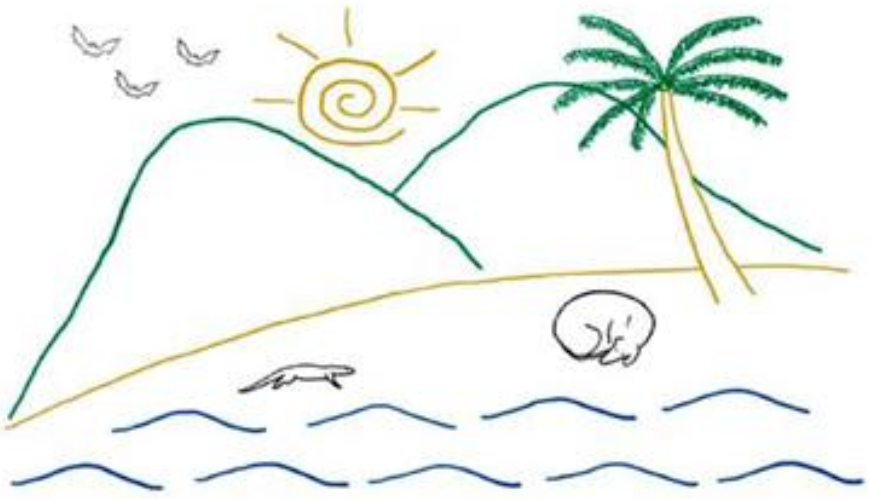
Abstract



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DR. GEORGE M. BAER AWARD

MOLECULAR EPIDEMIOLOGY OF RABIES VIRUS IN TRINIDAD AND CHARACTERISTICS OF THE 2010 OUTBREAK

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Trinidad lies 7 miles off the coast of South America and is the only Caribbean island with vampire bat-transmitted rabies. The link between bats and human paralytic rabies was established in Trinidad during the historical 1931 epidemic. Although no human cases have been reported since 1937, rabies continues to cause periodic outbreaks in other animal populations, with the most recent in 2010. We conducted an epidemiological analysis of the 2010 outbreak and used a Bayesian phylogeographic approach to investigate whether outbreaks in Trinidad are a result of local maintenance or regular re-introduction from the mainland. The investigation was based on a dataset of 189 sequences including 37 partial nucleoprotein gene sequences from equine, caprine and bovine species isolated between 1997 and 2010 in Trinidad. During the 2010 outbreak the laboratory confirmed 32 rabies cases (26 bovine, 6 caprine) from May to December. The first set of cases occurred in the southwest of the island (county St. Patrick) with subsequent spread in a northeasterly direction through Victoria and St. George counties. Phylogenetic analyses confirmed a vampire bat rabies virus origin. All of the 2010 viruses sequenced (n= 23) grouped together and were most closely related to sequences

from Uruguay (2007- 2008), Brazil (1999, 2004) and two earlier Trinidad sequences (2000, 2007). In contrast, a second distinct lineage integrating Trinidad sequences obtained in 1997-1998 and 2000 was similar to rabies virus circulating in Ecuador, Guyana and French Guiana. The Bayesian phylogeographic analyses suggest that the 2010 viruses arose from a common ancestor that existed in Trinidad approximately 3 years prior (95% HPD = 1.07 – 7.12), whose ancestors in turn were circulating on the mainland approximately 9 years prior to 2010 (95% HPD = 6 – 12.39). The data also suggest importations from South America previous to the 1997-98 and 2000 outbreaks, and provide evidence of limited *in situ* insular evolution. In light of the confirmed vampire origin of rabies viruses circulating within Trinidad, further studies should be conducted to investigate the relationship between rabies virus spatio-temporal dynamics and vampire bat population ecology, in particular any movement between the mainland and Trinidad.

RABIES EPIDEMIOLOGY & SURVEILLANCE

KNOWLEDGE, ATTITUDES AND PRACTICES AMONG PREGNANT AND CHILD-BEARING AGE WOMEN EXPOSED TO ANIMAL BITE INJURIES IN HAITI

Modupe O.V. Osinubi, Sergio Recuenco, Brett Petersen, Jesse Blanton, Charles E. Rupprecht

Haiti suffered a magnitude-7.0 quake on January 12, 2010. Rabies is endemic in Haiti which could be exacerbated by this natural disaster. Displaced people in combination with populations of free-ranging hungry dogs create opportunities for dog bites to occur, thus increasing the risk of transmission. One population vulnerable to such exposures is women that are pregnant or of child-bearing age. Little is known about how this population handles exposures from dog attacks and the threats that it poses to unborn children. To better understand the knowledge, attitudes, and practices of this population exposed to animal bite injuries in Haiti, a survey was carried out among this group at risk of bite injuries and rabies who live around the areas affected by the earthquake. Study participation was voluntary. Only women who were mature adults of child-bearing age were enrolled. Collected data were entered into a spreadsheet and analyzed using SAS version 9.2. Descriptive analyses were performed using a 2-tailed chi-square test or Fisher's exact test when applicable. Where necessary, data were computed as numbers, percentages and mean ± standard deviation. Sixty-two study participants were recruited. Thirty-two (51.6%) were pregnant or had been pregnant in the last two years. Mean age was 27.5 years (range 17 – 47) and mean education level was 4 years (range 0 – 16). Fifty-eight (93.6%) had little or no knowledge about rabies while 2(3.2%) had basic knowledge. Fifty-six (90.3%) did not know rabies was a severe disease, while 3(4.8%) thought rabies is a very severe or somewhat severe disease, 3 (4.8%) declined to answer the question. Twenty-five (40%) participants reported a history of an animal bite. Dogs and cats 21(33.9%) accounted for the highest number of bite cases, followed by pigs 2(3.2%), wildlife 1(1.6%) and other animals 1(1.6%). Of those bitten, 2 (8%) sought medical treatment, 8 (32%) washed the wound or took other measures, and 15 (60%) did nothing. Forty-five (72.6%) respondents thought money might be an obstacle preventing people from getting medical attention after a bite, while other responses included: no transportation (21%); lack of medicine (16.1%); don't know (14.5%); lack of facilities (9.7%); lack of trained personnel (6.5%); and other responses (3.2%).A majority of the respondents had little or no knowledge about rabies and as such did not handle bite exposures according to recommendations. Rabies educational outreach is strongly advocated for this vulnerable group and the entire community in general.

RABIES IN THE AMAZON: TIME FOR A PARADIGM SHIFT

Sergio Recuenco, Elizabeth Falendysz, Adan Monsalvo, Jorge Gomez-Benavides, Javier Guzman, Ana M. Navarro, Cesar Cabezas, Charles E. Rupprecht

After successful control of canine rabies in most of the Americas, vampire bat rabies remains the most important wildlife rabies variant in the Region. Most of the human rabies cases caused by vampire bats emanate from the department of Amazonas in Peru. Despite limited surveillance, rabies outbreaks and dozens of human deaths, mainly among children from indigenous communities, are reported annually. Available data from the Amazonas Department on human rabies outbreaks and a postexposure prophylaxis (PEP) campaign held as a response from 1975 to April 2011 were analyzed. Forty nine localities reported human outbreaks with a total of 161 deaths in four adjacent districts. Response intervention after outbreaks targeted 376 localities and resulted in the initiation of 22,000 rabies PEPs, with a rate of abandonment of ~ 31%. A suckling mouse brain rabies vaccine manufactured in country, requiring 7 daily subcutaneous doses and three boosters, was used in all interventions (without human rabies immune globulin). Only a cumulative ~23.4% of the population in the four affected districts received complete PEP, ~11% received incomplete PEP, and ~66% never received a single dose of a rabies vaccine, being considered naïve for rabies but with expected exposures to vampire bat bites. Initial comparative cost estimations for PEP intervention versus pre-exposure vaccination (Pre-EP) suggested the feasibility of rabies Pre-EP using cell culture rabies vaccines as an alternative to prevent human outbreaks in the Amazon. The cost per life saved in the Amazon with such a strategy should be less than the estimated costs for PEP in developing countries. Current animal rabies control strategies are inefficient for interventions on vampire bat rabies in the Amazon. Hence, it is necessary to re-examine priorities and adjust available strategies to the uniqueness of the Amazon environment. Shifting a paradigm for the use of Pre-EP among populations at risk appears to be one available alternative to tackle the vampire-bat associated rabies in Amazonia.

