CO.09
ENCEPHALITIS OF UKNOWN ETIOLOGY AND HUMAN RABIES IN THE UNITED STATES, 1999-2008

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Encephalitis is a severe neurologic syndrome caused by a variety of infectious and noninfectious pathologies. In many instances a definitive etiology of encephalitis is not identified, but a study of encephalitis in California found that 0.3% of cases referred for further evaluation to a specialized diagnostic facility (Glaser, Gilliam et al. 2003) was due to rabies. Under recognition of human rabies in the United States by healthcare providers may account for missed diagnoses of rabies. The purpose of this investigation was to estimate the number of encephalitis-related deaths in the United States and the proportion due to rabies specifically. We reviewed human mortality data in the United States between 1999-2008 and identified encephalitis-associated deaths using ICD-10 codes. Codes were categorized based on if an etiology of encephalitis was listed or not. We also reviewed all laboratory-confirmed human rabies cases within the United States and its territories that were reported to the Centers for Disease Control and Prevention (CDC) during this same time period. A total of 12,457 cases of encephalitis-associated deaths were identified, of which 8726 (70%) were of unknown etiology. Twenty (0.2%) cases were coded as rabies. During this same time period, 27 confirmed cases of rabies were reported to CDC. Of these 27 cases, 6 of the exposures occurred in countries other than the USA. Dogs were the most commonly reported exposure among imported cases. Of the 21 indigenous cases, 14 were attributable to bats, 4 to tissue/organ transplantation, 1 to a dog exposure in Puerto Rico and 2 from unknown sources. Our findings suggest that the majority of encephalitis-associated deaths in the United States were of unknown etiology. Among the confirmed cases of rabies, the majority who acquired rabies within the United States acquired the infection from wildlife. Nearly 10% of the indigenous cases occurred through an unknown exposure. Healthcare providers should consider evaluating for rabies when a patient develops acute progressive encephalitis of unknown etiology despite routine evaluation or if clinical history is suggestive. Glaser, C. A., S. Gilliam, et al. (2003). "In search of encephalitis etiologies: diagnostic challenges in the California Encephalitis Project, 1998-2000." Clin Infect Dis **36**(6): 731-742.

CO.10
HUMAN RABIES IN RIO CASCA MUNICIPALITY, 2012: CASE REPORT, SIX YEARS AFTER THE LAST RECORD IN MINAS GERAIS, BRAZIL

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Rabies is a viral disease that affects the central nervous system (CNS) of mammals and it's fatal in almost 100% of cases. In 2012, six years after the last record of a human case in Minas Gerais, the transmission has been confirmed through aggression by vampire bat. The objective of this report is to describe the clinical and epidemiological characteristics of this case. On 06/19/2012, the Health Department of Minas Gerais State has been notified by the Regional Health Department of Ponte Nova of a suspected case of human rabies from Rio Casca municipality. He was a 32-year-old male patient, farmer, residing in the rural area. The patient suffered bitten by a bat in his right hand after trying to remove it from the back of a calf in the month of May. At that time the patient did not seek medical care and the event was not notified to health service or the agriculture department. The calf died about 10 days after the incident and no material was sent for laboratory examination. Since 06/11/2012, the patient successively sought care because of a barbed wire cutting on the same hand attacked by the bat, which began to show dormancy. Then he developed lip deviation, fever and vomiting, and clinical progression toward paresthesia, insomnia, agitation, confusion, sore throat and drooling. Tetanus, muscle twisting and tonsillitis were diagnostic hypotheses raised by the municipal health services. The aggression was reported only after assistance at Ponte Nova municipality, and suspected human rabies was notified. On the same day, the patient was transferred to the referral hospital in Belo Horizonte municipality for specialized care. Patient sedation was maintained, with antiviral administration and control of symptomatic complications. The patient developed hypernatremia, ventilator-associated pneumonia and cardiac arrests. There was *antemortem* laboratory confirmation of rabies infection by Polymerase Chain Reaction (PCR) in cerebrospinal fluid and saliva sample, and genetic sequencing indicated that it was compatible with *Desmodus rotundus* genetic variant. Despite specific treatment, the patient died on 06/28/2012 due to refractory circulatory shock. *Postmortem* examination was performed by direct immunofluorescence reaction, with infection confirmation at CNS fragments. During the conduct of the case, epidemiological investigation was proceeded by medical reports and records reviewing, family interviews and active search of exposed. In association, health education, social mobilization and intersectoral work actions were developed. Through the described information it is evident the importance of health systems being able to properly suspect, notify, and investigate, added to need to maintain surveillance and control activities of rabies in Minas Gerais state.

