

CO.04**NOVEL LYSSAVIRUS FROM A MINIOPTERUS SCHREIBERSII BAT IN SPAIN.**

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In the frame of the Spanish Rabies Surveillance Program, a bat carcass was received on March 12th, 2012, in the Centro Nacional de Microbiología (National Center of Microbiology) (CNM). The bat was found in the city of Lleida, in July 2011, and it was taken to the Wildlife Care Center of Vallcalent (Lleida, Catalonia). The bat died soon after admission. Two different RT-PCR generic methods for the *Lyssavirus* genus (1,2) and two commercial rabies antisera for antigen detection were used for diagnosis. Brain smears were positive for both, FAT and RT-PCR, as well as the oropharyngeal swab for RT-PCR. The bat was morphologically and molecularly identified as *Miniopterus schreibersii* (3). To determine the identity of the virus, a fragment of the nucleoprotein gene (N) was sequenced. Dataset representative of all *Lyssaviruses*, including the recently described IKOV, was used for the phylogenetic reconstruction. The topology obtained by Bayesian Inference (BI) showed a new virus related more to IKOV and WCBV than any other lyssavirus included in phylogroups I and II. These results suggest a new virus, named *Lleida bat lyssavirus* (LLEBV), taking in consideration the locality where the bat was found. In Europe, from 1977 to 2011, a total of 988 cases of bat rabies were reported; *Eptesicus serotinus* and *E. isabellinus* which account for more than 95% of the cases are considered the major natural reservoirs of EBLV-1. Several *Myotis* spp. are reservoirs for EBLV-2, BBLV, ARAV, and KHUV(4). In Spain, EBLV-1 has only been described in *E. isabellinus* (5). Interestingly, the lowest nucleotide identity shown by LLEBV was with EBLV-1. The LLEBV has been detected on *M. schreibersii* such as WCBV. *Miniopterus* genus presently belongs to the *Vespertilionidae* family as the other bat genera linked to lyssaviruses in Eurasia (*Eptesicus*, *Myotis* and *Murina*). However, recent molecular analyses have postulated the group as an independent monospecific *Miniopteridae* family (6). *M. schreibersii* is a migratory bat, widely distributed throughout Southern Europe and Eurasia. This bat specie gathers in caves in large numbers (thousands) for wintering, moving in spring to different and sometimes distant summer roosts for reproduction. Due to its migratory habits and typically large size of populations of this bat, it is quite probable that once an infectious agent is introduced, it may spread quickly within populations. The evolutionary relationships between the new LLEBV with WCBV and IKOV need to be clarified to determine whether they will form one or more phylogroups. Further analyses are in process to assess this question and to establish a probable potential role as a human pathogen. More studies must be done in order to evaluate ecological aspects of LLEBV circulation. Nidia Aréchiga Ceballos is post-doctoral fellow granted by the Consejo Nacional de Ciencia y Tecnología (CONACyT) Mexico. This research was financially supported by the project SAF 2009-09172 of the General Research Program of the Spanish Ministry of Science and Education. **References:** 1. Echevarría JE, et al. J Clin Microbiol.; 39: 3678-83. 2001. 2. Vázquez-Morón S, et al., J Virol Methods. 135: 281-87. 2006. 3. Ibáñez C, et al., Acta Chiropterol.; 8: 277-297. 2006. 4. Schatz J, et al., Zoonoses Public Health. In press. 5. Vázquez-Morón S, et al., Emerg Infect Dis.; 17(3): 520-23. 2011. 6. Hooper, S. R y Van Der Bussche. Acta Chiropterol.. 5, supplement:1-63. 2003.

