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

Oliveira RN1, Castilho JG1, Batista HBCR1, de Paula FC1, Carnieli Jr P1, Lima JYO2, Macedo Cl1, Menozzi BD2, Carriere ML1, Kotait I1, Miranda CCP1, Brandão PE1 – 1Instituto Pasteur, 2Universidade Estadual Paulista – Faculdade de Medicina de Botucatu, 3Universidade de São Paulo – Faculdade de Medicina Veterinária e Zootecnia

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 Lasius. 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 Lasius. 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.

CELLULAR GROWTH IN DIFFERENT BIOREACTORS TO RABIES VIRUS PRODUCTION

Lantieri VS3, Medeiros FM3, Frazatti-Gallina NM3 – 1Instituto Butantan – Seção de Raiva, 2Instituto Butantan – Seção de Vírus

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, Cyctodex 1 (28L), 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...