CO.11
MEASURES OF RABIES IMMUNITY IN RELATION TO SUSCEPTIBILITY, DIAGNOSIS AND PREVENTION

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A number of reports describe limited numbers of animals inoculated intentionally with rabies virus that do not become ill and may have little to no detectable antibody while other conspecific individuals succumb to the same inoculum. This review will detail methods used in measuring immunity and optimal method validation, in relation to potential identification of baseline susceptibility, enhanced disease diagnosis, and optimal prevention practices for humans and animals. Potential factors for successful rabies virus infection include: 1) protection of infected neurons from host immunemediated destruction by the inhibition of virus-mediated apoptosis and overexpression of immune-subversive molecules resulting in killing or inactivation of 'protective' T cells migrating into the infected nervous system; 2) an hypothesis that lethality results from neuronal dysfunction inhibiting proteins required for neuronal maintenance; 3) a posit that virus phosphoprotein and host cell dynein light chain 8 interaction may regulate viral ribonucleoprotein linking to cell transport; 4) a thesis that innate immunity, paradoxically, favors rabies virus neuro-invasiveness through enhanced infiltration and promotion of CD8(+) T cell elimination; and then alternatively that ; 5) type I IFN produced in the rabies virus-infected nervous system reduces neuroinvasiveness and partially protects from fatal infection. It is clear that innate immune cells detect pathogens, most likely including rabies virus, via pattern recognition receptions, such as Toll-like receptors and others. Pathogen-associated molecular

patterns activate receptors which induce production of pro-inflammatory cytokines and signals to activate inflammation. In addition, these receptors are required for an adaptive immune response. Innate and adaptive immune responses act as two interlocking defense lines. Following a rabies exposure, virus may be initially suppressed by innate immunity accompanied by effector T cell recruitment for activation of adaptive immunity. Alternatively, the uniquely high mortality rate of successful rabies virus infection may be due to virus-host interactions that remain largely a mystery. The accurate and precise measurement of an adaptive immune response may be defined by experimental methods or well-described methods, such as the rapid fluorescent focus antibody test, as performed and fully validated in some laboratories. The interpretation of findings based on these various methods should be in relation to clinical observations and collaborative investigation of unique, isolated, novel findings. The fine-tuning, interaction, and timing of innate and adaptive host responses and the methods used for detection and measurement, will require dedicated investigation towards optimal disease prevention.

CO.12
DIFFERENCE IN INTRACELLULAR LOCALIZATION AND EXPRESSION LEVEL OF RECOMBINANT RABIES G-PROTEINS OF STREET VIRUS (KYOTO STRAIN) AND FIXED VIRUS (CVS-26 STRAIN) EXPRESSED IN HEK293T CELLS

