Estes aumentos podem coincidir com períodos de aumento de pluviosidade, cheias de rios e melhoria de pastagem. O coeficiente de incidência demonstrou uma grande variabilidade, dados que nos direcionam a novas pesquisas, assim como a falta do dimensionamento dos prejuízos econômicos e subnotificação de casos. Essa dificuldade em contabilizar os casos, somados às várias fontes de informação dificulta a obtenção de dados. Conclui-se que a raiva causa perdas econômicas e risco à saúde pública, porém não estimados. A ocorrência de raiva em herbívoros e atendimentos antirrábicos humanos coincide e apresenta aumentos nos períodos de fevereiro a março e setembro a outubro. Há relação dos casos com o mapa hidrográfico da região, direcionando a pesquisas a respeito dos fatores condicionantes. É difícil padronizar dados de diversas fontes, sugere-se a criação de banco de dados que permita compartilhar as informações entre as instituições.

PT.013
AVALIAÇÃO DE QUIRÓPTEROS POSITIVOS PARA RAIVA E SITUAÇÃO VACINAL DOS GATOS NO BLOQUEIO DE FOCO DE BOTUCATU-SP, EM UM PERÍODO DE 5 ANOS

Carvalho VM[1],[2],[3],[4],[5] – [1]Faculdade de Medicina Veterinária e Zootecnia – UNESP Campus Botucatu – Depto de Higiene Veterinária e Saúde Pública, [2]Prof. Dr. Cassiano Victória – Depto de Higiene Veterinária e Saúde Pública, [3]Prof. Dr. Carlos Roberto Padovanni – Depto de Bioestátistica, [4]Gabriella Koppany González – Equipe de Vigilância Ambiental em Saúde, Prefeitura de Botucatu, [5]Valdinei Moraes Campanucci da Silva – Equipe de Vigilância Ambiental em Saúde, Prefeitura de Botucatu

Palavras Chave: Quirópteros – Felinos – Raiva

Introdução: O presente trabalho avaliou a situação do risco de transmissão da Raiva em felinos levando-se em consideração a incidência de quirópteros positivos para a Raiva e o percentual da cobertura vacinal em gatos nas áreas de Bloqueio de Foco no município de Botucatu-SP, através de um estudo retrospectivo no período de 2005 à 2009. Para análise foram utilizados os relatórios de bloqueio de foco fornecidos pela Equipe de Vigilância Ambiental em Saúde (EVAS) da Prefeitura Municipal de Botucatu. **Resultados e Discussão**: No período avaliado, constatou-se 9 casos de quirópteros positivos para Raiva no município, de 4 espécies diferentes. Durante os 5 anos de Bloqueio de Foco, 10.625 residências foram visitadas e avaliado a situação vacinal e idade de 5.103 cães e 980 gatos. Posteriormente os dados levantados pelo Bloqueio de Foco foram comparados com os resultados obtidos na Campanha de Vacinação Antirrábica no mesmo período, para avaliar se os felinos domiciliados no perímetro do Foco estavam corretamente imunizados contra Raiva e se há risco real de transmissão da doença. **Conclusão**: Considerando que a média da cobertura vacinal nas Campanhas Antirrábicas no período de 2005 à 2009 foi 52,65%, índice abaixo do preconizado pela Organização Mundial da Saúde (OMS) e que na avaliação do estado vacinal dos gatos conferidos pelo Bloqueio de Foco indicam que 92% dos felinos não estavam previamente imunizados, concluindo que há risco de reintrodução da Raiva em humanos através do ciclo: quirópteros – felinos – humanos, caso as Campanhas de Vacinação não atinjam a Meta Vacinal para gatos no Município de Botucatu. **Agradecimentos**: Ao Depto de Higiene e Saúde Pública da FMVZ – Unesp Campus Botucatu, a Equipe de Vigilância Ambiental em Saúde da Prefeitura de Botucatu – SP e a agência FAPESP pelo financiamento da pesquisa.

PT.014
MONITORAMENTO DE MORCEGOS (QUIROPTERA) COMO ESTRATÉGIA DE VIGILÂNCIA DA CIRCULAÇÃO DO VÍRUS DA RAIVA NO RIO GRANDE DO SUL

Witt AA[1], Donini MAW, Predebon J, Diedrich G, Prato R – [1]Centro Estadual de Vigilância em Saúde/RS – Programa Estadual de Controle e Profilaxia da Raiva

