CO.37
DETECTION OF RABIES VIRUS IN ORGANS OF BATS OF GENUS ARTIBEUS BY MEANS OF HEMI-NESTED RT-PCR AND REAL TIME RTPCR TECHNIQUES

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Molecular techniques have been used increasingly as tools for the diagnosis by detecting the rabies virus genome. This study aimed to detect the presence of rabies virus in the wash of skull and in different organs of the genus *Artibeus* bats using the hemi-nested RT-PCR (hnRT-PCR) and Real Time RT-PCR molecular techniques. From approximately 4,000 specimens of bats received at the Institute Pasteur for rabies diagnosis, 30 bats of the genus *Artibeus* were selected, with records of positive results for rabies by the traditional techniques of direct fluorescent antibody test (FAT) and inoculation of murine neuroblastoma cell line (N2A). Salivary glands, urinary bladders, kidneys, lungs, and also the washes of the skullcaps of the specimens were collected. The scraping of the skull was performed with the aid of sterile pipette tips and then washed with 1,000µL diluent composed of 0.85% saline solution, supplemented with 2% Bovine Fetal Serum, free of rabies virus specific antibodies and containing 0.1% Gentamicin Sulfate. The urinary bladders were diluted using the same diluent mentioned above, to 1:20 (w/v) and other organs were diluted 1:10 (w/v). The extraction of total RNA was carried out using the TRIzol® and the reverse transcription was followed by PCR and hnRT-PCR using primers specific for the gene encoding the N protein. From the product derived by the reverse transcription, the Real Time RTPCR technique was run by using primers and probes specific for the antigenic variant 3 of rabies virus. When evaluated the total samples analyzed, the overall results of the sensitivity for both the hnRT-PCR and Real Time RT-PCR techniques was 86%. A comparison between the hnRT-PCR and Real Time RT-PCR techniques performed by Fisher's exact test has revealed that the proportion of positives detected for the washing of the skull was similar to that of the organs examinations (P> 0.05). In relation to the positive results found in hnRT-PCR and Real Time RT-PCR techniques were 100% in brain washes, 90% and 93.33% in the salivary glands, 83.33% and 90% in urinary bladders, 80% and 93.33% in kidneys, and 76.67% and 50% in lungs. These results suggest that both the hnRT-PCR and the Real Time RT-PCR techniques can be used as complementary methods for the rabies diagnosis and the techniques are sensitive enough for use in studies of pathogenesis. The Real Time RT-PCR technique performed in this study proved effective in detecting the rabies virus in different organs and extra neural tissues with the advantage of being a faster and more sensitive procedure.

CO.38
IDENTIFICATION OF THE SPECIES OF RESERVOIRS AND HOSTS OF THE RABIES VIRUS AND OTHER PATHOGENS BY SEQUENCING OF THE CYTOCHROME-B MITOCHONDRIAL DNA GENE

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The identification of animal species that transmit pathogens such as the rabies virus is of the utmost importance for public health and the natural history of infectious and contagious diseases. Diagnostic laboratories very often receive mauled or decomposing animal carcasses, particularly of bats, rendering morphometric identification unviable. The existence of different regional names for the same animal, morphological variability and the lack of staff trained in zoological identification constitute a serious problem for epidemiological surveillance. Molecular techniques are used routinely and effectively in systematics, evolution and ecology to identify species and can even be used to identify hybrids that originated from genetically close animals, in which the differences very often go undetected by morphometry. Some mitochondrial DNA (mt- DNA) genetic markers, such as control region sequences and the genes encoding cytochromes b and c, are frequently used in the genetic identification of species. Many of the genetic sequences for these genes are stored in public-domain websites such as GenBank, allowing new sequences to be compared with existing ones in databases. The objective of this study is to build a database with genetic sequences from the cytochrome b gene of rabies reservoir species for use in the identification of these species. mt-DNA fragments were amplified and sequenced as described previously by Carnieli et al. (2008), using the primers 5'- CGACTAATGACATGAAAAATCACCGTTG-3' (sense) and 5'- TATTCCCTTTGCCGGTTTACAAGACC-3' (antisense) described by Martins et al. (2007). Sixty-six mt-DNA samples from different species of wingless Brazilian mammals and fifty-four samples from different species of chiropterans were sequenced. Analysis of the genetic sequences from these wingless mammals highlighted the problem of genetic identification of species as only a few sequences of mt-DNA from wingless mammals of Brazil were found in GenBank. For example, there are seven species of marmosets (genus *Callithrix*) but mt-DNA sequences for only some of them are deposited in GenBank. However, the cytochrome b gene sequences obtained from bats in this study, together with morphometric identification carried out in parallel, allowed us to name the species with certainty. From the fifty-four mt-DNA samples from chiropterans, nineteen species from eight genera and four different families were identified. Thus, the method described here is efficient in the identification of animal species and the search for samples of mt-DNA in Natural History Museums and Zoos may complement and certify unequivocally the sequences in the database under construction. Financial Support: Instituto Pasteur, Brazil

CO.39
PHYLOGENETIC ANALYSIS OF RABIES VIRUS IN THE STATE OF RIO GRANDE DO SUL, SOUTHERN BRAZIL

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Rabies is a worldwide zoonosis caused by rabies virus (RABV), a member of the *Lyssavirus* genus, family *Rhabdoviridae*. In nature, RABV is maintained in cycles with distinct natural reservoirs. In the urban cycle, the main reservoir for the virus is the domestic dog, on the other hand, in the sylvatic cycle different species can be the reservoir. In Latin America, the main natural RABV reservoir is the haematophagous bat *Desmodus rotundus*. However, RABV lineages adapted to different bat species, including insectivorous and frugivorous bats, have been frequently reported. The RABV lineages isolated from non haematophagous bats are genetically distinct from the RABV lineages whose natural

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 detecto 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 endemia en quirópteros. Desde el año 2000 al 2011, se analizo 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, Lasiurius 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 Astroviruses 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