CO.05**HUMAN RABIES IN THE UNITED STATES: EVALUATION OF CLINICAL FINDINGS FROM 1960-2010**

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Background: Clinical diagnosis of human rabies is challenging in the United States (U.S.) due to the rarity of human cases, non-specific symptoms, and infrequent attainment of a potential exposure history. The traditional presentations of rabies, furious and paralytic, are reported in regions of endemic canine rabies, but have not been systematically characterized among rabies cases in the U.S. This study aims to examine the clinical characteristics of patients in the U.S. that are associated with rabies to aid in diagnosis and surveillance. **Methods:** Patient data were extracted from an existing database associated with samples submitted for rabies diagnosis to the Poxvirus and Rabies Branch at the CDC. A de-identified dataset consisting of records for all patients diagnosed with rabies from 1960-2010 and patients ruled-out for rabies from 2007-2010 was provided. Patients with at least one symptom reported were included in analysis. Chi-square was used for tests of association. **Results:** Of the 252 patients in the dataset, clinical symptoms were reported for 246; 103 (41.9%) of 246 were diagnosed with rabies. Symptoms significantly associated with rabies ($p < 0.01$) were localized pain or paresthesia (OR 10.4, 95% CI 5.6-19.1), hydrophobia (OR 9.9, 95% CI 4.3-22.2), dysphagia (OR 3.1, 95% CI 1.8-5.2), localized weakness (OR 2.9, 95% CI 1.7-5.1), and aerophobia (OR 15.3, 95% CI 1.9-121.3). The presence of agitation or a focal neurologic sign (dysphagia or localized pain, paresthesia, or weakness) had a combined specificity of 95% and a likelihood ratio of 1.8 for rabies. Symptoms significantly associated with patients for whom rabies was ruled-out by laboratory diagnosis included: confusion (OR 2.9, 95% CI 1.5-5.4), malaise (OR 3.3, 95% CI 1.9-5.6), seizure (OR 3.1, 95% CI 1.8-5.4) and headache (OR 4.1, 95% CI 2.4-8.8). **Conclusion:** Focal neurologic signs, hydrophobia, and aerophobia should be emphasized in the evaluation of patients for rabies and the submission of samples for laboratory diagnosis. These clinical associations may also be applied to a formal case definition to improve reporting of human rabies where laboratory diagnosis is limited. Ongoing analysis of clinical data from patients tested for rabies may be utilized to develop algorithms of signs and symptoms predictive of rabies diagnosis. **Topic:** Human Rabies or Epidemiology and Surveillance of Rabies **Honors and Financial Assistance:** Jennifer Bass performed this work as a fellow in the CDC Experience Applied Epidemiology Fellowship. Brett Petersen initiated this project as an officer in CDC's Epidemic Intelligence Service.

CO.06**CLINICAL FEATURES OF DOG- AND BAT-ACQUIRED RABIES IN HUMANS**

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Clinical differences in rabies due to canine and bat rabies virus variants have been noted, but no detailed studies have been reported to support these observations. Using PubMed and the MMWR we identified 120 case reports of rabies from the USA, Canada, Europe, and Asia. We systematically abstracted selected clinical features, results of investigations, incubation times and durations of illness. Details about clinical features were recorded. Cases were classified as dog- or bat-acquired based on reported animal exposure or viral

variant typing by molecular or monoclonal antibody characterization. Categorical variables were summarized as frequency (%), and continuous variables were summarized as median (interquartile range [IQR]). We compared bat- and dog-acquired cases using chi-square or Fisher's exact tests for categorical variables, and Mann Whitney U tests for continuous variables. Of 120 cases, 38 (32%) were dog-acquired and 54 (45%) were bat-acquired. Survivors and cases acquired from aerosolized viral exposure or tissue/organ transplantation were excluded. The median incubation times for dog- and bat-acquired rabies were 63 (IQR 42.75, 108) and 52.5 (IQR 27.25, 92.5) days, respectively ($p=0.074$). The median durations of illness for dog- and bat-acquired rabies were 17 (IQR 11.75, 23) and 14 (9.25, 18.5) days, respectively ($p=0.201$). There was no difference in patients with bat- and dog-acquired rabies in terms of the presence of fever, prodromal malaise, encephalopathy, sore throat, cranial nerve abnormalities, hemiparesis or seizures. Clinical manifestations that were more common in bat- than dog-acquired rabies included a local prodrome of sensory or motor symptoms ($p=0.026$), hemisensory abnormalities ($p=0.042$), tremor ($p=0.003$), and myoclonus ($p=0.009$). Neither tremor nor myoclonus was observed in patients with dog-acquired rabies. Aerophobia and facial or pharyngeal spasms were more common in dog- than bat-acquired rabies ($p=0.007$ and $p=0.029$, respectively). Hydrophobia was more common in dog-acquired rabies ($p=0.054$). There was no difference between dog- and bat-acquired rabies in terms of results of diagnostic investigations such as skin biopsy, salivary analysis or the detection of antibodies in serum and cerebrospinal fluid (CSF). The CSF protein was higher for bat rabies (79; IQR 52, 109 mg/dL) than dog rabies (31; IQR 26, 48, mg/dL; $p=0.012$). In summary, bat-acquired rabies is associated with more local symptoms, tremor, and myoclonus, whereas dog-acquired rabies has more hydrophobia, aerophobia, and pharyngeal or facial spasms. We speculate that these clinical differences may reflect differences in the route of viral entry of the rabies virus variants into the nervous system because fundamental differences in the neuropathology or viral distribution have not been identified. Bat rabies virus variants may also have greater effects on the blood-CSF barrier by affecting endothelial cell permeability through unknown mechanisms.