Morcegos são animais comuns em áreas urbanas no Rio Grande do Sul, principalmente em grandes cidades. Atualmente, dentre os animais sinantrópicos observados nessas áreas os morcegos são, provavelmente, os que causam maior preocupação por parte das autoridades de saúde pública. Segundo o Ministério da Saúde, desde 2004, os morcegos são os principais agentes na disseminação do vírus da raiva no Brasil. Diante desse cenário, a Secretaria Estadual de Saúde através do Centro Estadual de Vigilância em Saúde (CEVS), instituiu o Programa de Monitoramento de Morcegos, com o objetivo de estudar a importância dos quirópteros no ciclo urbano da raiva. O monitoramento de raiva através de amostras de morcegos era realizado até o ano de 2011, de forma passiva, onde os morcegos encontrados em situações não habituais (caídos no chão, dentro de casa, etc.), eram enviados para investigação laboratorial sem terem sido identificadas e catalogadas as espécies envolvidas neste processo. Sendo assim, o CEVS passou então a identificar e catalogar os animais enviados pela população, com o objetivo de traçar estratégias para o manejo de morcegos em áreas urbanas no Estado. Além disto, foi estabelecida rotina para coleta de morcegos, para obtenção de amostras de saliva, sangue e tecido cerebral de indivíduos em colônias de diversas regiões do Estado. A maior parte dos morcegos é anilhada e solta para verificar deslocamentos entre cidades e regiões positivas para raiva. No ano de 2011 foram enviadas para análise 268 amostras de quirópteros, das quais apenas seis indivíduos de morcegos nãohematófagos resultaram positivos. A maioria das amostras de morcegos pertence à família Molossidae, onde se observam espécies bem adaptadas à vida nas cidades. Com base neste monitoramento o Rio Grande do Sul está investindo na prevenção e investigação da circulação do vírus rábico nas áreas urbanas, onde atualmente, o morcego é considerado o principal agente transmissor.

PT.015
DETECTION OF B LYMPHOCYTES IN THE CENTRAL NERVOUS SYSTEM OF CATTLE AND HORSES NATURALLY INFECTED WITH RABIES VIRUS.

Achkar SM[1], Fernandes ER[1], Carrieri ML[1], DUARTE MIS[2] – [1]Instituto Pasteur – Laboratório de Diagnóstico da Raiva, [2]Faculdade de Medicina da Universidade de São Paulo – Departamento de Patologia

Humoral immunity has an essential protective function in the course of rabies virus infection. Virus-neutralizing antibodies, under the control of T helper cells, play a critical role in immunoprotection. Therefore, our aim was to detect the presence of B lymphocytes in samples of central nervous system (CNS) from cattle and horses and compare the findings between species. For this were selected four samples of cattle and four samples of horses. Fragments CNS (cortex, hippocampus, cerebellum and brain stem) were analyzed by immunohistochemical reaction. All immunostained cells were quantified with the aid of the graticule of 1 cm2 coupled in light microscope with 10x eyepiece and 40 x objective, were quantified 40 fields in every fragment of the CNS. Results were expressed in number of cells per mm2. When comparing the population of B lymphocytes in cattle and horses, we found a statistically significant increase of these cells in cattle in relation to horses (p = 0.0006). By analyzing

the different brain areas in samples obtained from bovine not was found statistically significant differences between areas, but in samples of horses was found a greater presence of these cells in the brain stem (p = 0.0266). We could also observe that the meningeal and perivascular environments were where we find more immunostaining for B lymphocytes. The detection of B lymphocytes in CNS samples of cattle and horses was more pronounced in meningeal and perivascular environments, suggesting that these cells would be entering the CNS by breaking the blood brain barrier, however, the induction of specific antibodies for viral clearance is delayed, occurring only when the animal already have in severe neuronal damage. Although we have detected B cells *in situ* in the samples studied, these cells were in small amounts mainly in samples of horses. The collaboration intercellular between CD4 + T lymphocytes and B lymphocytes for activation of these cells and consequently induction of specific antibodies to the virus may be impaired because CD4 + T lymphocytes when entering the CNS may undergo apoptosis through its association with infected neurons that up expressing FASL and bind to CD4 + T cells expressing FAS occurring so the death of these immune cells essential for protection against rabies virus. These findings are important for understanding how the immune response is manifested in these animal species and also to improve understanding of the pathogenesis of rabies in cattle and horses.

