four in Colombia (two in 2008 and two in 2012) and one in Ecuador (2009). In each case, the epidemiological investigation implicated a cat as the vector. The antigenic and genetic analyses identified variants maintained by the hematophagous bat *Desmodus rotundus*. Fruit-eating bats in the genus *Artibeus* may also be affected by a variant similar to that of *D. rotundus*. Such fruit bats may be found in urban areas. Such affected species can transmit rabies virus to felids, which are important predators of bats. Therefore, in cases of human rabies following aggression by cats in areas that are otherwise free of canine rabies (variants 1 and 2) but where there are rabies epizootics in sentinels such as herbivores, the hypothesis of secondary transmission of bat rabies viruses should always be investigated.

CO.32
STANDARDS AND ASSAYS FOR RABIES SEROLOGY

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Several immunoassays are currently used to measure humoral immunity to the rabies virus. A standard or reference serum is a consistent component of all assays to standardize the results and control the assay performance. Two human international standard rabies immune globulin (SRIG) reference serum preparations are recognized by the World Health Organization (WHO): the first international WHO SRIG(WHO 1) with a potency of 59 IU and the second WHO SRIG(WHO 2) with a potency of 30 IU. The WHO 1 SRIG is also known as RIG Lot R-3 in the United States, distributed by Center for Biologics Evaluation and Research (CBER). The WHO 2 SRIG is distributed by National Institute for Biological Standards and Control (NIBSC) in the UK. These standards are used globally to promote uniform potency measurement of RIG products used for prophylaxis, individual vaccine response, and disease diagnosis in a standard international unit (IU/mL). Because it is important to have consistent and accurate assignment of IU/mL values for rabies immune globulin (RIG) products and reliable vaccine response measurements for the evaluation of vaccines, the SRIG in use for a particular method should be routinely evaluated for potency against a recognized international standard. Previous studies in 1997 and 2006 have indicated that WHO 1 SRIG has lost potency in comparison with WHO 2 SRIG. Further potency comparison studies have supported this finding. To determine the difference in potency between the two SRIG preparations and their potencies in different assays, a comparison study was performed at KSU. Three potency levels of each SRIG and four rabies virus neutralizing antibody (RVNA) positive serum samples were tested in two rapid fluorescent focus inhibition assays (RFFIT), differing in challenge virus strain and cell type, and two ELISA assays, one indirect and one blocking. Statistical analysis revealed there is no significant difference overall in the measurements when either WHO 1 or WHO 2 are used as the SRIG in the RFFIT assays. However, a trend was clearly seen in higher IU/mL values obtained when WHO1 was used as the SRIG to obtain the IU/mL values. Additionally, for some of the samples a significant difference in IU/mL was found. The comparison of indirect ELISA results, where the kit standard is used to calculate the EU/mL values, revealed a significant difference between WHO 1 and WHO 2 measurements at potency level 2.0 IU/mL; with WHO 1 higher in EU/mL value than WHO 2. The potential for obtaining incongruent measurements with use of different SRIG preparation and the performance of each SRIG in different methods should be considered when selecting assays standards/controls and in the interpretation of rabies serology results.

CO.33
EVALUATION OF AN ELISA TO DETECT RABIES ANTIBODIES IN WILD (FOXES AND RACCOON DOGS) AND DOMESTIC CARNIVORES (DOGS AND CATS)

