CO.29
DETECTION OF THE DOG/COYOTE VARIANT OF RABIES VIRUS IN THE MEXICO-US BORDER

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Currently in the border states of northern Mexico, samples from skunks and dogs that have the V-1 antigenic variant of the rabies virus have been identified, however, genetic analysis indicates that they are strains of skunks. In 2010, 4 cases of human rabies were registered in Mexico, while in Nuevo Leon there was 1 case of canine rabies transmitted by insectivorous bats. It has been almost a decade since the apparent non-transmission of the (V-1) variant dog/coyote, in the border between Mexico (Coahuila and Tamaulipas) and USA (Texas). Surveillance data suggest that this variant of canine rabies virus is not longer in circulation in the United States of America, however, the last detection was registered in March 2004 in the US-Mexico border. In addition, one dog carrying the rabies virus was detected in June 2011 in the town of Sabinas Hidalgo, NL., which is about 130 km from the US-Mexico border. This finding is very important because the (V-1) variant (dog/coyote) could be present on the border of both countries. The geography of this region is very similar to Texas, which may permit the free movement of carnivorous species on both sides of the border. Therefore, the objective of this study was to identify the (V-1) variant of rabies virus in the dog from Sabinas Hidalgo, NL. By using direct immunofluorescence, antigenic characterization with a panel of 8 monoclonal antibodies, RT-PCR and nucleotide sequencing techniques, the 709pb (751nt to 1460nt) and 420pb (992nt to 1411nt) fragments from the semi-variable region of the viral N protein were analyzed. According to the antigenic characterization, the variant found was the V-1, while the molecular study was also positive for this variant. Our results suggest the same lineages published by Velasco-Villa et al., 2005 in the analyzed sample. This study demonstrates the actual prevalence of the V-1 variant (dog/coyote) in the US-Mexico border and warns about the risk for transmission of the V-1 variant to humans, as well as to domestic and wild animals. Acknowledgements: We are grateful to Miguel Angel Zuniga, Isabel Aguilar Tavitas and Alma Liliana Lizarán Meneses for their support in the diagnosis of rabies virus. Financial support: This work was supported by the Rabies Program of the Ministry of Health of Nuevo Leon and Health Services of Nuevo Leon.

CO.30
IMPORTANCIA EPIDEMIOLÓGICA DE LOS MURCIÉLAGOS INSECTÍVOROS EN LA TRANSMISIÓN DEL VIRUS RABIA A FELINOS Y OTROS MURCIÉLAGOS CASEROS EN EL VALLE DEL CAUCA, COLOMBIA

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En los últimos diez años se ha destacado la importancia epidemiológica de los murciélagos insectívoros como transmisores de la rabia a felinos y otros murciélagos insectívoros/caseros en Colombia. En junio del 2012, después de 21 años sin registrarse casos de rabia en humanos en el Departamento del Valle del Cauca, se presentan dos muertes en mujeres mordidas por gato infectado con la variante antigénica 4 (VAg4), cuyo reservorio es el murciélago insectívoro *Tadarida brasiliensis*. **Objetivos.** Determinar las asociaciones entre especies de murciélagos, su comportamiento, la transmisión del virus entre ellos y su significado epidemiológico con énfasis en riesgo para la población humana y mascotas. **Metodología.** Durante el periodo diciembre 1999 a junio 2012, fueron capturados más de 1.321 murciélagos por el programa de vigilancia de rabia en el departamento del Valle del Cauca. El diagnóstico de rabia se hizo por inmunofluorescencia directa e inoculación en ratones, utilizando tejido encefálico de los murciélagos capturados. La tipificación viral se hizo por inmunofluorescencia indirecta usando anticuerpos monoclonales. **Resultados.** Se detectaron dos ejemplares de *Eptesicus brasiliensis* positivos para rabia en los años 2000 y 2002, y dos casos más en especímenes de *E. brasiliensis* y *Molossus molossus*, en el 2008. Se encontró virus rabia VAg3 en gato año 2009 y VAg4 en humano mordido por gato año 2012. Se encontraron distintas especies de murciélagos, como *E. brasiliensis*, *M. molossus*, *Myotis nigricans*, *Glossophaga soricina*, *Noctiliio albiventris* y *Carollia perspicillata*, compartiendo refugios en casas. Se detectaron virus rábicos de VAg 3 y 4, en murciélagos *M.molossus* y *E.brasiliensis* así como en gato y dos humanos. **Conclusiones.** En Valle la presencia de las VAg 3 y 4 del virus rábico en murciélagos insectívoros/caseros, probablemente, ha sido facilitada por la deforestación de los hábitats naturales de estas especies; así como el estilo de arquitectura urbana que provee un hábitat artificial que posibilita el contacto físico entre las especies, aumentando la probabilidad de transmisión de rabia entre estas y en las personas que habitan las casas invadidas. Ante las dificultades para controlar la rabia en murciélagos y la falta de herramientas adecuadas, la vigilancia continua de la enfermedad en los murciélagos, basada en el diagnóstico y la tipificación de los virus rábicos por laboratorio, en los asentamientos humanos y alrededor de ellos, la vacunación preventiva en animales domésticos y de producción, así como la educación de la comunidad para la concientización del riesgo y la recolección pasiva de muestras para su análisis, se convierten en las mejores herramientas para prevenir la transmisión a humanos. **Agradecimientos y Financiación.** Secretarías de Salud Departamental Valle y Municipal Cali, Unidad Ejecutora de Saneamiento Valle, Laboratorios del Instituto Nacional de Salud y Universidad del Valle. Núñez MC. Infectio. 2012; 16(1): 23-29.

CO.31
SECONDARY TRANSMISSION OF RABIES IN LATIN AMERICA

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Compared to primary pathways among reservoirs, secondary transmission of rabies virus has not received much attention from researchers or public health professionals, because spillover of infection from hematophagous and non-hematophagous bats to a potential vector is believed to be uncommon. This review seeks to remind those working on Latin American rabies control programs of the possibility of a bat-cathuman pathway. Following the increase in the control of canine rabies in most Latin American countries, epidemiological surveillance also focused on hematophagous and non-hematophagous bats and the use of molecular techniques in the characterization of rabies. At least eight cases of secondary transmission to humans were identified in Latin America from 2001 to 2012: one in Brazil (2001), two in Costa Rica (2001),

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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