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Virulence of rabies virus (RABV) has been mainly studied with fixed viruses (laboratory strains) having different degrees of pathogenicity, however, virulence is much different between fixed viruses and street viruses (wild strains). Highly attenuated fixed strains of RABV does not cause lethal infection with profound inflammation accompanied with apoptosis and neural degeneration in the central nervous system (CNS). Induction of innate immune responses in CNS is a hallmark of infection with highly attenuated strains, whereas neural damage is absent or minimal and innate immune responses are not induced in animals infected with street virus. In the street virus infected cells, intracytoplasmic virion maturation taken place in the ER/Golgi apparatus is commonly observed and budding of virus from cellular plasma membrane is less frequent than in the fixed virus infected cells. Since RABV G-protein is critical for induction of virus neutralizing antibody, profound expression and budding of virions on cellular surface in fixed virus infected cells might be a major target of the host immune system. Thus different pathogenicity between street virus and fixed virus might be associated with different localization of virion maturation. However, little is known about molecular mechanism of virion maturation both of fixed and street viruses. To elucidate this, we have expressed G-proteins of CVS-26 strain (fixed virus) and Kyoto strain (street virus) in HEL293T cells upon transfection of the pCAGGS (CXN2) plasmid bearing G-genes of CVS-26 and Kyoto strains, respectively. Intracellular expression and localization of G-protein of each strain was then examined by fluorescence antibody technique and Western blot analysis using anti-G mAb (No.#7-1- 9, kindly provided from Dr.Kawai). Confocal laser scanning microscopy showed CVS- 26 G-protein was mainly localized on plasma membrane, while Kyoto G-protein was predominantly localized at perinuclear membrane region. CVS-26 G-protein was shown to be expressed in abundance than Kyoto G-protein in HEK293T cells by Western blot analysis. These results indicate maturation site of infectious RABV solely determined by localization of G-protein. Four amino acids in a signal peptide (SP) of G-protein of CVS-26 were distinct from those of Kyoto. The number of putative N-linked glycosylation sites was 3 and 2 on G-proteins of CVS-26 and Kyoto, respectively. Further analysis is required to elucidate whether these differences affect intracellular localization and expression level of RABV G-protein.

CO.13
ROLE OF MITOCHONDRIA IN RABIES VIRUS-INDUCED OXIDATIVE STRESS

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Neuronal process degeneration occurs in an experimental mouse model of rabies with hindlimb footpad inoculation of the challenge virus standard-11 (CVS) strain. CVS infection of primary mouse and rat dorsal root ganglion (DRG) neurons has associated axonal degeneration with axonal swellings and neurite outgrowth reduction. The CVSinduced axonal swellings feature protein adducts of 4-hydroxy-2-nonenal (4-HNE), a marker for lipid peroxidation, indicating a critical role of oxidative stress. Western immunoblotting analysis indicated that adducts of 4-HNE expression is also increased in the CVS-infected rat adrenal medulla (PC12) cell line. Mitochondrial dysfunction is one of the most important causes for overproduction and accumulation of reactive oxygen species (ROS). We investigated the effects of CVS infection on several mitochondrial parameters in different cell types (DRG primary neurons, PC12, mouse neuroblastoma (MNA), and baby hamster kidney (BHK-S13) cells) at 72 hrs post infection. The biochemical activity of electron transport system (ETS) complexes (I, IIIII, and IV) and Krebs cycle enzymes (citrate synthase and malate dehydrogenase) were evaluated using a spectrophotometric approach. Krebs cycle enzyme activities were not affected in CVS- versus mock-infected cells. Complex I activity was significantly increased in all CVS-infected cells versus mock-infected controls. Complex I was increased by 30-35% in CVS-infected DRG and PC12 cells, whereas it was increased by 65-75% in MNA and BHK-S13 cells. These values were proportional to the susceptibility of the cells to CVS infection suggesting a direct effect of the CVS infection on Complex I. Complex II-III activity was normal in the infected cells. Complex IV activity was upregulated in all types of CVS-infected cells. However, the increase did not relate to the susceptibility of the cells to the infection, suggesting an indirect effect. We postulate that enhanced Complex IV activity in CVS-infected cells may play a role in avoiding apoptosis. NADH, which is a Complex I-substrate, level was significantly higher in CVS-infected versus mock-infected PC12 cells. NAD+ level in CVS-infected PC12 cells was similar to that in mock-infected controls. Despite the increased activity of ETS complexes, CVS infection reduced the intracellular level of ATP in PC12 cells. The reduced ATP level in CVS-infected DRG neurons may explain, at least in part, the reduction in the neurite outgrowth that was previously observed. We predict that a high mitochondrial inner membrane potential is generated in CVS infection because of increased proton pumping across the mitochondrial inner membrane due to higher activity of Complex I and IV, and decreased proton consumption as indicated by reduced intracellular ATP level. Induction of a high mitochondrial membrane potential promotes electron leakage, primarily at the Complex I site, leading to ROS overgeneration and oxidative stress.