FIRST EUROPEAN INTER-LABORATORY COMPARISON FOR TETRACYCLINE AND AGE DETERMINATION ON RED FOX TOOTH SAMPLED IN THE FRAME OF ORAL VACCINATION PROGRAMMES FOLLOW UP.

Robardet, Demerson, Andrieu, Cliquet

First European inter-laboratory comparison for tetracycline and age determination on red fox tooth samples was organised by the European Union Reference Laboratory (EURL) for rabies. Performances and procedures of this technique, widely used to control bait uptake in the frame of oral vaccination campaigns monitoring in Europe, were compared between Member States. A panel of five red fox jaws including positive, negative, juvenile and adult samples as well as a technical questionnaire were sent to 12 voluntary participating laboratories. Considering tetracycline

determination, results revealed that only 3 laboratories (25%) found concordant results on all samples. False negative results were significantly more frequently detected in juvenile samples (58%) while they were infrequent in adult samples (4%). Considering age determination, results revealed that 4 laboratories (44%) presented concordant results on all samples. False age identification was observed in both adult and juvenile samples with respectively 11% and 17% of discordant results. Analysis of technical questionnaires in parallel to the test results suggested that every laboratory using jaw section between canine and first premolar presented false results. Behave the same, all laboratories using longitudinal section rather than transversal and not using mounting medium showed false results. Width of the section appeared also to be a factor affecting the results as no error was found in laboratories using width under 150µm. These factors potentially having an impact on laboratories success were discussed and recommendations were also proposed. Such inter-laboratory study underlines the importance to use harmonised procedure of biomarker detection in the frame of oral vaccination campaigns against rabies. There are indubitable tools to assist the improvement of analysis quality and increase the comparability of bait uptake frequencies between Member States.

EMERGING THREAT OF RABIES POSED BY CAT BITES IN SRI LANKA

Amila Gunesekera, W.K.C.P Werawatte, S.K.N.A Subasinghe, Dushantha Karunanayake

The Feline family is represented by five members in Sri Lanka: The Leopard, Jungle Cat, Fishing Cat, Rusty Spotted Cat and the House Cat. Out of which the House Cat poses the most significant challenge for the quest of eradication of human rabies.

The disease is mainly linked with dogs and the threat posed from cat bites is not yet recognized. Even the second most commonly spoken language of the country translates the word Rabies as Mad Dog Disease. Several factors can be implicated in the increasing threat of rabies following cat bites. Almost every cat has his own house either adopted or occupied on his own. Many people will not recognize cat as their pet and vaccination rate against rabies is very low. The objective of the study is to assess the risk caused by cat bites on Human Rabies.This study was designed as a descriptive comparative study of patients who had undergone Post Exposure Treatment for Rabies at The National Hospital, Colombo, Sri Lanka. The sample population was drawn from patients who had undergone treatment for Cat Bites for the last 12 months going back from July 2011. Stratification was done from a total sample of 10,661 and the numbers of cat bites analyzed were over 2,301 and the percentage of cat bites were 21.58%. The comparative sample was drawn from 12 months going back from July 2006 and the corresponding total sample size was 11,904 out of which cat bites were 1,547 and the percentage of cat bites were 13 %. The results showed that there is a significant increase in the percentage of cat bites presented showing an increase over 8 %

(1.6 times). The WHO Category III exposures in 2005/06 were 265 patients and in 2010/11 it has increased to 669 patients which is 2.5 fold rise. The emerging trend in the disease patterns shows an increased incidence of cat bites and signifying a "Clear and Present Danger" in the fight against Rabies. All the stakeholders in the prevention activities focus exclusively on dogs where the threats posed by the Cats are ignored.

RABIES SURVEILLANCE OF TRAPPED FOXES IN A CHANGING ARCTIC

K Jartsten Hueffer, Katie Kokx, Kimberlee Beckman, Brian Person

Background: In the Arctic the northward range expansion of the red fox (Vulpes vulpes) into the historical habitat of the arctic fox (Vulpes lagopus) may lead to a change in major rabies hosts in the Arctic due to a changing climate. Understanding the ecology of rabies virus as well as host species will be important in understanding and modeling of rabies dynamics in a changing Arctic. Fur trapping is still common in Alaska and some trappers believe that rabid foxes are very unlikely to be trapped and rabies does not pose a significant threat to fur trappers. However this assumption is not based on surveillance data. We tested trapped foxes to assess this believe and to gain a better understanding of rabies prevalence in free ranging foxes in Alaska. We also use this as a proof of principle to use fur trapping and fox control programs as enhanced rabies surveillance tools in the Arctic. Methods: We obtained foxes from fur trapper (red foxes) and USDA fox control programs (Arctic foxes). In total over 450 foxes were tested for rabies using the direct Rapid Immunohistochemistry Test (dRIT). Results: We found rabies positive foxes among both Arctic as well as red foxes in Alaska. The prevalence rate showed variation between years as could be expected in a cyclical disease like rabies in the Arctic. We will discuss the findings in the context of a changing Arctic and other parameters collected form the trapped foxes. Conclusions: This study showed that rabies should be a concern in fur trappers in Alaska and the North American Arctic. These findings will be communicated to trapper organizations throughout the state with special emphasis on endemic areas. The results are also preliminary results for a bigger study on rabies ecology in a changing Arctic and we will discuss plans for host population structure and mobility to better model rabies in the Arctic.

HUMAN RABIES INEQUATORIAL GUINEA: EMERGENCE OF A NEW CANINE RABIES VIRUS CLADE IN WEST AFRICA

David D., Willhoughby R.E. Jr, Niezgoda M, Rupprecht C.E., Rubin J.

Canine rabies is endemic in Africa, within the second greatest burden of disease after Asia. The objective of project was a diagnostic investigation and viral characterization of 7 human rabies cases (EG1-EG7), in 4 children and 3 adults which occurred from 2007 and 2010 in West African country of Equatorial Guinea (EG). The 7 patients consisted of 3 males and 4 females, aged between 4 and 80 years old. All were bitten by dogs with the exception of one patient (EG7) exposed a cat. None of the 7 patients received rabies post exposure prophylaxis (PEP). The incubation periods varied between 1 and 3 months.

The main clinical symptoms upon hospital admission included difficulty in swallowing, pruritus, hydrophobia and priapism. All patients were treated according to the Milwaukee protocol, but without success and they died between 3 and 22 days after hospital admission. For diagnosis samples of saliva, CSF, serum and a nuchal skin biopsy were sent to the Rabies Laboratory at the Kimron Veterinary Institute Israel. Rabies virus-specific amplicons were detected in saliva and skin biopsy of all 7 patients by hemi nested RT-PCR. Rabies virus antigens were detected in all 7 cases by examination of frozen sections of the nuchal skin biopsy by direct fluorescent antibody test, as corroborated by CDC. .Rabies virus neutralizing antibodies (VNA) were not detected in 4 cases (EG2-EG4 and EG-6), whereas in two cases (EG1 and EG5), VNA were detected on days 14 and 12 of hospitalization respectively. In one case (EG7) VNA detected on day 1 of hospitalization. Rabies virus was isolated in families of suckling mice from the 4 human cases: in 3 cases (EG4-EG6) from saliva, and in one case (EG1) from a skin biopsy suspension. A molecular analysis of the complete N gene and G-L intergenic region sequences was performed. The EG isolates belonged to the sub-lineage Africa 1A, that circulates in North Eastern Africa, and West Africans countries, suggesting a very broad distribution. In addition, phylogenetic analysis revealed a new canine rabies virus clade in EG closely related to a Gabonese isolate. Trans-boundary spread between the neighboring countries is a likely explanation for the possible emergence of these viruses. Ante mortem diagnosis is essential if human rabies therapy anticipated. Improved regional cooperation and local community health communication is needed, with more focused attention on canine vaccination, avoidance of exposure, and prompt PEP after animal bite.

