

**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 mm<sup>2</sup>. 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≤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<sup>-1</sup> and 0.023 h<sup>-1</sup> in the industrial and 30 L bioreactors respectively. The percentage

averages of cellular loss in the first day after cell inoculation were 37% ( $\pm$  16%) in the industrial bioreactor and 52% ( $\pm$  21%) in the bioreactor of 30 L. The analyze of the data found to cell specific grow rate and cellular loss in the rabies virus production cycles showed that the spread and growth cellular were not affected by the blades of the impeller of industrial bioreactor. In conclusion, the expansion of Vero cell growth for rabies virus production in bioreactor of 150L was satisfactory because in this system the values of cellular loss and cell specific growth rate were similar or better than the values found in the 30 L bioreactor. Financial support: Fundação Butantan

#### PT.087

### AVALIAÇÃO DAS VACINAS ANTIRRÁBICAS VETERINÁRIAS, NACIONAIS E IMPORTADAS REALIZADAS PELO MINISTÉRIO DA AGRICULTURA PECUÁRIA E ABASTECIMENTO DO BRASIL NO PERÍODO DE 2009 A 2011

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**Introdução e objetivo:** No Brasil as vacinas a serem usadas na profilaxia da raiva animal são submetidas a controle por órgão governamental. Em cada lote de vacina nacional e importada são realizadas amostragens destinadas à análise fiscal. Dentre os testes aplicados estão o de esterilidade, inocuidade, inativação viral, pH e o teste de NIH. Para o teste de NIH faz-se a determinação da dose efetiva (DE<sub>50</sub>) capaz de proteger 50% dos camundongos vacinados com a vacina a ser testada e uma vacina referência. A partir da comparação estatística entre a DE<sub>50</sub> das duas vacinas, calcula-se a potência relativa da vacina teste. A análise fiscal das vacinas, realizada pelo MAPA no Lanagro-SP, é de extrema importância, uma vez que, promove os interesses nos objetivos da saúde pública, economia e para a indústria produtora indica pontos críticos que são merecedores de melhorias. Deste modo, foram realizadas estatísticas descritivas dos resultados obtidos dos lotes de vacinas amostradas entre 2009 a 2011. O objetivo deste trabalho foi avaliar o desempenho das mesmas, além de comparar as vacinas nacionais e importadas. **Resultados:** Durante este período foram realizados 227 testes, nos quais foram avaliadas amostras de lotes de 14 tipos de vacinas importadas e 18 nacionais (com seus respectivos lotes). As vacinas nacionais totalizaram 592 partidas e as importadas 154. O teste de inocuidade, que avalia a toxicidade da vacina antirrábica, inativação viral e a mensuração do pH, não apresentaram reprovação. Por sua vez, o teste de esterilidade apresentou em média 0,5% de reprovação nas vacinas nacionais. No teste de NIH as vacinas apresentaram em média 9% de reteste interno (RI). Em 36% dos testes a DE<sub>50</sub> da VRN estavam fora dos limites estabelecidos e em 16% a DL<sub>50</sub>. A avaliação pelo NIH mostrou que 22% das vacinas nacionais não apresentaram nenhuma reprovação, nas importadas 86% apresentaram-se satisfatórias. Para diferenciar o desempenho das vacinas, quanto à potência relativa, estas foram separadas nos grupos: “vacinas com 100% de aprovação” e “vacinas com alguma reprovação”. As potências médias de cada vacina, por grupo, foram comparadas por um teste estatístico de comparações múltiplas de médias. O 1º. Grupo foi constituído por 11 tipos de vacinas importadas e cinco nacionais, com destaque a um tipo de vacina importada que é distinto dos outros 15, devido a sua variabilidade da potência (superior) relativa e sua média. No 2º. Grupo foi composto por 13 tipos de vacinas nacionais e três importadas, diferenciando-se em oito tipos de comportamento com relação a potência relativa. **Conclusão:** Os produtos avaliados atenderam aos critérios de aceitabilidade de acordo com a Portaria Ministerial nº288, contudo

considerando os diferentes produtos e espécies a que se destinam, as vacinas importadas demonstraram o maior índice de aprovação. Ressalta-se a necessidade de uniformidade e consistência nos produtos a fim de se obter vacinas de elevada capacidade imunogênica, assim como, desenvolvimento de métodos para avaliar a qualidade do antígeno e concentração proteica nas vacinas avaliadas. **Agradecimento:** MAPA.

#### PT.088

### DOGS AND CATS RESCUED IN A SMALL BRAZILIAN CITY: POTENTIAL RABIES TRANSMITTERS

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Domestic dogs and cats are the most important rabies transmitters to men in Brazil. Besides the annual vaccination realized by the public service, many animals remain unprotected and exposed to rabies. Stray animals are even more vulnerable to rabies due to sanitary conditions which they are exposed, such as contact with bats, bite injuries caused by other stray dogs and cats potentially infected, and absence of vaccination. Towards these facts, the population of domestic dogs and cats is an important aspect in rabies prevention. The present study aimed to analyze the number of dogs and cats rescued in a small Brazilian city. The study was realized in the city of Botucatu (22°53'09"S 48°26'42"W), São Paulo State, Brazil, which comprises an area of 1,482.87 km<sup>2</sup>, and has a human population of approximately 127,370 hab.. The city realizes annual vaccination campaign against rabies since 1968, and the last positive diagnosis for rabies in a dog was in 1985. Although canine/feline rabies remain controlled since this date, the infection is present in bats, bovines and horses, as confirmed by several positive diagnosis performed annually. The animal rescue is performed by the Environmental Health Surveillance Department of the Municipal prefecture, and includes stray or unwanted animals. The last group consists mostly of dogs and cats abandoned by their owners, usually declaring a severe disease, lack of physical space, excessive aggression, among others. Data were recorded from the department's files. The number of rescued animals was as follow: in 2006 – 2,298 dogs and 560 cats; in 2007 – 2,066 dogs and 533 cats; in 2008 – 1,271 dogs and 421 cats; in 2009 – 688 dogs and 149 cats; in 2010 – 655 dogs and 204 cats; and in 2011 – 641 dogs and 199 cats. These results show a great number of animals rescued by the public service in Botucatu. A decrease in the number of animals was observed along the years, especially after 2009. This fact is a result of a municipal law (number 12.916) established in 2008, which defined new criterions for animal euthanasia, and consequently reduced the amount of this procedure. As a consequence, the Environmental Health Surveillance Department adopted a new policy, and started to rescue only stray animals causing public disturbance or visibly sick. Unwanted animals, which were frequently ordered to be euthanatized (by their owners) before 2008, were also not allowed to be rescued. Thus, the reduction of rescued animals led to a decrease in euthanasia procedures, which is in accordance with the new law. The results of this new measure, as well as the law, were not evaluated, and one must consider the possible increase of stray animal population, and also the risk of rabies infection. The responsible ownership is an important aspect that must be evaluated after the implement of this new policy, once that unwanted animals are not being allowed to be abandoned and sent to the Environmental Health Surveillance Department.