

reservoirs are haematophagous bats. In the State of Rio Grande do Sul (RS), southern Brazil, urban rabies has not been detected since 1988. Nevertheless, rabies remains endemic in haematophagous and non haematophagous bat species. The present work reports the first phylogenetic analyses on RABV isolates from the State of RS, for that, a total of 30 rabies virus (RABV) isolates sent to rabies diagnosis were analyzed. The isolates were recovered from different bat species (*Tadarida brasiliensis*, *Myotis nigricans* and *Histiotus velatus*), from herbivores (bovines and buffalo) and carnivores (domestic dog and cat). The bat species were identified with the aid of a morphological dichotomous key. For the phylogenetic analysis, total RNA was extracted from original brains (herbivores and carnivores) or infected mice (bats) with Trizol and submitted to reverse transcription/polymerase chain reaction (RT-PCR) with primers targeting a initial portion of the nucleoprotein gene (N). Phylogenetic analysis of the sequenced fragments revealed the occurrence of four RABV lineages, named after its natural hosts: *Desmodus rotundus* (haematophagous bat), *Tadarida brasiliensis* (insectivorous bat), *Myotis nigricans* (insectivorous bat) and *Histiotus velatus* (insectivorous bat). All RABV isolates from herbivores belonged to the haematophagous bat *Desmodus rotundus* lineage. The two RABV isolates from carnivores clustered within the *Tadarida brasiliensis* lineage, revealing two occasional spillovers from insectivorous bats to domestic pets, thus not compromising the status of “urban rabies free” of the area. These findings highlight the importance of the identification of RABV lineages and its value as an aid to support rabies surveillance. Financial support: Instituto Pasteur.

CO.40

SITUACIÓN EPIDEMIOLÓGICA DE LA RABIA EN CHILE. 2000-2011

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En Chile, el año 1990, se detectó el último caso de rabia identificado como variante canina desde entonces esta variante no circula en el país, la importancia de los animales silvestres en la transmisión de la rabia fue reconocida en 1985, cuando se detectó por primera vez rabia en murciélagos insectívoros de la especie *Tadarida brasiliensis*. El reconocimiento de los murciélagos como reservorio de la enfermedad hizo que se ampliaran las acciones de vigilancia epidemiológica hacia esas especies caracterizándose el patrón epidemiológico de la rabia por una epidemia en quirópteros. Desde el año 2000 al 2011, se analizaron un total de 32802 muestras para diagnóstico de rabia, de estas 979 fueron positivas (3,0%), 976 murciélagos insectívoros, 2 gatos y 1 perro. Según la distribución geográfica de casos, estos se registraron en las regiones centrales del país, y no se han encontrado muestras positivas al virus rábico en las regiones extremas. A través de tipificación antigénica y genética se han identificado 4 variantes virales que son las responsables de la transmisión de la rabia, los principales reservorios silvestres circulando en el país son murciélagos de la especie *Tadarida brasiliensis*, *Myotis chiloensis*, *Lasiurus cinerius* y *borealis* y finalmente *Histiotus macrotus*. La especie *Tadarida brasiliensis* representa el 91,1% de los casos positivos. Los estudios de caracterización antigénica y genética nos han permitido tener un conocimiento más amplio de la epidemiología de la rabia. El Programa de Control de Rabia contempla la educación de la población para evitar el contacto con murciélagos y el reporte de cualquier mamífero sospechoso, la eliminación de colonias de murciélagos se realiza solamente en casos de detección de especímenes positivos, en razón del importante rol que esta especie desarrolla en la mantención del equilibrio ecológico y dado el bajo porcentaje de positividad a rabia (alrededor de 2%) en capturas masivas de esta especie.

CO.41

MOLECULAR CHARACTERIZATION OF RABIES VIRUS AND OTHER VIRAL AGENTS ISOLATED FROM BATS IN VENEZUELA.

