

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 ( $-70^{\circ}\text{C}$ ), 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

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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

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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 TCID<sub>50</sub>) 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 antirabies- 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 isothiocyanateconjugated 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 TCID<sub>50</sub>), viral inhibition was 100% for all the lineages tested. When higher virus concentrations were used (10 and 100 TCID<sub>50</sub>), inhibition varied according to the viral load and lineage of rabies virus used. With an infectious dose of TCID<sub>50</sub>, inhibition varied from 82.7% to 100% for the lineages tested. With a 100 TCID<sub>50</sub> 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

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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

biology, behavior and adaptation of bats in urban environments. The objective of this study was to identify the several specimens of bats received for rabies diagnosis at the Pasteur Institute, meet the positive index of them and assess their distribution on the feeding habits in relation to the seasons. In the period between January and December 2011, specimens of bats were received for the rabies diagnosis and submitted to identify the family, genus and/or species, according to identification keys, journals and scientific books. We also recorded the feeding habits and month of capture of animals. The period of this study was divided according to the seasons. During this period, 2846 specimens of bats were identified, being 77.6%, 11.4% and 11% belonging to the *Molossidae*, *Vespertilionidae* and *Phyllostomidae* families, respectively. Of the total of bats identified 88.53% had habits insectivorous, 6.82% frugivorous, 4.39% sucking nectar and 0.28% hematophagous. In the warmer seasons of the year (spring and summer) it was received 1808 bats being 1727 insectivorous, 48 frugivorous, 29 sucking nectar and two hematophagous. In the colder seasons (autumn and winter), it was received 1038 bats being divided into 791 insectivorous, 145 frugivorous, 95 sucking nectar and 6 hematophagous. In relation to the rabies diagnosis, 37 specimens (1.3%) were diagnosed as positive, including the families *Vespertilionidae* (17), *Phyllostomidae* (13) and *Molossidae* (7). According to feeding habits bats were classified into 30 insectivorous and seven frugivorous specimens. The positive rate in the winter, were four insectivorous and one frugivorous, and it seems smaller than other stations. The positive rate in the summer was higher in *Vespertilionidae* than in *Molossidae*. This result was different that was observed by Constantine in the USA. He observed the higher rate positive in *Molossidae*. The differences observed in this study suggested the importance of feeding habits and breeding season of bats, considering principally the supply of food and warm seasons. The knowledge of bats and seasonal identification studies can contribute to improve the epidemiological surveillance program used in rabies management in determining the existence of bats of different families and/or feeding habits in different seasons.

**PT.044**  
**STUDY AND DISTRIBUTION OF RABIES VIRUS IN NON NEURONAL ORGANS IN BATS SENT TO LABORATORY DIAGNOSIS IN PASTEUR INSTITUTE**

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Bats are considered important reservoirs of rabies virus, which is paramount in the study of the pathogenesis of this zoonosis disease, through the use of sensitive systems. The presence of viral antigen in these species shows that non-neuronal viral spread is efficient in different organs that participate effectively in the elimination of rabies virus, such as, salivary glands and bladder. This study aimed to investigate the presence of rabies virus in samples of bats submitted for laboratory diagnosis, as well as, to study the pathogenesis of the disease through the use of laboratory animals. 3,930 routine diagnostic specimens of bats were processed during the period between January 2011 and May 2012 by direct immunofluorescence (DIF) and viral isolation on murine neuroblastoma cells (VICC) techniques. 58 samples were diagnosed rabies positive from 37 bats in 2011 and 21 samples were diagnosis rabies positive in 2012, representing a positivity rate of 1.80%. We randomly selected 28 bats from rabies positive which were submitted to collect organs for preparation of inoculum in the proportion to the ratio of 1:10 for salivary glands and tongues, and 1:20 for bladders, which were inoculated in volume of 0.03 mL by the intracerebral route in post-weaning Swiss mice (21 days old and weighing 11g and 14g). Clinical observation was performed during 30 days and the presence

of the virus was verified by the DIF technique in diseased and dead animals. 60.7%, 50% and 42.8% of animals selected for the study were rabies positive by viral isolation in the salivary glands, tongue and bladder, respectively. The minimum incubation period was seven days and maximum incubation period varied between 17 and 21 days. The present study demonstrated the presence of rabies virus in non-neuronal organs (salivary gland, bladder and tongue) in 67.8% rabies positive animals in central nervous system (CNS). The detection of rabies virus in non-neuronal organs by DIF and virus isolation has been observed in several studies. For studies of pathogenesis of rabies in bats, these results demonstrate that the use of mice is still a good alternative. Due to lack to use CNS in routine practice in bats for reasons of poor preservation of the specimen, it may be necessary to use nonneuronal organs in order to obtain the positive rabies diagnosis.

**PT.045**  
**EFFECTS OF ONRAB IN SELECT NON-TARGET WILDLIFE SPECIES**

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ONRAB<sup>®</sup> is a recombinant rabies vaccine used to as an oral vaccine in wildlife species such as: fox (*Vulpes vulpes*), raccoons (*Procyon lotor*), and striped skunks (*Mephitis mephitis*). The viral vector in the ONRAB<sup>®</sup> vaccine is human adenovirus type 5 (HAd5) with the gene for rabies glycoprotein incorporated into its genome. HAd5 is a relatively safe and well-studied virus, which is used in many vaccine formulations. Canadian researchers (e.g., Knowles et al. 2009) have conducted vaccine efficacy and safety studies using ONRAB<sup>®</sup> in 18 species of animals. Our research expands on the species previously evaluated. We studied the vaccine as it relates to its safety in wildlife species likely to contact the ONRAB<sup>®</sup> vaccine during oral rabies vaccine (ORV) campaigns in the United States. We investigated the effects of high doses of the ONRAB<sup>®</sup> vaccine in wood rats (*Neotoma spp.*), eastern cottontail rabbits (*Sylvilagus floridanus*), Virginia opossums (*Didelphis virginiana*), Eastern wild turkey (*Meleagris gallopavosilvestri*), and fox squirrel (*Sciurus niger*), whose range overlaps with ORV target species in the United States. After inoculation of the animals we performed realtime PCR on fecal swabs, oral swabs, and tissues to detect viral DNA. Our preliminary results mostly concur with the findings of Knowles et al. (2009). By 7 days postvaccination, turkeys, opossums, and cottontails had all stopped shedding viral DNA. One woodrat and five fox squirrels still had detectable levels of viral DNA in fecal swabs on 7 days post-inoculation. However, 45% of fox squirrels were co-infected with *Leptospira interrogans*, which may be a confounding factor to the prolonged detection of viral DNA in fecal swabs from these animals. There were no significant findings on gross histology of liver, kidney, small intestine, large intestine, and lung in any of the species studied. We are currently completing PCR analysis of the tissues listed above as well as nasal turbinates. Initial results suggest low likelihood of persistence of ONRAB<sup>®</sup> in the environment or in individual animals that contact the vaccine. Our preliminary conclusions suggest that non-target species will not be negatively impacted by the distribution of ONRAB<sup>®</sup> as part of ORV programs in the United States.