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The international organizations (OIE, WHO and European Commission) have promoted the use of serological testing in addition to other specific requirements as an alternative to the quarantine for free movements of pets in certain countries. They have also recommended that countries involved in oral vaccination programmes against rabies should monitor the efficacy of the campaigns by testing a certain number of field samples collected from the target species to check their immunity against rabies. The WHO/OIE reference tests (the FAVN test and the RFFIT) are time-consuming, expensive, require highly-trained technicians, the maintenance of cell cultures, laboratories with a high containment level and vaccinated technicians to handle live rabies virus. In addition, since they are based on cell cultures, they are sensitive to any cytotoxic products and contaminating agents present in samples. We have evaluated the performances of a commercial ELISA (BioPro Rabies ELISA Ab kit, Czech Republic), a blocking ELISA that detects rabies virus antibodies, in reference to the FAVN test. The specificity assessed on 315 samples from unvaccinated dogs and cats was 100%. A total of 701 samples from vaccinated dogs and cats were tested using the FAVN test and the ELISA. The overall agreement between the two tests was found equal to 86%. Considering samples from wildlife, a total of 188 sera from foxes and raccoon dogs were sampled in a rabies-free country. The specificity reached 100% in those sera taken from naïve animals. Overall, a high concordance (95%: 648 out of 682 sera) was observed between the BioPro ELISA and the FAVN test, which was similar in sera from red foxes (95.1%: 388 out of 408 sera) and raccoon dogs (94.9%: 260 out of 274 sera). The concordance between tetracycline and seropositivity results was also evaluated. The overall agreement with tetracycline results was excellent in the fox for both the BioPro ELISA (95.9%) and the FAVN test (91.8%). Concordance was slightly lower in the raccoon dog, with a value of 82.8% for the BioPro ELISA and 78.4% for the FAVN test. Rabies antibodies were equally detected with the BioPro ELISA in animals vaccinated with different types of vaccines (SAG2 or VRG vaccine baits) and in highly haemolysed sera. In our hands, the BioPro ELISA is a valuable alternative to the FAVN test for assessing rabies antibody titres in vaccinated pets and in fox and raccoon dog populations for the follow up of oral vaccination campaigns efficacy. An inter laboratory collaborative study is planned in the next future to assess the reproducibility of the BioPro ELISA for laboratories involved in the monitoring of oral vaccination programmes.

CO.34
DETECTION OF RABIES VIRUS – SPECIFIC ANTIBODIES IN WILD MAMMALS FROM A RAINFOREST AREA, SÃO PAULO, BRAZIL USING RFFIT, SFIMT AND ELISA TECHNIQUES

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The emergent importance of rabies in wild animals in Brazil demonstrates the necessity of continuous epidemiological surveillance in these animal species aiming the development of better strategies for the prevention and control of the disease. The use of blood serum samples from several wild species captured in a native Rainforest area in the North coast of São Paulo State, Brazil, was an excellent opportunity for the research of rabies virus circulation among wildlife in the region, and also to compare different techniques for antibodies detection. In this study we used the "Rapid Fluorescent Focus Inhibition Test – RFFIT, the Simplified Fluorescent Inhibition Microtest – SFIMT and the Enzime Linked Immunosorbent Assay – ELISA techniques for the detection of rabies virus-specific antibodies in terrestrial wild mammals. Out of 139 samples, 15 (10,8%) presented positive titers for RFFIT ("gold standard" for detection of rabies virus neutralizing antibodies), 50 (35,9%) positive titers for SFIMT and 02 (1,43%) positive titers for ELISA. When comparing RFFIT and SFIMT, 100 (72%) samples presented concordant results when considering positive and negative titers. These results are an evidence of rabies virus circulation between the wild animal species (mainly opossums, capuchin-monkey and coati) in the studied area, even when considering the low concordance between RFFIT and SFIMT. The discordant results between ELISA and RFFIT or ELISA and SFIMT, (99,3%), can be due to the fact that the ELISA kit used was developed for vaccinated foxes, and when considering the Brazilian fauna, which present a great species variety without the use of oral vaccination, the efficacy of the technique could be affected. This result indicates the importance of continuous research regarding a better knowledge of the role presented by wild animals in rabies circulation and transmission in Brazil. Epidemiologic studies in different regions of the Country could provide a valuable information regarding the prevention and control of the disease, and also aiming the standardization and validation of the different diagnostic serologic techniques, especially considering the great and unique variety of animals present in our Country. Acknowledgments: Gaia Consultoria Ambiental, Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