HUMAN RABIES, EXPORTED FROM HAITI TO NEW JERSEY, USA, JULY 2011

Danielle M. Tack, Faye E. Sorhage, Dori Prasek, Miranda Chan, Olena Stepanyuk, Colin Campbell, Charles E. Rupprecht, Christina Tan

On 30 June 2011, a 73 year-old Haitian female with history of hypertension and diabetes mellitus type II presented to a New Jersey hospital with right shoulder pain, chest pain, headaches and increased blood pressure. Ischemic heart disease was suspected, but ruled out. She was released with pain medication, but returned to the hospital the following day with shortness of breath, insomnia, and hallucinations. She was subsequently hospitalized due to deteriorating mental status. On 7 July, health officials were contacted as rabies was now a differential diagnosis. There was no known history of animal exposure, and after Northeastern United States' arboviruses, herpes simplex virus and other potential etiologies were negative, clinical samples were sent to CDC for testing. Rabies virus antigens were identified in a nuchal skin biopsy on 18 July and genetic sequencing identified a canine rabies virus variant. The patient's neurologic status continued to deteriorate leading to her death on 20 July. After diagnosis, additional patient history revealed that she had received a minor dog bite during April in Haiti, prior to travel to the US. Risk assessments were conducted for 246 health-care staff, with 9 (3.7%), along with 4 social contacts, receiving PEP. This is the third report of human rabies in the US acquired in Haiti and highlights the importance of a detailed history of a patient with travel from a rabies-endemic country, and the utility of consultation with medical and public health professionals related to animal bites regardless of perceived severity.

THE UNUSUAL SUSPECTS: 10 YEARS OF INFREQUENTLY REPORTED RABID ANIMALS IN THE UNITED STATES

Jesse D. Blanton, Marlene Williamson, Jillian Fitzpatrick, Charles E. Rupprecht

The majority (92%) of rabid animals reported in the United States to the Centers for Disease Control and Prevention (CDC) each year are among recognized wildlife reservoir species (i.e. raccoons, bats, skunks, foxes, and mongoose). The remaining 8% of rabid animals are reported among domestic species, with the majority being domestic pets and livestock. Approximately 1-2% of rabid animals represent spillover infection to infrequently reported species. We evaluated rabies diagnostic submission data for the US from 2000-2010 for reports of infrequently submitted species. Uncommon species were considered any animal submitted for rabies diagnosis that excluded the following: raccoons, bats, skunks, foxes, mongoose, cats, dogs, cattle, horses, donkeys, sheep, and goats. We also excluded records where the reported animal species was unknown. Uncommon species were further categorized as other domestics, other wildlife, and rodents and lagomorphs. A spatial and temporal analysis was conducted to detect changes in the submission rates for these species and compared to overall rates reported in the previous decade. More than 68,000 uncommon animals were tested for rabies. A total of 1,065 rabid uncommon animals were reported from 2000-2010 among 29 species. On average, 96 rabid uncommon animals were reported each year during the study period. The majority of reported rabid uncommon animals were other wildlife species (54.9%), followed by rodents and lagomorphs (43.0%), and other domestic animals (2.1%). Bobcats (Lynx rufus) and coyotes (Canis latrans) accounted for the majority (73.2%) of other wildlife species. The majority (89.7%) of rabid rodents and lagomorphs were reported in groundhogs (Marmota monax). Pigs (Sus scrofa) and ferrets (Mustela putorius furo) accounted for 63.6% of rabid other domestic species.Small mammals, such as insectivores and rodents, have never been documented as rabies virus reservoirs in the US. Rabies among uncommon animals reflects spillover infection from regional reservoir species. While the majority of rabid animals reported in the US are reported among bats and four mesocarnivores, it is important to consider spillover into uncommon species and the rare occurrences where human exposure might be implicated. Exposures to uncommon species where rabies prophylaxis is not generally recommended should be carefully evaluated where the animals exhibited unusual behavior or signs of neurologic illness. Reported cases of rabies in other mesocarnivores should also be evaluated for their potential to represent host shifts of circulating rabies virus variants and local sustained transmission from established sources, particularly among canids such as coyotes, given their historic role as a rabies reservoir in the US and the potential of reintroduction of the coyote/dog rabies virus variant at the US-Mexico border, or the opportunity to perpetuate among new hosts, such as bobcats.

RETROSPECTIVE STUDY OF THE ORAL RABIES VACCINATION PROGRAM IN WEST VIRGINIA, 2001-2010

Xiaoyue Ma, Jesse D. Blanton, Dennis Slate, Charles E. Rupprecht

As part of the nationwide collaborative effort to halt the westward spread of raccoon rabies, an oral rabies vaccination (ORV) program was initiated in West Virginia during September 2001. From 2001 to 2010, the prevalence of raccoon rabies significantly decreased in the ORV area, whereas rabies cases in the non-ORV areas were maintained. In the ORV area, no rabid raccoons were reported from most central West Virginia counties since 2003. Alternatively, cases in the West Virginia northern panhandle counties have raised concern about increased risks for raccoon rabies virus transmission to Mid-western states. In this study, rabies surveillance data from ORV and non-ORV areas were analyzed to evaluate the impact of the ORV program on rabies in West Virginia during 2001-2010. Raccoon rabies seroconversion data from this period were compared for the northern panhandle region and central counties in the West Virginia ORV area. There was no substantial difference in rabies virus neutralization antibody (VNA) titers between male and female raccoons. However, serology test results were significantly different between adult and juvenile raccoons. Among tested raccoons, approximately 75% were adult and 25% were juvenile. Seroprevalence for juvenile raccoons was approximately half of the rate for adult raccoons. In northern panhandle counties, such as Brook County, less than 15% of raccoon sera collected post-ORV had detectable VNA titers, compared to greater than 30% in other ORV counties, such as Lewis County. This retrospective study points to the potential need for targeted vaccination strategies in northern panhandle counties and underscores the need to field test alternative baits and oral rabies vaccines that may enhance overall seroconversion rates to better assure continued success in West Virginia and nationally.

