

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.

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#### PT.016

#### EFFICACY OF RECOMBINANT ADENOVIRUS EXPRESSING G PROTEIN OF RABIES IN MICE

Yang DK<sup>1</sup>, Kim HH<sup>1</sup>, Song JY<sup>1</sup> – <sup>1</sup>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 cytopathic 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 TCID<sub>50</sub>/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 TCID<sub>50</sub>/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 CVS2c 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<sup>1</sup>, Harris MB<sup>2</sup>, Hueffer K<sup>3</sup>, Schulte MK<sup>2</sup> – <sup>1</sup>University of Alaska Fairbanks, <sup>2</sup>University of Alaska Fairbanks, <sup>3</sup>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 AChBP. 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 ( $k_{on} = 100-300 \text{ 1/M}\cdot\text{s}$ ) with off rates ( $k_{off} = 0.01-0.004 \text{ 1/ M}\cdot\text{s}$ ) corresponding to binding with a dissociate rate ( $K_d$  of 25.4-60.3 micromolar). Voltage clamp electrophysiology data obtained using *Xenopus* oocytes shows similar  $K_i$  values for inhibition of acetylcholine induced responses on  $\alpha 4/\beta 2$  nAChR.

#### PT.018

#### RABIES IN IRAN

Baghaipour MR<sup>1</sup> – <sup>1</sup>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,

foxes, raccoons, coyotes, wolves may transmit the disease as well. More than 50 000 people die of rabies every year. The virus spreads through the central nervous system and progressive, fatal inflammation of the brain and spinal cord develops. After an incubation period of 1–3 months (days to years) the initial symptoms start with fever and often pain or paraesthesia at the wound site. In about 35% of the patients, Rabies has a longer course. The muscles gradually become paralyzed, starting at the site of the bite or scratch. A coma slowly develops, and eventually death occurs. It is named the paralytic form and its diagnosis is difficult. The majority of patients show a furious form and exhibit signs of hyperactivity, excited behavior, hydrophobia and sometimes aerophobia. After a few days, death occurs by cardio-respiratory arrest. No tests are available to diagnose rabies infection in humans before the onset of clinical disease. For suspected animals, taking tissue samples (often brain tissue) and using immunofluorescence or immunological techniques to detect the virus is the mainstay of diagnosis. A person exposed to rabies should ideally be treated within 12 hours of the exposure and definitely within 48 hours for the best outcome. They are usually treated with rabies immune globulin and rabies vaccine initially and then get several additional vaccine injections. The number of infected person has been decreased during past decade in Iran. Most of them are males and under ten years old. More than 250 centers provide post exposure treatment for Rabies and Pasture Institute have produced IG and Vaccine of Rabies since 85 years ago.

#### PT.019

### PROGRAMAS DE EDUCAÇÃO EM SAÚDE – ALCANCE, ESTRATÉGIAS, METODOLOGIA.

Reichmann MLB<sup>1</sup>, Nunes VFP<sup>2</sup>, Santos MB<sup>3</sup>, Viaro O<sup>4</sup> – <sup>1</sup>Instituto Pasteur – Assistente Técnico, <sup>2</sup>Prefeitura do Município de Jundiá – Jardim Botânico, <sup>3</sup>Ministério do Meio Ambiente – Instituto Chico Mendes, <sup>4</sup>Secretaria Municipal de Saúde de São Paulo – Centro de Controle de Zoonoses

**Introdução:** A Educação em Saúde deve ser estruturada em ações programáticas. Os profissionais da área devem conhecer a realidade local a ser trabalhada, estratégias específicas que favoreçam a incorporação de princípios de promoção da saúde, preservação do meio ambiente, a interação saudável com animais de estimação, efetivando a guarda responsável. Os interlocutores devem ser estimulados a compreender o assunto, analisar as informações, aplicar os conteúdos aprendidos e experimentar mudanças em seus hábitos e comportamentos, transferindo e replicando os conceitos adquiridos e incorporando-os a sua cultura e a seus valores. **Objetivos:** Divulgar os trabalhos de educação em saúde do Estado de São Paulo – SP, em 2011. **Materiais e Métodos:** Pesquisa documental nos programas de educação e promoção da saúde nos municípios que utilizam metodologia compatível com as pesquisas científicas, gerando um roteiro, descrevendo atividades e resultados obtidos. **Resultados:** O roteiro utilizado incluiu resumidamente: conhecer e visitar o local habitado pelos interlocutores, identificar problemas na interação com animais, estabelecer objetivos e estratégias compatíveis com aquilo que deve ser trabalhado, avaliar periodicamente os resultados e corrigir vieses. As ações programáticas geraram instrumentos que serviram de normas e diretrizes para informar, nortear e educar interlocutores de diferentes classes socioeconômicas e educacionais, fortalecendo vínculos para uma melhor qualidade de vida. **Conclusão:** A incorporação de programas de educação em saúde por profissionais das áreas de Saúde, Meio Ambiente e Saúde Animal, desde o início de suas carreiras, utilizando metodologia compatível com suas atribuições propicia maior efetividade nos resultados.