CO.07

IMMUNE RESPONSES IN HUMAN CNS DURING RABIES VIRUS INDUCED ENCEPHALITIS

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Understanding the mechanisms of rabies virus clearance from the CNS will be a significant step towards the treatment of clinical rabies. Although a few animal studies have provided insight about immune responses in the CNS during rabies encephalitis, data about the same are very scarce for humans. In our study, formalin-fixed, paraffinembedded, central nervous system (CNS) tissues from patients who succumbed to rabies following infection with RABV variants common to the tricolored bat (*Perimyotis subflavus*) in the United States, canine RABV present in Haiti, and canine RABV from Afghanistan, as well as tissues from a patient who recovered from clinical laboratory confirmed rabies following cat bite in Colombia, but succumbed as a result of secondary medical intervention, were subjected to comparative immunohistological evaluations identifying particular immune cell populations associated with rabies encephalitis. A non-encephalitic brain from an influenza patient was used as a negative control. Populations of B-cells (CD20), T-cells (CD3),

and macrophages (CD68), as well as the presence of rabies virus antigens were compared using a semi-quantitative scale. In addition, gene expression analyses, using the Human Antiviral Response RT² Profiler™ PCR Array, focusing on the expression of 84 key genes involved in the innate antiviral immune response, were performed. No rabies virus antigens were detected in the brain tissue of the patient who survived clinical rabies or in the control brain. T-cells and macrophages were abundant in the parenchyma in all rabies patients, but B-cells were detected only in the perivascular tissue of the putative rabies survivor, and rabies patients infected with canine RABVs. Few T- and B- cells, and only local microglia cells, were detected in the influenza patient. Differences in the expression of multiple genes associated with innate immunity, as well as inflammatory responses, were identified, suggesting the importance of their role in rabies encephalitis and viral clearance from CNS tissue.

CO.08

FIRST MYOTIS LUCIFUGUS RABIES VIRUS VARIANT DETECTED IN A HUMAN

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A 63 year old male from Barnstable County, MA was evaluated at Massachusetts tertiary care facility for possible stroke and encephalitis. Although the patient's first symptoms were joint stiffness, within 2 days the patient was exhibiting signs of hydrophobia, and acute progressive encephalitis. Serum, CSF, nuchal (skin) biopsy, and saliva samples from the patient were sent to CDC for rabies diagnostic testing. No rabies virus IgG or IgM antibodies were detected in serum and CSF by the indirect fluorescent antibody (IFA) test, and no viral neutralizing antibodies were detected in the serum or CSF samples by the rapid fluorescent focus inhibition test (RFFIT). Rabies virus antigen was detected in nuchal biopsy samples using direct fluorescent antibody (DFA) test. Nested (and heminested) RT-PCR amplicons were produced from skin and saliva using multiple rabies virus nucleoprotein gene primers sets. Sequence analysis of the entire nucleoprotein gene and comparisons with samples in the CDC database and Genbank indicated that the rabies virus variant was associated with *Myotis sp* bats. Further analysis of phylogenetic trees (1000) by Neighbor Joining, Maximum Parsimony and Maximum Likelihood indicated the variant was most parsimonious with the common "little brown bat" *Myotis lucifugus*. Postmortem brain tissues were positive for rabies virus antigen by the direct fluorescent antibody test. Antigenic typing with monoclonal antibodies to the rabies virus nucleoprotein was consistent with the previous results of a bat rabies variant, but lacked the resolution of genetic typing methods. Sequence analysis of the RT-PCR amplicons from the complete nucleoprotein gene were consistent with the previous findings of the variant seen in *M. lucifugus*. Although *M. lucifugus* is common in the US and frequently has had known encounters with humans and animals, this is the first documented case of this rabies virus variant in a human. In contrast to this unique finding, the rabies virus variant associated with a solitary bat with rare known human or animal encounters *Lasiurus noctivagans* (silver-haired bat) has been responsible for most human rabies cases in the USA over the last 2 decades.