MORPHOLOGY AND MOLECULAR GENETICS OF DESMODUS ROTUNDUS (GEOFFROY, 1810) (CHIROPTERA, PHYLLOSTOMIDAE): TOOLS FOR ANALYZING THE EPIDEMIOLOGY OF RABIES TRANSMITTED BY THIS SPECIES IN THE EASTERN BRAZILIAN AMAZON

Fernanda Andrade, Marcus Emanuel Barroncas Fernandes, Wilson Uieda, Eduardo Sousa Varela, Marcelo Nazareno Vallinoto de Souza

Background: In the context of the pattern of rabies transmission by vampire bats to humans and bovines in rural areas of the Brazilian Amazon, the present study is the first attempt to integrate epidemiological data with information on the genetics and morphology of populations of Desmodus rotundus. Methods: 775 specimens of D. rotundus from 76 sites were donated by public health authorities from the states of Amapá, Amazonas, Tocantins, Maranhão, and Pará. The records of cases of rabies in humans and bovines for the 10-year period between 1999 and 2008 were obtained from the Pará State Public Health Ministry (SESPA). Measurements of 39 phenotypic traits (16 external and 23 cranial) were taken from 644 adults (329 males and 315 females), while 258 specimens (47% males and 53% females) were screened for genetic variation at 10 microsatellite loci. High-risk areas for rabies infection were identified based on ecological, biological, socio-economic, and use and land cover parameters, all georeferenced. Results: The morphometric analysis identified two principal groups (females only), one to the north (Lower Amazon, Marajó, and Northeast), and the other located in southern Pará. By contrast, the genetic

analysis indicated the presence of a number of different groups on Marajó, with an elevated absolute number of alleles (A), effective number of alleles (Ne), and allelic richness (Ra). In addition, the matrix of genetic distances (Fst) suggested the presence of three principal groups – Lower Amazon, Marajó, and the Northeast. However, the Hardy-Weinberg equilibrium observed in 93.5% of the loci vs. groups comparisons indicated the presence of a typically panmictic population, in which 96% of the variability is intrapopulational (AMOVA) rather than between geographic groups. Within this highly homogenous population, there is a certain tendency for the subdivision of genetic groups mainly in the Marajó and in the Northeast, which were associated with medium and high-risk areas for human rabies, respectively. Conclusions: The transmission rates of rabies to bovines and humans by hematophagous bats, in rural areas of eastern Amazonia, were highest in the northeastern region of the state of Pará. Although not statistically significant, the population of D. rotundus formed genetically distinct local groupings. Similarly, analysis of morphological characters showed homogeneity in the population studied and this feature may be maintained by the reproductive and adaptive strategies adopted by this species in eastern Amazonia.

PATHOGENESIS

RABIES VIRUS – INDUCED OXIDATIVE STRESS AND THE ROLE OF THE TRANSCRIPTION FACTOR NUCLEAR FACTOR-KB (NF-KB)

Alan C. Jackson, Wafa Kammouni, Leena Hasan, Paul Fernyhough

Background: Recent studies in an experimental model of rabies have shown that there are major structural changes in the brain involving neuronal processes that are associated with severe clinical disease. Cultured adult mouse dorsal root ganglion (DRG) neurons are a good in vitro model for studying the mechanisms involved in rabies virus – induced degeneration of neurites (axons), because unlike other neuronal cell types, these neurons are permissive to rabies virus infection. DRG neurons infected with the CVS strain of rabies virus show axonal swellings and immunostaining for 4-hydroxy-2-nonenal (4-HNE), indicating evidence of lipid peroxidation associated with oxidative stress, and also reduced axonal growth in comparison with mock-infected DRG neurons. Treatment with the antioxidant N-acetyl cysteine prevented the reduction in axonal outgrowth that occurred with CVS infection. Because the transcription factor nuclear factor-κB (NF-κB) plays a central role in oxidative stress, we have investigated its role in CVS-induced oxidative stress. In the inactivated state NF-κB is located in the cytoplasm complexed with the inhibitory protein IκBα. A variety of extracellular signals can activate IκB kinase that results in translocation of activated NF-κB into the nucleus, where it exerts its effects. Methods: We have evaluated the role of NF-κB using the NF-κB peptide inhibitor SN50. The expression and localization of NF-κB subunits were evaluated by Western immunoblotting and immunofluorescence, respectively, in mock- versus CVS-infected DRG neuron cultures. We have evaluated the activity of NF-κB by quantitatively evaluating p50 signals in the nucleus and cytoplasm of CVS-infected versus mock-infected neurons. Results: SN50 elevated oxidative stress in CVS-infected DRG neuron cultures versus mock-infected cultures, suggesting that NF-κB plays a neuroprotective role in CVS infection. We have found increased expression of the p50 subunit of NF-κB after CVS infection versus mock-infection. We have observed activation of p50 (with high nucleus:cytoplasm ratios) at 24 hours, but loss of activation at both 48 and 72 hours post-infection with high significance ($p<0.005$). Conclusions: CVS infection is associated with only transient enhancement of NF-κB activity (assessed by nuclear localization) followed by loss of this NF-κB activity that is associated with oxidative stress with the development of degenerative axonal changes. Further investigations are needed to gain a better understanding of the basic mechanisms involved in the oxidative damage associated with rabies virus infection. This information may prove helpful in the design of future therapeutic effects for this dreaded disease.

COMPARATIVE POST-MORTEM HISTOPATHOLOGY OF IMMUNE RESPONSES IN HUMAN RABIES ENCEPHALITIS

Richard Franka, Brigid Batten, Wun-Ju Shieh, Michael Niezgoda, Xiaoyue Ma, Sherif Zaki, Charles E. Rupprecht

To identify particular immune cell populations associated with rabies encephalitis, we performed comparative immunohistological evaluations upon the brain stem, cerebellum, hippocampus and cortex collected from a patient who succumbed acutely to rabies (15 days after symptoms first appeared) and a patient who recovered from clinical rabies (illness developed due to the lack of appropriate prophylaxis), but succumbed as a result of secondary medical intervention. 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, necrosis, and apoptosis were compared using a semi-quantitative scale. No rabies virus antigens were detected in the brain tissue of the patient who survived clinical rabies. In contrast, T-cells and macrophages were abundant in the parenchyma in both rabies patients, but B-cells were detected only in the perivascular tissue of the putative rabies survivor. Almost no T- and B- cells and only local microglia cell were detected in influenza patient. Although further analyses of additional cases are required, our study suggests that the early and prolonged presence of B-cells in the CNS of patients with rabies encephalitis may be a critical determinant of effective viral clearance.

COMPARISON OF LYSSAVIRUS DISEASE IN THE MOUSE MODEL FOLLOWING PERIPHERAL INOCULATION

Derek Healy, A.R. Fooks, A.C. Banyard, S. M. Brookes, A. Nunez

Rabies is almost invariably fatal once clinical signs are observed. Following peripheral inoculation, clinical signs of rabies virus disease and distribution of viral antigen in the mouse brain were examined. Statistical analysis of the mean incubation and morbidity periods for each of the viruses was undertaken using the Bartlett test of homogeneity of variances, and a parametric ANOVA test was used to compare the means between groups. The distribution of antigen in the murine brain for each of the virus strains at clinical scores 1, 2 and 3 were assessed using the Kruskal-Wallis non-parametric ANOVA. Data representing histopathological scores for 14 different areas of the brain in four groups of mice infected with each of the virus strains: CVS (n=6), EBLV-2 (n=32), EBLV-1 (n=29) and a wtRABV (n=26) were also assessed statistically. The appearance of clinical disease varied both within and across the different lyssavirus species with differences in incubation periods and weight loss throughout disease progression. The distribution of viral antigen throughout the regions of the brain examined was similar for each of the isolates during disease progression. Specific regions of the brain, including the cerebellum, caudal medulla, hypothalamus and thalamus showed notable differences in the proportion of virus antigen positive cells present. These data suggest that the presence of virus antigen in different regions of the brain may contribute to the clinical presentation observed following rabies virus infection.