#### PT.020

### AVALIAÇÃO DOS ESTRATOS DA POPULAÇÃO CANINA DE MUNICÍPIOS DO INTERIOR DO ESTADO DE SÃO PAULO – BREVE AVALIAÇÃO DA LITERATURA.

Reichmann MLB<sup>1</sup>, Alves MCGP<sup>2</sup>, Matos MR<sup>3</sup>, Dominguez MHS<sup>4</sup>, Dominguez MHS<sup>4</sup> – <sup>1</sup>Instituto Pasteur – Assistente Técnico, <sup>2</sup>Secretaria de Estado da Saúde de São Paulo – Instituto de Saúde, <sup>3</sup>Secretaria de Estado da Saúde de São Paulo – Superintendência de Controle de Endemias, <sup>4</sup>Profissional autônoma

**Introdução:** Uma das principais questões relacionadas ao cuidado e proteção da saúde humana e animal consiste na determinação do número de animais e de suas demais características demográficas. Esta avaliação sempre foi possível em um dos estratos, a de cães com proprietários, uma vez que a presença nos domicílios permite sua contagem. A Técnica Pasteur São Paulo (TPSP) foi um modelo de avaliação de populações caninas de áreas urbanas, desenvolvido em 2001, que permitiu a estimação da população segundo os estratos de restrição e dependência. Metodologia: Em 2002, a TPSP foi aplicada nos municípios do interior do Estado de São Paulo para estimar o número de cães existentes segundo estratos e conhecer aspectos relacionados à vacinação e dinâmica populacional. A amostra foi composta de 100 setores censitários, distribuídos em 41 municípios. Resultados: Foram obtidos dados que se situavam acima da relação até então disponíveis, indicando uma proporção de um cão para cada quatro habitantes. Salientase a baixa idade dos cães, em média, de quatro anos, indicativa da velocidade da reposição populacional. A maior concentração (59%) de cães foi de até três anos, gerando preocupações com a introdução continua de animais susceptíveis e os possíveis prejuízos no controle da leishmaniose e demais zoonoses. Estas dificuldades ficam presentes quando se observa a alta percentagem (20%) de proprietários que desconheciam se seus animais foram vacinados contra a raiva nos 12 meses anteriores e quantas crias foram geradas nos partos ocorridos no mesmo prazo de tempo. Conclusões: Mesmo para cães com proprietário, parcela teoricamente mais protegida, a convivência com animais de estimação não tem se revestido da responsabilidade legal para prevenção de doenças que envolvam as pessoas e os animais. Recomenda-se a inserção de programas suplementares, como os de educação, de registro e concessão de licenças e a interação com profissionais de diversas especialidades para obtenção de resultados concretos no equilíbrio ecológico de cães mantidos em domicílios.

#### PT.021

### UTILIZAÇÃO DO GEOPROCESSAMENTO COMO FERRAMENTA NO MONITORAMENTO E CONTROLE POPULACIONAL DOS MORCEGOS HEMATÓFAGOS NAS ILHAS FLUVIAIS DO MUNICÍPIO DE ANANINDEUAPARÁ/ BRASIL.

Ramos OS, Barreiros MA, Souza EM, Souza SMF, Melo TIS

É crescente a importância do ciclo silvestre envolvendo os morcegos hematófagos na região, tem sido observada uma mudança no perfil epidemiológico da raiva humana no estado do Pará, tornando os morcegos os principais responsáveis pela doença. O presente trabalho visa fornecer subsídios possibilitando um banco de dados geográficos dos principais eventos, para gerar mapas de áreas propícias para o ataque espoliativo de morcegos hematófagos, fazendo o controle populacional dos quirópteros, cadastrando e georreferenciando abrigos e as propriedades dos moradores ribeirinhos das ilhas Sasunema, João Pilatos, Santa Rosa e Viçosa onde os animais (suínos, bovinos e