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Bats (Chiroptera) are reservoirs for zoonotic diseases, including Rabies, Hendra, Nipah, SARS-CoV, Ebola virus. Hence their importance as a potential reservoir hosts of viruses affecting human and animal health. In our country, there is no knowledge of bats as reservoir for viruses except rabies. The aim of this investigation was the molecular characterization of rabies virus and other viral agents, isolated from bats in Venezuela. The molecular characterization was based on: viruses with impact in public health, persistence in hosts and endemic areas. A total of 54 bats were collected in different states and years. Those were identified and classified into: 12 vampires, 29 frugivorous and 13 insectivorous belonging to different families, genera and species. They were autopsied to collect tissues from different organs including brain tissue of livestock positive to rabies virus. Different systems were used for PCR to detect DNA and RNA viral genomes. Samples were amplified, molecularly characterized and sequenced to identify the phylogeny of each virus. We were able to detect 8 Herpesviruses and 4 Polyomaviruses in trachea and lungs samples from different bat species and one Astrovirus in an intestine of an insectivorous bat. Eight Rabies isolates were grouped in the genus *Lyssavirus* genotype 1. Four of them characterized as antigenic variant 3 (*Desmodus rotundus*). The detection of these viral agents in the Venezuelan bats is the first and paramount information for the study of these unknown agents, which could pose great risk to humans and livestock health in our country. **Acknowledgements:** MCTI-Misión Ciencia, Venezuelan Institute of Scientific Investigation (IVIC): Molecular Virology Laboratory, National Institute of Agricultural Research (INIA): Rabies Laboratory, National Institute of Integral Agricultural Health (INSAI). **Funding:** IVIC. **Almeida M**, *Rev.Inst.Med.trop.S.Paulo*; 53:31, 2011; **Calisher C**, *Rev.Med.Vir*, 17:67, 2007; **Chen Zhue**, *J.Gen.Virol*, 90:883, 2009; **De Mattos C**, *J.Clin.Microbiol*, 34:1553, 1996; **Olivier D**, *PLoS ONE*, 4:e2057, 2008; **Richter R**, *J.Gen.Virol*, 90:44, 2009; **Wong S**, *Rev.Med.Vir*, 17:67, 2007.

CO.43

THE SPATIAL AND TEMPORAL DYNAMICS OF RABIES IN CHINA

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Background and Objectives: Recent years have seen a rapid increase in the number of rabies cases in China and an expansion in the geographic distribution of the virus. In spite of the seriousness of the outbreak and increasing number of fatalities, little is known about the phylogeography of the disease in China. In this study, we report an analysis of a set of Nucleocapsid sequences consisting of samples collected through the trial Chinese National Surveillance System as well as publicly available sequences. This sequence set represents the most comprehensive dataset from China to date, comprising 210 sequences (including 57 new samples) from 15 provinces and covering all

epidemic regions. Using this dataset we investigated genetic diversity, patterns of distribution, and evolutionary history. **Results:** Our analysis indicates that the rabies virus in China is primarily defined by two clades that exhibit distinct population subdivision and translocation patterns and that contributed to the epidemic in different ways. The younger clade originated around 1992 and has properties that closely match the observed spread of the recent epidemic. The older clade originated around 1960 and has a dispersion pattern that suggests it represents a strain associated with a previous outbreak that remained at low levels throughout the country and reemerged in the current epidemic. **Conclusions:** Our findings provide new insight into factors associated with the recent epidemic and are relevant to determining an effective policy for controlling the virus.

CO.44 PLAYING THE ODDS: PRIORITIZING HUMAN RABIES BIOLOGICS IN LIMITED SUPPLY SCENARIOS

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Limitations in the availability and access to human rabies biologics in enzootic regions result in most rabies deaths in the developing world. Efforts to supply modern rabies vaccines and immune globulin (RIG) have improved availability, but cost and the lack of structured programs in many countries remain major obstacles to providing optimal care. Proposed policies to provide rabies post-exposure prophylaxis (PEP) at no cost to the patient through government programs are challenged by the limited supply of biologics that providers are able to obtain. In many cases, the demand for biologics exceeds the limited supplies and national rabies programs are therefore forced to ration, resulting in delays or complete failures in provision of adequate PEP. Optimal PEP involves the use of rabies immune globulin and vaccine. While WHO recommendations for PEP are comprehensive, those recommendations offer no guidance on management of rabies exposures when there are limited supplies of biologics in the country nor if there is only vaccine available but no RIG. Complex operationalization issues, such as to how to approach prioritization when both nervous tissue and modern vaccines coexist in a country, or how to optimally integrate private distribution of biologics, are not part of the WHO guidance documents. We present a proposal on how to develop recommendations and guidelines to deal with these scenarios accounting for local rabies epidemiology, patient age and body size, delays after exposure, and cultural and social issues. Several Old and New World country cases are presented to highlight how these challenging circumstances might be managed and overcome.