Financial support: INSTITUTO PASTEUR/FACULDADE DE MEDICINA DA USP

PT.016
EFFICACY OF RECOMBINANT ADENOVIRUS EXPRESSING G PROTEIN OF RABIES IN MICE

Yang DK[1], Kim HH[1], Song JY[1] – [1]QIA – Viral disease

Background: Since rabies case occurred again in 1993, a number of animal rabies had been reported up to 2011. Even though animal rabies cases seemed to be decreased, the continuous outbreak was identified at some counties of Gangwon Provinces of Korea. Although national mass vaccination program with live and inactivated vaccines to domestic and pet animals has blocked dog-to-dog transmission, most of rabies cases are related with to animal bitten by rabid raccoon dogs and rabies in wild animals are not eradicated. A safe and effective vaccine is needed for the immunization of wild animals and dogs. Human adenoviruses have been studied as viral vector. In this study, we constructed three kinds of recombinant adenovirus expressing rabies proteins and checked efficacy of the constructs in mice. **Material and Methods**: Rabies virus (RABV) circulating in Korea was isolated using neuroblastoma cell (NG108-15) in 2009. The RABV designated as KRVB0910 strain was propagated in the NG108-15 cells for the cloning of genes. In order to analysis the glycoprotein (G) and nucleocapsid (N) genes of the strain, the G and N genes were amplified with three kinds of primers and cloned into pENTR/D-TOPO cloning vector respectively. After cloning three genes (Nfull, Gfull,G-TMCD), each plasmids containing the genes were transfected into TOP10 competent cells. The purified plasmids were mixed with pAd/CMV/V5-DEST gateway vector and the mixtures had reaction with LR Clonase II enzyme to catalyze the LR recombination reaction. After confirming the expression clones, the clones were digested with Pac I to expose the ITRs and transfected into the 293A cell lines to construct recombinant adenovirus (reAdV) expressing N and G genes of RABV. The 293A cells transfected with the clones showed specific cytophatic effect. For 6 days after inoculation, the cells were stained with monoclonal antibodies and FITC conjugated goat anti human IgG+IgM and examined by fluorescent microscopy. To check efficacy of three kinds of reAdVs, the reAdVs containing 108.0 TCIID50/ml was inoculated into 4 weeks old Balb/C mice. Survival rate and change of body weight of the mice were checked for 17 days after challenge. **Results**: We successfully reconstructed three kinds of reAdVs (Nfull, Gfull, G-TMCD) in 293A cells. The titer of reAdVs ranged from 107.7 to 108.0 TCID50/ml. Four groups of mice (Gfull, G-TMCD, Nfull+Gfull, Nfull+G-TMCD) were inoculated with 0.2 ml reAdV and half of mice in each group were challenged with CVSN2c strain intramuscularly 21 days after inoculation. All mice did not show any typical rabies symptoms and showed complete protection. On the other hand, half of mice in three groups (Gfull, G-TMCD, G-TMCD+Nfull) did not show complete protection against challenge by intracranial (IC) route. However, the one group inoculated with Nfull+Gfull reAdVs revealed 100% survival rate. These data demonstrated the potential of the reAdV as a safe rabies vaccine. **Conclusion**: We constructed three kinds of reAdVs in 293A cells. The combination of two kinds of reAdVs (Nfull+Gfull) may be a useful tool in search of rabies vaccine candidate for animals and further study related to oral vaccination of dogs and raccoon dogs is needed in the near future.

PT.017
INTERACTION OF RABIES VIRUS GLYCOPROTEIN FRAGMENTS WITH THE NICOTINIC ACETYLCHOLINE RECEPTOR

Rideout SA[1], Harris MB[2], Hueffer K[3], Schulte MK[2] – [1]Univeristy of Alaska Fairbanks, [2]University of Alaska Fairbanks, [3]University of Alaska – Veterinary Medicine

The rabies virus glycoprotein (RVG) interacts with Torpedo and muscle nicotinic acetylcholine receptors (nAChR). The field of Ligand Gated Ion Channels, such as the nicotinic receptors, has benefited greatly over the last decade due to the discovery of non-membrane bound Acetylcholine Binding Proteins (AChBP). Since nicotinic acetylcholine receptors and the ACHBP share significant sequence and structural homology in the neurotoxin binding domain, the AChBP could provide a useful model for studying the molecular basis of the RVG/nAChR interaction. In this study we investigated the interaction between RVG neurotoxin like peptide fragments and the AChBP. Surface Plasmon resonance (SPR) was used to assess binding kinetics to the AChPB. Electrophysiology experiments were used to compare these results to interactions between these RVG fragments and human nicotinic acetylcholine receptor subtypes. RVG fragments were shown to bind with micromolar affinity to the Lymnaea AChBP. SPR permits determination of on and off rates for binding of all 6 fragments. Our data show slow on rates (ka= 100-300 1/M•s) with off rates (kd = 0.01-0.004 1/ M•s) corresponding to binding with a dissociate rate (Kd of 25.4-60.3 micromolar). Voltage clamp electrophysiology data obtained using Xenopus oocytes shows similar Ki values for inhibition of acetylcholine induced responses on alpha4/beta2 nAChR.

PT.018
RABIES IN IRAN

Baghaipour MR[1] – [1]Milad Hospital

Rabies is a disease caused by a virus, Lyssavirus rabies that affects the nervous system and usually results in death unless treated quickly. Rabies is found in mammals in all regions of the world. The disease infects domestic and wild animals, and is spread to people through close contact with infected saliva via bites or scratches. Dogs are the main host and transmitter of rabies but bats,