COMPARATIVE PATHOGENESIS OF EXPERIMENTAL RABIES VIRUS INFECTION IN RED FOXES (VULPES VULPES)

James A. Ellison, William C. Carson, Felix R. Jackson, Amy S. Turmelle, Charles E. Rupprecht

Each variant of rabies virus (RABV) occupies a unique ecological niche. The opportunity for re-emergence of rabies in meso-carnivores and the likelihood of sustained transmission of RABV in different populations is influenced in part by host and variant-specific factors. Such attributes are critical when conducting risk assessments of the epizootiological potential of specific viruses in different hosts for management considerations. In an attempt to identify potential differences in susceptibility, neurovirulence and neurotropism of RABV variants, experimental infections were conducted in red foxes (Vulpes vulpes). In this study, 30 red foxes were divided into six groups of five animals, and each group was inoculated bilaterally into the gastrocnemius muscle with a standardized dose of an antigenically distinct RABV variant (silver-haired bat; cosmopolitan dog; gray fox; raccoon; north central skunk;south central skunk). Standard immunohistochemical and immunoflourescent techniques were used to assess relative antigen distribution and intensity throughout sections of brainstem, cerebellum, hippocampus, and cerebrum. No differences in overt susceptibility were detected, with 97% (29/30) mortality observed among infected foxes. However, time after infection to clinical presentation varied significantly by isolate ($\chi2=40.8$, $p <0.05$). Foxes infected with a canine RABV variant had the shortest incubation periods. Clinical signs were diverse and not specific to the RABV variant used for infection. Moreover, 20% (n=6) of foxes progressed rapidly without display of any clinical signs. Significantly, distribution of RABV antigens was different throughout brain sections ($\chi2=16.88$, $p <0.05$ and $\chi2=16.88$, $p <0.05$) for the hippocampus and cerebrum, respectively. Transmission potential varied by group: infectious virus was recovered from the submandibular salivary glands of only one fox infected with a skunk RABV variant, and all foxes (n=5) infected with a gray fox RABV variant. No infectious virus was recovered from the salivary glands of foxes infected with a bat, canine, raccoon, or most skunk RABV. This study confirms the observation that red foxes are highly susceptible to heterologous RABV infection. These data also support the concept that spillover infections of biologically relevant North American RABV among red foxes may be transient or dead ends, but dependent upon the isolate, dose, and route, could result in sustained transmission when epizootiological conditions are ideal.

LYSSAVIRUS TRANSMISSION: EXPERIMENTAL MODELS AND NATURAL ROUTES

Ashley C. Banyard, Derek M. Healy, Nicholas Johnson, Anthony R. Fooks

A major unknown in lyssavirus biology remains mechanisms of transmission and viral persistence in infected animals. Numerous non bite routes have been postulated from both experimental studies and natural transmission events. Classically, lyssaviruses are transmitted via a mechanical route: the bite of a rabid dog; virus invasion via minor scratches; crossing

of the dermal barrier through pre existing cuts and scars following licking by an animal excreting virus; or transplant of infected organs. Several of these mechanisms have been experimentally studied with variable results. Other mechanisms of horizontal transmission, such as through the aerosolisation of virus or through vertical transmission from parent to offspring have also been suggested but remain poorly understood. In particular, the role of vertical transmission in bat species remains of interest whilst remaining controversial for human infection. We have attempted to asses the ability of virus to be transmitted both horizontally through excretion studies and vertically in the mouse model. We conclude that whilst mice appear to act as a dead end host for lyssavirus excretion in saliva, transmission during pregnancy may occur.

IMMUNOLOGY

AN INTEGRATED APPROACH TO UNDERSTANDING THE HOST RESPONSES INVOLVED IN THE CLEARANCE OF A LETHAL RABIES VIRUS INFECTION OF THE BRAIN

Bernhard Dietzschold, Milosz Faber, Craig Hooper

Post-exposure treatment (PET) of mice with the live-attenuated TriGAS vaccine but not mock treatment resulted in virus clearance from the brain and survival of the majority of mice that were intranasally infected with a lethal dose of the highly pathogenic rabies virus DOG4. TriGAS treatment induced a robust virus-neutralizing antibody (VNA) response that did not prevent the DOG4 RV from invading and replicating in the brain during the initial phase of the infection, indicating that in addition to VNA other factors induced by the TriGAS PET must be involved in the clearance of RV from brain tissue. Whole transcriptome analysis of normal mouse brain and brains of DOG4 RV-infected mice that received either mock or TriGAS PET revealed substantial differences in RNA expression profiles at day 6 after infection. Many of the genes that were found to be activated in the brain of the mock-treated DOG4 RV-infected mouse represent type I IFN response genes. This indicates that the DOG4 RV infection induces an early type 1 IFN response that evidently is not able to control the infection by itself. In contrast, most of the activated genes in the brain of the TriGAS-treated DOG4 RV-infected mouse play a role in adaptive immune responses including the regulation of T cell activation, T cell differentiation, and regulation of lymphocyte and mononuclear cell proliferation. This suggests that TriGAS induces responses in the brain that provide immune effectors access to the sites of infection and contribute to their virus-clearing activity. The tanscriptome analysis data were confirmed by qPCR array analysis data which showed at 4 and 6 days p.i. a significantly higher activation of 4 genes, chemokine ligand 3, natural killer cell activator 2 , E-selectin, and Granzyme A, in brains of TriGAS than in the brains of mock-treated mice. The early activation of these genes, which are known to play key roles in the regulation of lymphocyte and mononuclear cell proliferation, T cell differentiation, and trafficking of immune cells,

EXPRESSION OF MURINE INTERFERON-Γ BY RECOMBINANT RABIES VIRUS HIGHLY ATTENUATES PATHOGENICITY AND INCREASES IMMUNOGENICITY

Darryll Barkhouse, Milosz Faber, Bernhard Dietzschold, Craig Hooper

Background: Previous studies of rabies virus immunology in mouse models have implicated the TH1 arm of the response as crucial for the efficient clearance of rabies virus from the central nervous system. The TH1 response is characterized by the production of interferon-γ, a cytokine with direct anti-viral, immunostimulatory and immodulatory effects. These traits, along

with recent findings that upregulation of interferon-γ in the brains of rabies-infected mice strongly correlates with immune effector entry into the brain, led us to hypothesize that insertion of interferon γ into the rabies genome would enhance the host immune response to the virus. Methods: Using reverse engineering technology, we constructed two rabies viruses expressing murine interferon γ. SPBNγ was constructed using the pathogenic SPBN vector, while GASγ has the attenuated GAS rabies virus as its backbone. To study pathogenicity, groups of mice were infected intranasally with varying doses of SPBNγ or control virus. The safety and efficacy of GASγ to induce a robust immune response to rabies was analyzed by: 1) intracranial infection of suckling mice 2) intramuscular immunization followed by intracranial challenge with highly pathogenic DOG4 rabies virus, and 3) intracranial mixed infection with GASγ and DOG4. All GASγ experiments included a highly attenuated rabies vaccine virus as control. Morbidity and mortality were monitored and quantitative PCR was used to measure viral replication in the brain. Results: We report that the SPBNγ virus is highly attenuated, having an LD50 at least 2.6 logs greater than that of its control. This is reflected in a 10x decrease in viral load in SPBNγ-infected mice at 8 days post-infection. Additionally, the GASγ virus exhibits a better safety profile and induced a more protective immune response against lethal challenge with DOG4 rabies virus than the control vaccine virus. In fact, the ED50 of GASγ is 7.6x lower than the control vaccine strain during a mixed infection with DOG4 and more than 2x lower for challenge after immunization. Conclusions: Interferon-γ expression by a rabies virus has the ability to attenuate the virus and induce a more protective immune response in the host. The mixed infection results, in particular, suggest that an interferon-γ-expressing rabies virus may have the potential to clear a pre-existing rabies infection from the brain.