CO.35
ANOMALIES IN THE RABIES INDIRECT FLUORESCENT ANTIBODY TEST CONFOUND ACCURATE ANTEMORTEM DIAGNOSIS OF HUMAN RABIES

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The antemortem diagnosis of rabies in humans employs techniques that require accuracy, speed and sensitivity. A combination of histochemical, *in vitro* virus isolation, immunologic and molecular amplification procedures are utilized in an effort to diagnose the disease. Present day technology offers a potentially life-saving treatment for a disease that was considered invariably fatal once clinical signs develop. This new development adds to the need for a rapid diagnosis as early in the course of clinical signs as possible. The techniques offering diagnosis within hours are the direct fluorescent antibody test on skin and the indirect fluorescent antibody procedure on cerebral spinal fluid and serum. We describe examination by indirect fluorescent antibody assay of cerebral spinal fluid and serum taken from patients with viral encephalitis or a presumed viral infection from an agent other than rabies virus. A total of 135 CSF samples from viral encephalitis patients were tested by the rabies indirect fluorescent antibody procedure. A majority of the spinal fluids tested, from patients with encephalitis, presented immunoglobulins that bound to antigens present in cell culture substrate. Most notable were the reactions on kidney cells provided from sera or spinal fluid obtained from patients diagnosed with the flavivirus infections Powassan Virus or West Nile Virus. The majority of reaction patterns were recognizably different than what is seen with specific anti-rabies antibodies. However, results indicate that false positive results could occur when interpreting the rabies indirect fluorescent antibody procedure. A staining pattern appearing similar to specific anti-rabies staining was observed in 7 of the 135 spinal fluids examined. The potential for false positive results documented in this work offers weight to the argument that tandem positive results from two diagnostic test platforms are essential when diagnosing rabies in the human patient.

CO.36
UNIQUE RABIES VIRUS VARIANT AND GENETIC LINEAGE IN INSECTIVOROUS BATS *Histiotus velatus*, BRAZIL.

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Bats represent approximately one-third of the Brazilian mammal fauna and the Rabies virus has been isolated from 41 of the 167 species of bats present in the country. A *Rabies virus* independent species-specific variant was detected in 16 insectivorous bats *Histiotus velatus* in the Southeast of Brazil from 1995 to 2009. The antigenic characterization was made by monoclonal antibodies panel from Centers for Disease Control and Prevention (CDC – Atlanta, USA) and the genetic characterization was performed by sequencing of carboxi-terminal portion of nucleoprotein followed by Maximum Likelihood (ML) genetic analysis with GARLi software. The antigenic characterization made in 12 of these samples showed a unique profile previously described only for the insectivorous bats *Histiotus velatus* (positive reactivity only with MAb C12 from the utilized panel). The entire 16 samples positive to rabies virus were genetically characterized and they were segregated in the independent monophyletic cluster supported by high bootstrap values. These sequences showed a minimal average intrinsic distance whiting group (1.3%) but they presented low similarity when compared to other lineages circulating in bats and other wild animal lineages from Brazil and worldwide with a range of 8.8% to 25.4%. The antigenic site of the nucleoprotein at residue 377 to 379 (based on PV strain) analysis showed a residue TEV (Thr-Glu-Val) like a some insectivorous bats and different to vampire bats lineage, marmosets lineage and terrestrial cycle related samples. The PV strain shows the amino acids residues TDV (Thr-Asp-Val), *D. rotundus* isolates show AET (Ala-Glu-Thr) and Marmoset lineage show the amino acids residues TEA (Thr-Glu-Ala). This antigenic variant and genetic lineage has been identified in a large area covering various kilometers and different biomes for at least 14 years between the states of Minas Gerais and Sao Paulo exclusively in this bat species. Surprisingly the system documentation not describe this antigenic variant and genetic lineage found before in other bat species and the *Histiotus velatus* bat species never ever found before with a different antigenic variant and genetic lineage. The fact of this lineage has been isolated only in this species besides long temporal space and geographically distal to each other, associated with phylogenetic results and previously antigenic data suggest strongly that this rabies virus lineage is associated to *Histiotus velatus*.