

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

Moore S<sup>1</sup>, Hanlon C<sup>1</sup> – <sup>1</sup>Kansas State University – KSVDL/Rabies Laboratory

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)

Cliquet F<sup>1</sup>, Guiot AL<sup>2</sup>, Schereffer JL<sup>1</sup>, Tribout L<sup>1</sup>, Wasniewski M<sup>1</sup>, Mähar K<sup>3</sup> – <sup>1</sup>Nancy laboratory for rabies and wildlife, France, <sup>2</sup>CPB – Conseils en Pharmacie et Biologie – France, <sup>3</sup>Estonian Veterinary and Food Laboratory, Virology-Serology department – Estonia

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

Araujo DB<sup>1</sup>, Wasniewski M<sup>2</sup>, Rodrigues CS<sup>3</sup>, Campos ACA<sup>3</sup>, Martorelli LFA<sup>4</sup>, Kataoka APAG<sup>4</sup>, Cunha EMS<sup>5</sup>, Durigon EL<sup>3</sup>, Favoretto SR<sup>3,6</sup> – <sup>1</sup>Universidade de São Paulo – Núcleo de Pesquisas em Raiva – Laboratório de Virologia Clínica e Molecular, <sup>2</sup>Nancy Laboratory for Rabies and Wildlife, <sup>3</sup>Universidade de São Paulo, <sup>4</sup>Centro de Controle de Zoonoses de São Paulo – COVISA, <sup>5</sup>Instituto Biológico, <sup>6</sup>Instituto Pasteur de São Paulo