LYSSAVIRUS RESERVOIRS, ANTIBODIES, AND RABIES

Phyllis Catharina Romijn

Not so long ago, we used modified Lyssavirus genotype 1 particles for rabies prophylaxis in herbivores (ERA vaccine). This product resulted in antibody production, and almost none of the individuals that were injected did develop rabies upon challenge, for at least 36 months. In this case, it is well known that only muscular cells capture this strain of virus, consequently were infected and produced virus particles very similar to the injected strain. The infection was thus restrained to the muscular tissue, until the immune system of the host got rid of them. Once or twice, a “vaccinal accident” happened, considered normal in populations, and clinical disease was expressed (in these situations, virus was found also in the brain). Several articles report that Lyssavirus may be present in the saliva of certain mammals (bats, dogs, hyenas) before there is any clinical evidence of the infection. In other words, it may be that, depending on the genetics of a population or of a single individual, certain cells in an organism could be producing Lyssavirus without compromising the nervous system (no clinical signs). Clinical signs only appear when its immune system did not eliminate the infected cells and

certain vital cells of the brain are infected and produce infectious virus. Active monitoring of Lyssavirus circulation is rare, and this means that *absence of evidence* should not be considered the same as "*evidence of absence*". In most rabies diagnostic laboratories, evidence of infection in the recent past (presence of specific antibodies) is not taken into consideration unless there are clinical signs when a case is notified. Laboratories mainly receive and examine brain tissue suspected of producing the virus. My speculation is to find several stray dog, bat and carnivore wild animal populations with anti-Lyssavirus antibodies, and some individuals of these populations with virus in their saliva and even other organs. Stress of several origins reducing the immune system's capacity of maintaining an intern equilibrium may be the kick-off for development of clinical disease. When carrying out research, or even routine diagnosis, shouldn't we look for Lyssavirus in other tissues then the brain, and always for antibodies in animal populations such as bats, wild mammals in general, straw dogs and cats? This may lead to an understanding of the viral infection BEFORE the appearance of clinical signs, and perhaps allow discoveries which could contribute to ways of preventing this terrible disease

SURROGATE MODEL USING VOLEPOX VIRUS IN *PEROMYSCUS CALIFORNICUS* TO EVALUATE THE EFFECT OF PREVIOUS ORTHOPOXVIRUS INFECTION ON THE HUMORAL RESPONSE TO AN ORAL VACCINIA-RABIES RECOMBINANT VACCINE

Nadia F. Gallardo-Romero,* Sonja L. Weiss, Richard Franka, Yoshinori J. Nakazawa, Andres Velasco-Villa, Scott K. Smith, Charles E. Rupprecht, Inger K. Damon, Kevin L. Karem, and Darin S. Carroll

Indigenous North American orthopoxviruses include volepox, raccoonpox and skunkpox. These viruses are divergent from Eurasian orthopoxviruses, but share serologic and genetic similarities. In the USA, for disease control among mesocarnivore reservoirs, the national oral rabies vaccination program involves the annual deployment of tens of millions of doses of a recombinant orthopoxvirus, a vaccinia-rabies glycoprotein (V-RG) virus vaccine. Wildlife populations naturally exposed to native orthopoxviruses may be refractory to immunization with the V-RG vaccine. Volepox virus is stable within the local Pinyon mouse and California vole populations in the California bay area. The California mouse (*Peromyscus californicus)* is both geographically and ecologically sympatric with Pinyon mice and California vole populations and therefore resides in geographic areas where volepox virus has been found. In this study we examined the impact of volepox virus infections on the vaccination-induced immunity to rabies virus, following immunization with V-RG in the California mouse. Sixty six adult *P. californicus* mice were divided into 4 groups. Groups 1 and 2 were inoculated intranasally with 10 ul of volepox virus at either 1.6X10^3 (group 1) or 120 PFU (group 2). Groups 1, 2 and 3 were

vaccinated *per os* with 150 ul of 1.5X10^8 PFU/ML of V-RG on day 28 (group 1) or day 7 (group 2) post-volepox inoculation. Group 4 was administered PBS as a negative control group. Based upon previous orthopoxvirus animal models, we used intranasal inoculation because this route may mimic natural orthopoxvirus exposure in the wild, where the virus infects by direct contact with the external mucosal of the naïve host. Orthopoxvirus immune induction was measured using a standardized ELISA method, Western blotting and plaque reduction assays. Rabies virus antibodies were detected by a rapid fluorescent focus inhibition test. The percentage of mice that developed rabies virus neutralizing antibodies included: 78% in Group 3, 58% of in Group 2, only 20% of Group 1, and none in Group 4. The results of this study indicate that survivors of high dose infection with volepox virus display immune induction that interferes with subsequent V-RG vaccination at day 28 pi, as evidenced by absence of immune response to rabies virus. However, the group inoculated with the low dose of volepox virus did not prevent an anti-rabies virus immune response by V-RG given 7 days post infection.

EVALUATION OF ONRAB® ORAL RABIES VACCINE (RABIES VACCINE, LIVE ADENOVIRUS VECTOR {ADRG1.3}) SAFETY IN FERAL DOGS (CANIS LUPUS FAMILIARIS)

Bender, S.C., Beresford, A.V., Baker, R.J., Kyle, C., Hausig, K., Slate, D., Bergman, D. L.

ONRAB®, a human adenovirus rabies construct vaccine, has been used in extensive field trials in Canada for several years to determine the safety and efficacy of the vaccine in target species (striped skunk, raccoon, and red fox). These trials also determined that the most common non-target species that may ingest baits are dogs. In support of use of the vaccine in the United States, the Navajo Nation Veterinary Program, Navajo Nation Department of Agriculture, Navajo (First) Nation applied to the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Center for Veterinary Biologics for a research and evaluation permit to evaluate the safety of ONRAB® vaccine in feral dogs. Twenty feral dogs, kept in isolation, were offered ONRAB® vaccine contained in "Ultralite" baits (Artemis Technologies Inc., Guelph, Ontario, Canada). General health status was monitored and sera collected at day 0 and at 2 week intervals for the duration of the study, with seroconversion against HAd5 and rabies to determine vaccine uptake. Oral swabs collected at 2, 12, and 24 hours post-bait consumption, and rectal swabs at 48 and 72 hours post-bait consumption were assayed for AdRG1.3 by Real Time Polymerase Chain Reaction (PCR) to evaluate potential environmental virus shedding. Two human subjects (investigation veterinarian and one animal caretaker), two non-study, investigator-owned dogs and one unvaccinated dog kept in intimate contact with four vaccinated dogs, were assessed pre- and post-study for HAd5 and rabies titers or seroconversion to monitor any potential horizontal transmission. AdRG1.3 was titrated from one unused bait to assess any degradation due to shipping and storage of the vaccine during the study. Uptake of the ONRAB® vaccine by titer was observed in a majority of the dogs, with no adverse health affects from vaccination noted during the course of

the study. While shedding was detected by the swabs, no indication of a horizontal transmission to humans, non-study dogs or the dog in intimate contact was observed. No shipment degradation was indicated in the returned unused bait. No adverse affects were observed by the vaccination of dogs using ONRAB® oral Rabies vaccine.

WHAT IS BEST MEDICINE FOR MEASURING IMMUNITY TOWARDS OPTIMAL HUMAN AND ANIMAL RABIES PREVENTION?

Cathleen A Hanlon, Chandra Gordon, Susan M Moore

Background: The rapid fluorescent focus inhibition test (RFFIT) is the "Gold Standard" for measuring rabies virus neutralizing antibody titers in humans and animals. But the World Health Organization guidance of 0.5 IU/ml as an acceptable level differs from the US Advisory Committee on Immunization Practices recommendation of "complete neutralization at a dilution of 1:5." Is it important to understand that this level when converted to IU/ml can be at the threshold of detection depending upon the run of the assay and the SOPs of a specific laboratory. Identifying, understanding, and the control of method variables is critical. It is essential to validate test performance characteristics according to carefully characterized standards, reference sera, and cut-off values, and also consider the robustness of quality programs, especially for highly specialized assays limited to a few laboratories. Methods: An informal proficiency exchange is coordinated by our laboratory between independent laboratories conducting the RFFIT method. As part of our quality program, we track virus dose, performance of the international reference sera and related internal controls, cell passage number, and lot numbers of virus stock, and other critical reagents. Results: In summary of recent exchanges, all participating laboratories found IU/mL values in agreement with theoretical IU/mL based on dilution factors used to prepare the samples with a range of 0.9942 to 0.9998 in 2009 and 0.6902 to 0.9978 in 2010, with further details to be reported. As expected with a biological assay, and in compliance with regulatory standards for this type of assay, we report variability according to the particular run of the assay, which may vary, but in our laboratory is well within the coefficient of variability allowed for these types of assays. The tracking of our internal standards and data on specific samples will be presented, in detail, specifically in regard to precision, accuracy, dilutability, specificity, lower limit of quantitation, and stability.
Conclusions: There remains a need for proficiency testing and advancement of quality control practices to optimize human and animal rabies serologic and diagnostic practice. Rabies prevention is "One Health and One Medicine in action," as such, if it is best medicine to measure titers on pre-exposure vaccinated humans rather than empirical booster doses of vaccine every two year, one must contemplate the pros and cons of measuring titers in animals versus empirical boosting on a set timeline that may not be appropriate for every individual.

