*. However, RABV lineages adapted to different bat species, including insectivorous and frugivorous bats, have been frequently reported. The RABV lineages isolated from non haematophagous bats are genetically distinct from the RABV lineages whose natural