

la correcta realización e interpretación de estos ensayos. Las elevadas temperaturas, como las que se registran en las provincias del norte de Argentina, pueden ocasionar el deterioro de los cadáveres de los animales investigados, provocando que las muestras de cerebro presenten desde una licuefacción leve hasta un avanzado estado de descomposición. Estas condiciones afectan la sensibilidad de las pruebas diagnósticas dado que provocan la degradación de la estructura viral y la producción de toxinas bacterianas. Asimismo, si los aislamientos de RABV no se conservan a muy bajas temperaturas (-70°C), pierden rápidamente su viabilidad lo que ha provocado la pérdida de muchas colecciones de RABV en laboratorios que carecen de la infraestructura adecuada. Se evaluó una técnica de RTPCR de un paso para el diagnóstico y caracterización molecular en muestras de tejido cerebral en avanzado estado de descomposición y en aislamientos antiguos. Se tomó un grupo de 10 cepas de rabia aisladas en cerebro de ratón lactante, de las variantes de mayor circulación en nuestro país, 3 cerebros caninos expuestos a descomposición controlada y 14 cepas antiguas. La caracterización antigénica se realizó mediante la técnica de inmunofluorescencia indirecta usando un panel de 19 anticuerpos monoclonales (CDC, USA). La caracterización molecular de una región de 159 nucleótidos correspondiente al gen de la nucleoproteína fue analizada y se confeccionó un árbol filogenético. La caracterización antigénica y molecular se correspondió en todos los aislamientos. En este estudio pudo efectuarse la caracterización molecular de los aislamientos de mayor circulación en Argentina, en muestras en avanzada descomposición y en cepas antiguas en forma directa, con una técnica que utiliza una pequeña porción del gen de la nucleoproteína viral en el 100% de las muestras.

PT.041 ANTIGENIC VARIANTS OF RABIES VIRUS IN VENEZUELA. 2000-2012

PEREZ M¹, HIDALGO M¹, BOYER L¹ – ¹National Institute of Agricultural Research (INIA) – Rabies Laboratory

Rabies is a fatal zoonotic disease, caused by the rabies virus, the prototype of the genus *Lyssavirus* of the *Rhabdoviridae* family, with a single-stranded negative-sense RNA genome, surrounded by a bullet shape capsid. In Venezuela for many years rabies has occurred in endemic and epidemic form, constituting a socioeconomic problem that affects human health and causes losses in livestock. It is distributed throughout the country. The detection of rabies antigen and antigenic characterization of field strains allowed the identification of animal species that serves as a reservoir responsible for an outbreak of rabies in a given area. The aim of this study was to perform the antigenic characterization of 34 fields isolates of rabies virus from different animal species, states and years, to know which antigenic variants were circulating in our country. The detection of rabies antigen was performed by direct immunofluorescence test of nerve tissue imprints of animals with symptoms of the disease. The viral amplification was performed by inoculation in suckling mice. Antigenic characterization was performed by indirect immunofluorescence impressions brains of mice inoculated with field strains that had obvious symptoms of the disease. Only variants 1 and 3 were found. It was concluded that the antigenic variant 1 (canine) was located exclusively in Zulia State, while variant 3 (vampire) was present in several states, so the common vampire bat *D. rotundus* was the main transmitter of rabies for livestock in that period. **Acknowledgement:** National Institute of Agricultural Research (INIA), National Institute of Integral Agricultural Health (INSAI). **Funding:** INIA. DeMattos C. OMS pg 30(1989). Hidalgo M. Rev. Fac. Cs Vets UCV 46:33. (2004) Hidalgo M. Rev. Fac. Cs. Vets. UCV. 49(2):121.(2008). Meslin FX WHO 476p (1996). Hidalgo M. Med.Vet al día. 1:19 (2011).

PT.042 EXPERIMENTAL ANTIVIRAL THERAPY AGAINST DIFFERENT RABIES VIRUS LINEAGES USING TRANSFECTION WITH ANTI-RABIES ANTIBODIES

Castilho JG¹, Batista HBCR¹, Rodrigues AC¹, Carnieli Jr P¹, Oliveira RN¹, Silva ACR², Caporale GMM², Carrieri ML¹, Kotait I¹, Brandão PE³ – ¹Instituto Pasteur – Virologia, ²Instituto Pasteur – Imunologia, ³Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

The aim of this study was to develop a new mechanism for antiviral therapy against rabies based on the introduction by transfection with a cationic reagent (lipofectamine 2000) of antibodies into neuronal cells infected with the rabies virus. N2A cells were infected using 96-well plates and different viral concentrations (0.1, 1.0, 10 and 100 TCID₅₀) of three lineages of rabies virus circulating in Brazil (dog, *Desmodus rotundus* and *Eptesicus furinalis*). After incubation for 24 h, the cells were transfected with anti-rabies- virus polyclonal antibodies and lipofectamine 2000. These cells made up the treatment group (TG). The cells in the negative control group (CG) were treated with only Minimum Essential Medium. After 11 hours, the plates were fixed with 80% acetone and analyzed by direct immunofluorescence using a fluorescein isothiocyanate-conjugated antinucleocapsid rabies antibody. The effectiveness of the transfection and subsequent neutralization of the virus was determined by calculating the percentage inhibition of fluorescent foci. This was done by measuring the difference in the number of fluorescent foci in the two groups (CG and TG). The results show that for lower viral concentrations (0.1 and 1.0 TCID₅₀), viral inhibition was 100% for all the lineages tested. When higher virus concentrations were used (10 and 100 TCID₅₀), inhibition varied according to the viral load and lineage of rabies virus used. With an infectious dose of TCID₅₀, inhibition varied from 82.7% to 100% for the lineages tested. With a 100 TCID₅₀ dose, inhibition was 90.7% for the *D. rotundus* lineage, 90.3% for the dog lineage and 67.0% for the *E. furinalis* lineage. It can be concluded from these results that, irrespective of the viral load the patient is exposed to, transfection with antibodies is an efficient mechanism for use in antiviral therapy against rabies in cases where the transmitter is the hematophagous bat *D. rotundus* or the dog as inhibition only varied from 89.2% to 100% when these lineages were used. However, if the patient has been exposed to the lineage associated with the insectivorous bat *E. furinalis*, inhibition varies with viral load. These findings show that transfection with antibodies is a promising mechanism that could be used to develop an antiviral therapy against rabies. Further studies are required to assess the efficiency of transfection with antibodies *in vivo*. Financial Support: FAPESP

PT.043 CLASSIFICATION AND POSITIVITY RATE OF BATS RECEIVED FOR RABIES DIAGNOSIS

Lima JYO¹, Scheffer KC¹, Achkar SM¹, Kotait I¹, Carrieri ML¹ – ¹Instituto Pasteur

The diversity of bat species in Brazil is great, and there are 172 species distributed among nine families. The *Phyllostomidae* family is the most numerous, followed by *Vespertilionidae* and *Molossidae*. According to feeding habits, the majority of bats are insectivorous, followed by frugivorous, nectarivorous, carnivorous and hematophagous. As these animals are considered reservoirs of rabies virus it is essential to correctly identify the species and knowledge of the