DIAGNOSTIC METHODS

EVALUATION OF BLOCKING ELISA TEST FOR DETECTION OF RABIES ANTIBODIES FROM THE VIEW AS ALTERNATIVE TO VIRUS NEUTRALIZATION TESTS.

Miroslav Mojžiš, Peter Korytár, Slavomír Jerg

The pet travel scheme has been created to allow animals to travel easily between EU member countries and certain third countries without undergoing lengthy quarantine. The crucial requirement in the frame of this scheme is rabies vaccination followed by determination of seroneutralization antibody levels. Up to now, only virus neutralization test (FAVN or RFFIT) is recommended to control sufficient rabies antibody levels after vaccination. Aim of our study was to evaluate possibility to replace rather time consuming, laborious virus neutralization tests with some simpler alternative such as ELISA test. For that purpose we used blocking ELISA, developed at our institute, which has been routinely used for many years in frame of monitoring efficacy of oral vaccination campaigns in wild foxes (as presented on the poster at RITA in 2008).Validation study was based on comparison performances of blocking ELISA with FAVN test as a reference test. On the basis of serial dilution of WHO reference serum and defined dog sera, cut off 70% of blocking and higher has been established for ELISA to correspond with protective level 0,5 IU/ml in FAVN test. The specificity has been estimated on panel of dog rabies negative sera and reached 100%.

Comparison of ELISA with FAVN test on panel of dog sera obtained in frame of EU proficiency testing showed very good correlation. Other evaluation has been made on panel of 1546 dog and cat sera selected from samples tested in frame of pet travel scheme in FAVN test, were we have obtained 86,4% agreement with diagnostic sensitivity of 85,5%. From 210 discrepancy sera only 1 FAVN negative serum (0,38 IU/ml) had percentage of blocking higher than 70% (70,9%). This finding showed that evaluated ELISA was very safe in term of possibility to produce false positive results. Most of the FAVN positive samples which were negative in ELISA had rather low FAVN titer and in ELISA ranged between 40 and 70 percentages of blocking. Very good correlation on this panel of sera between ELISA and FAVN test has been proved with ROC curve analysis. Results presented in this study showed that blocking ELISA under certain conditions in combination with virus neutralization test could be promising tool used for pet travel scheme.

RT-PCR FOR DETECTION OF GENETIC VARIANTS OF RABIES VIRUSES IN DIFFERENT HOST

Fernando G. Bastida-González, Isaac Velázquez-Quiroz, Juan Manuel Jimenez-Estrada, MA. Dolores G. Ramírez-Hernández, Paola B. Zarae-Segura

Introduction :Rabies virus (RV) is the most widespread and epidemiologically important member of the genus and the only taxon documented in the Americas. Several specific RV variants have been characterized from different mammal hosts. Nowadays, many molecular studies have been performed targeting the N gene, this data have permitted insights to virus-reservoir relationships, patterns of transmission and dissemination, as well as viral evolution. The objective of this study was to design a new method for molecular characterization of RV by genetic variant depending on the host found in the State of Mexico. Methods:Brain samples from the host animals used in this study were tested positive by the Fluorescent Antibody Test (FAT) and the antigenic variants collected were determined with the Monoclonal antibodies (MABs) detection. nRT-PCR and RT-PCR SYBR Green detections were done with our own primer design in complete sequence of the N gene Rabies Virus (Genomic Databases from NCBI). To confirm detection, PCR products were sequenced and identified with the Basic Local Alignment Search Tool (BLAST) from the NCBI database. Results:
All of the brain samples from rabid animals, diagnosed as positive in the FAT test, signaled positive in the nRT-PCR and RT-PCR SYBR Green. To confirm the specificity of the both methods, they were carried out with the additional genotype 1 prototype laboratory strain CVS, used as a positive control. Real-time RT-PCR used primers of internal end-point nRT-PCR. In the characterization of the antigenic variants (AgV) with MABs in the dog sample, the dog primers identified the Variant dog (AgV1). This result matched with both the nRT-PCR and SYBR Green primers a 100%. Similarly, the skunk samples matched the same percentage with the skunk primers. On the other hand, in the bovine samples where the MABs detection identified the skunk Variant (AgV8), the determined host by nRT-PCR and SYBR Green diagnosed as positive with the vampire primers. The last two samples, the one determined as atypical and the one not determined with the MABs, the nRT-PCR and SYBR Green diagnosed as positive with the bat primers; proving thus the specificity of the RV variant detection depending on the host with the present study proposal. nRT-PCR and SYBR Green detection matched a 100% with the FAT and 80% with the MABs results. Conclusion: By using the tests described above with the brain samples from the studied host animals, this technique allowed molecular characterization faster than other conventional procedures.

EVALUATION OF AN INDIRECT RAPID IMMUNOHISTOCHEMISTRY TEST FOR THE DIFFERENTIATION OF RABIES VIRUS VARIANTS

Jessie Dyer, Michael Niezgoda, Lillian Orciari, Pamela Yager, Charles Rupprecht

Cost effective diagnostic tests are needed in rabies virus (RABV) enzootic areas to study the prevalence, distribution, and transmission of RABV among reservoir hosts. To reduce the associated costs of acquiring and maintaining specialized laboratory equipment, an indirect rapid immunohistochemistry test (IRIT) for the detection and differentiation of RABV variants was evaluated by traditional light microscopy. In this preliminary study, 71 rabies virus samples, confirmed by the direct fluorescent antibody test, were tested by the IRIT. The IRIT utilizes brain touch impressions, fixed in 10 percent buffered formalin for 10 minutes, a panel of murine anti-nucleoprotein monoclonal antibodies (MAB-N) and commercially available biotin- labeled goat anti-mouse antibody. Preliminary results on antigenic variation were also obtained using anti-glycoprotein MAB for canine RABV variants that could not be delineated using a conventional MAB-N panel. Results obtained revealed a distinct reactivity

pattern associated with reservoir hosts, similar to the results obtained with a panel of MAB-Ns in an indirect immuno-fluorescence antibody test using a commercially available fluorescein isothiocyanate (FITC)-labeled goat anti-mouse antibody. These results should be useful in studying the epizootiology of rabies, and inferring the source of infection when unknown. Evaluation of suspected RABV samples through IRIT does not require specialized equipment and is possible to perform in a field setting. Additionally, commercially available labeled secondary antibodies permit the use of a standard panel of unlabeled primary MAB, without the need for fluorescent microscopy, and should augment existing attempts at antigenic characterization, particularly throughout Latin America.