CO.45 IMMUNE RESPONSE OF BALB/C MICE IMMUNIZED WITH VERO CELL RABIES VACCINE AND BpMPLA-SE ADJUVANT

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The prophylaxis is an important strategy to control of human and animal rabies disease. The vaccine from Vero cellular culture for human use is efficacy and safety. However, because the technology used to produce this vaccine is expensive this product costs about ten dollars. This cost makes them impossible the use of this vaccine type in poor countries where the animal rabies control is inefficient and there many cases of human rabies. Rabies disease

is responsible for about 55,000 deaths per year in the world. The objective of this study was evaluate the humoral immune response of mice (Balb/c) immunized with three different doses of Vero rabies vaccine associated with the BpMPLA-SE adjuvant. This adjuvant is a product obtained from Bordetella pertussis. Three groups of ten mice were immunized with two doses of 500µl (G1), 250µl (G2) or 125µl (G3) of Vero cell rabies vaccine (IB-lot 1103075) mixed with BpMPLA-SE (10µg/dose). Three groups control (Gc) received only rabies vaccine. The immunization occurred on days 0 and 21 and samples were taken ten days after the last dose injected and on days 60, 120 and 180 to determine the titers of neutralizing antibodies for rabies virus in BHK21 cells (RFFIT). The averages of the neutralizing antibodies titers found in the samples from each group ten days after finished the immunization were 39.2, 33.1, and 20.4 IU/ml for groups G1, G2 and G3 respectively. The results obtained on day 180 were 17.1 IU/ml (G1), 10.6 IU/ml (G2) and 9.8 (G3). In the control groups the averages of the antibodies titers were: 29.7 (Gc1), 26.9 (Gc2) and 22.2 IU/ml (Gc3) after immunization and 10.7 (Gc1), 9.5 (Gc2) and 8.5 IU/ml (Gc3) on day 180 (Gc3). These data show that the adjuvant BpMPLA-SE increased the humoral immune response for rabies vaccine in Balb/c mice independent of the volume of vaccine utilized to immunize the animals. The results found are very important to reduce the number of doses and the volume of Vero cell rabies vaccine utilized in the immunization against rabies. Financial Support: Butantan Foundation

CO.46 SAFETY AND IMMUNOGENICITY OF THE PURIFIED VERO RABIES VACCINE NEXT GENERATION IN CHINESE PEDIATRIC (≥ 10 YEARS) AND ADULT POPULATIONS

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Background: The Purified Vero cell Rabies Vaccine Next Generation (PVRV-NG) is a highly purified vaccine developed with innovative technology and human and animal origin components-free medium. It was shown to be at least as immunogenic as Verorab[™] and presented a similar safety profile in a phase II clinical study conducted in France (pre exposure regimen). A phase III clinical study was performed in Chinese pediatric (≥ 10 years) and adult populations in simulated post-exposure regimen to further document PVRV-NG in comparison to Verorab[™]. **Methods:** This was a randomized, blind-observer, controlled study in healthy subjects aged 10 to 17 years (pediatric cohort) or ≥ 18 years (adult cohort). Participants received five doses by intramuscular route of PVRV-NG or Verorab[™] (ratio 2:1 in each age group) at Do, D3, D7, D14 and D28 as per recommendation for post-exposure prophylaxis (Essen schedule). No rabies immune-globulins were administered concomitantly with the first vaccine dose. Immunogenicity was evaluated at Do, D14 and D42 by measuring the level of rabies virus neutralizing antibodies (RVNA) using the rapid fluorescent focus inhibition test. Testings were performed at the National Institute for Food and Drug Control (Beijing). Safety was evaluated with a list of predefined solicited injection site and systemic reactions during the period between Do and D14 and during the seven days after the 2 last doses; any adverse events until 28 days after the final dose and any SAE until 6 months after the final dose were also recorded. **Results:** 816 participants were enrolled; 408 in each age group corresponding to 272 in PVRV-NG group and 136 in Verorab[™] group. The predefined criterion for noninferiority in terms of proportions of participants with RVNA titers ≥ 0.5 IU/mL at D14 (before the 4th injection) was met in the per-protocol analysis set and confirmed in the full-analysis set population,