PT.084
A REVIEW OF THE CLASSIFICATION OF RABIES VIRUS LINEAGES MAINTAINED BY INSECTIVOROUS BATS IN BRAZIL

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Little was known about the importance of nonhematophagous bats in the epidemiology of rabies in Brazil and most of Latin America until the 1980s. From that decade on, as canine rabies came under control in many municipalities and molecular and antigenic typing was incorporated in surveillance programs, the importance of nonhematophagous bats in the epidemiology of the disease began to be appreciated in these countries. In Brazil, genetic studies based on gene N have shown that different lineages are circulating in insectivorous bats from the species *Tadarida brasiliensis*, *Nyctinomops laticaudatus* and genus *Myotis*, *Eptesicus*, *Molossus*, *Histiotus* and *Lasiurus*. In most studies, the characterization of these lineages is based on only 264 nt of the carboxyterminal region of the viral nucleoprotein, when the ideal would be to use the complete N gene. The aims of the present study was to review the genetic classification of the RABV isolated from insectivorous bats from Brazil based on current literature, Genbank dataset and new partial DNA sequencing of the nucleoprotein comparing the phylogenetic analysis of N gene based on 1218 nucleotides (nt 203 to nt 1420) with that based on 264 nucleotides (nt 1157 to nt 1420), corresponding respectively to amino acids 45 to 450 and 363 to 450 of the viral nucleoprotein. Phylogenetic analysis demonstrated the existence of at least eleven lineages of RABV associated with different genera and species of insectivorous bats. Nine of these lineages have already been described in literature while two of them were herein characterized for the first time and associated to the genus *Myotis* and *Lasiurus*. There were no differences in the classification of Brazilian strains by comparing the two alignments used, but changes were observed in phylogenetic relationships between the clusters, with bootstrap values always greater regarding the 1218 nt tree. Two sequences of RABV from the genus *Myotis* from Uruguay and Chile did not keep the same classification after the analyses with the two alignment lengths. These findings should be taken into account in molecular epidemiology of rabies, as sources of infections might be determined in a more accurate way and also in the correct use of fragments of the N Gene for the classification of lineages of RABV.

PT.085
IN SITU EVALUATION OF CYTOTOXIC IMMUNE RESPONSE IN CENTRAL NERVOUS SYSTEM OF HUMAN RABIES TRANSMITTED BY DOG AND VAMPIRE BAT

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Introduction: CD8 (+) T cells and natural killer (NK) cells are immune effectors that, by cytokine production or cytotoxicity, help to contain a viral infection. **Objective:** To quantify and compare the T CD8 lymphocytes, natural killer cells and B granzyme expression in central nervous system lesions of human rabies transmitted by dog and vampire bat. **Methods:** Five fragments of central nervous system (CNS) were selected (cortex, hippocampus, basal ganglia, cerebellum and medulla oblongata) from each specimen of the four human rabies cases transmitted by dog and four cases by vampire bat (*Desmodus rotundus*). The fragments were subjected to immunohistochemistry with antibodies for CD8, CD57 and B granzyme. For each specimen, cells were quantified by counting the number of immunolabelled cells in thirty fields considering the parenchyma. For normalizing, a x10 ocular lens was used with a square grid in a x40 objective marking an area field of 0.0625 mm2. Statistical analysis was performed by Graph Pad Prism version 5.0 for Windows (Graph Pad software, San Diego, Ca, U.S.A.) using the nonparametric Mann-Whitney test. Samples were considering different at the 95% ($p \leq 0.05$) level of significance. **Results:** The number of CD8+ T lymphocytes in human rabies transmitted by dog was lower ($p<0.0001$) than in those with human rabies transmitted by vampire bat. No significant difference in the number of CD57+ natural killer cells ($p>0.05$) and the number of B granzyme-expressing cells ($p>0.05$) was observed between samples evaluated of the human rabies transmitted by dog and vampire bat. **Discussion and Conclusions:** In the present study, we compared lesions in CNS of human rabies transmitted by dogs and vampire bats by quantitative examination of the "in situ" cytotoxic immune response. Rare NK cells and B granzyme-expressing cells in cerebral parenchyma were observed, but there were no significant statistical difference between the human rabies transmitted by dog and vampire bat. This could reflect an immune evasion mechanism triggered by rabies virus, preventing these cells arrive at the site of injury, or that their cytotoxic function would be altered. CD8+ T lymphocytes were more abundant in the human rabies transmitted by vampire bat, which appeared related to the viral variant type involved in infection, however eventually the function these cells may be impaired. So, we can speculate if this fact also could be due to longer survival of these patients compared to those bitten by dog.

PT.086
CELLULAR GROWTH IN DIFFERENT BIOREACTORS TO RABIES VIRUS PRODUCTION

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The scaling up of virus production process involves different challenges, mainly when is used an animal cells origin with a substrate. The growing of the animal cells in high densities depends on the beads and these cells present high susceptibility to the shear stress that occurs in the process realized in bioreactors. The objective of this study was to evaluate the growing of Vero cells in the scaling up process of rabies virus production in bioreactor. Two bioreactors were used in this study, one of 30 L (Bio Flow 4500, NBS) and other of 150 L (Bio Flo PRO Industrial, NBS). These bioreactors have different agitation systems: while the 30 L has a "Cell Lift Impeller", the industrial, one STR, has pitched blade impellers. This difference was important to select the velocity of agitation necessary to maintain the beads in suspension and to minimize the shear stress and bead collisions. Vero cells added to solid microcarriers, Cytodex 1 (2g/L), infected with PV rabies virus (MOI 0,02) were cultivated in serum-free medium VP SFM AGT in the two bioreactors. Were realized seven cycles in each bioreactor type and the initial cellular concentration was 13-14 cell/microcarrier. Supernatants of these cultures were harvested on days 2 and 3 after start the cycle of production. Samples of these cultures were taken every day during the production cycle to determine the cellular concentration. It was studied too the cellular loss in the first day after the cell inoculation to analyze the cell difficulty for spread on the microcarriers. The averages of the values of cell specific grow rate found before the harvest beginning were 0.025 h-1 and 0.023 h-1 in the industrial and 30 L bioreactors respectively. The percentage