EVALUATION OF FLUORESCENT-ANTIBODY TEST AND MOUSE INOCULATION TEST IN RABIES LABORATORY DIAGNOSIS FOR SAMPLES FROM DIFFERENT ANIMAL ORIGIN

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Rabies is one of the most important zoonotic disease that, even with symptoms quite characteristic, has no pathognomonic clinical signs, so post mortem laboratory diagnosis is the only reliable and definitive confirmation of animal rabies infection. The fluorescent-antibody test (FAT) is considered the most sensitive method for the detection of rabies antigen in fresh tissue identifying rabies-infected samples sent to diagnosis, with an agreement of almost 100% with the Mouse Inoculation Test (MIT). FAT positive results confirms the clinical suspect, but, if the predictive value of a negative result obtained by a competent laboratory did not approach 100% and because the importance of the laboratory diagnosis for a medical decision, the virus isolation is an important routine backup procedure. Although FAT is described as the gold test for rabies diagnosis, its accuracy depends upon the expertise of the examiner, the quality of anti-rabies conjugate and the fluorescence microscope, so that, many reports traditionally still considered the MIT as the golden technique for rabies diagnosis using it to confirm results of other techniques. The purpose of this research was to evaluate the sensitivity, specificity and concordance coefficient of FAT compared to MIT in two Brazilian laboratories of rabies diagnosis. A total of 17,850 samples from different species were included: 7,960 from dogs, 2,771 from cattle, 616 from horses and 6,503 from bats.16,436 samples resulted negative and 1,286 positive by both techniques, 14 were positive by IFD and negative in MIT and 114 were negative by IFD and positive by MIT. Considering all the samples, regardless of species, IFD presented 91.86% of relative sensitivity, 99.91% (99.59 to 99.96%) of relative specificity and 94.87% of Kappa concordance coefficient. However, the relative sensitivity and Kappa concordance coefficient varied according to the species of the sample, had corresponded to 96.44% and 97.69% respectively for dog, 94.92 and 96.04% for cattle, 74.22 and 81.05% for horses and 70.33 and 79.76% respectively for bat samples. These results showed that, MIT proved to be more sensitive and for some animal species like horses and bats, the IFD cannot be used as a guide for medical decision of using or not post

exposition treatment. This is one of the reasons why, in Brazil, bats are considered high-risk animals an all people bitten by this species must receive prophylactic treatment.

PEACE-TIME TO OUTBREAK: A LYSSAVIRUS MOLECULAR DIAGNOSTIC PORTFOLIO

Denise A. Marston, L. M. McEhinney, Graeme Harkess, David T.S. Hayman, Ashley C. Banyard, Anthony R. Fooks

Lyssavirus infection is invariably fatal, therefore the availability of highly sensitive and specific techniques that enable a rapid differential diagnosis to be made is crucial where infection is suspected. We have developed a portfolio of pan-lyssavirus molecular assays that are fully validated for day to day diagnostic analysis. Importantly, these assays are also easily scaled up should an out-break occur. These molecular assays have been specifically designed to be: 1) pan-lyssavirus, enabling the detection of any lyssavirus currently characterized; 2) differential, ensuring immediate confirmation of the lyssavirus species present; 3) sensitive, ensuring confidence that detection of low levels of viral nucleic acid is achieved; 4) rapid, ensuring swift diagnostic evaluation and enabling a rapid policy driven response; 5) quality controlled, ensuring specific reaction criteria are met and finally 6) protected in a closed-tube environment against sample contamination. In addition, we have developed and validated a non-phenol based RNA extraction method in parallel with the molecular assays which can be scaled up to a 96-well plate format using an extraction robot. Here we present data from two real-time based molecular assays developed at AHVLA: 1) a SYBR® Green based RT-PCR that uses a chelating dye to detect amplification; 2) a Taqman based RT-PCR with probes specific for each lyssavirus species to enable rapid differentiation between lyssavirus species. Both assays use the same primer sets, on the same platform under the same cycling conditions. Finally, we discuss maintenance and optimization of the current hemi-nested (hn)RT-PCR assay, to improve turnaround times and prevent potential contamination. The latter assay enables genetic sequencing of isolates and therefore remains an essential part of our diagnostic portfolio. Whilst not currently recommended by the WHO or OIE for standard testing, molecular techniques are an important tool in diagnostic virology. Although this molecular tools portfolio has been compiled and validated in the UK, it is readily transferable to any lyssavirus laboratory with molecular capabilities and in-house quality systems.

HUMAN RABIES TREATMENT AND PROPHYLAXIS

GLOBAL AVAILABILITY OF RABIES IMMUNE GLOBULIN AND VACCINE FOR TRAVELERS SURVEY: INITIAL FINDINGS

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Background: Rabies, which is endemic in most of the world, poses a risk to international travelers. During periods of limited rabies vaccine (RV) supply, travelers seeking pre-exposure vaccination are deprioritized to ensure adequate supplies of postexposure vaccine for persons with high-risk exposures. To better form recommendations for international travelers, we assessed the global availability of RV and rabies immune globulin (RIG). Methods: To ascertain the availability of RV and RIG by country, we conducted a 20-question online survey distributed via e-mail listserv to travel medicine providers and other clinicians worldwide. The survey, available in English, Spanish, and French, was accessible during February 1-March 30, 2011. One completed survey was randomly selected from each responding clinic. All identifying information was deleted before analysis. Results were compiled by country and region. Data were analyzed in SAS. Results: Of 341 respondents who began the survey, 41 were excluded because of lack of responses (n=5) or duplicate clinic information (n=36). Of the 300 remaining respondents, 250 (83%) clicked over to the final question; not all respondents answered each question. Respondents from 58 countries participated; the most common regions of respondents were North America (47%), Western Europe (16%), Australia/Pacific (10%), East Asia (5%), and Southern Africa (4%). The median number of patients administered postexposure prophylaxis per clinic in 2010 was two patients (range: 0-30,000). One hundred eight (44%) of 246 respondents stated that patients seldom or never presented with adequately cleansed wounds due to an animal exposure. RIG was always accessible for 58% (n=60) of respondents in North America, 64% (n=28) in Western Europe, 68% (n=13) in Australia/Pacific, and 38% (n=5) in East Asia. RV was always accessible for 72% (n=72) respondents in North America, 79% (n=33) in Western Europe, 100% in Australia/Pacific (n=19), and 92% (n=12) in East Asia. For all regions, 31% (n=69) and 26% (n=58) of respondents felt the cost was too high for RIG and RV, respectively. Regional analyses are ongoing. Conclusions: The availability of RV and RIG varied by geographic region. Hence, all travelers should be informed that RIG and RV might not be readily available at their destination and that they should consider travel health

and medical evacuation insurance prior to departure. Travelers should be educated to avoid animal exposures, to clean all animal bites and scratches thoroughly with soap and water, and to seek medical care immediately, even if overseas.

SURVIVAL FROM HUMAN RABIES WITHOUT NEUTRALIZING ANTIBODY – CALIFORNIA

Wiedeman, J., Glaser, C., Willoughby, R., Slupsky, C., Rupprecht, CR.

Background: Rabies is almost uniformly fatal encephalitis. Protection from and clearance of rabies is primarily effected by neutralizing antibody as detected by RFFIT or similar culture-based assays. Two survivors of serologically proven human rabies, with a moderate clinical phenotype, have been reported in the United States and India. We report a third patient. Method: Case report. Results: A 9 year old girl was diagnosed with rabies following a progressive, asymmetric paralytic syndrome. Possible exposures in an area endemic for skunk and bat rabies included a downed horse and feral cats at her school. The patient had no history of rabies prophylaxis; serum antibodies became positive by indirect fluorescent antibody testing at 2 reference laboratories; CSF tested positive at one laboratory. Testing of saliva and skin were repeatedly negative. The patient was treated according to the Milwaukee protocol after receiving corticosteroids for empirical treatment of bacterial meningitis. Serological response was limited to low concentrations of non-neutralizing antibody. Initial MRI showed predominantly cortical encephalitis. Investigation for alternative infectious causes of encephalitis and autoimmune disorders including limbic encephalitis was negative. CSF metabolomics clustered the patient with 26 normal controls, away from 44 rabies samples including those from 2 survivors . Her clinical course was benign. Sedation was tapered after stable titers were evident, with residual left leg paresis and continued rehabilitation at time of discharge.Discussion: This girl is the third vaccine-naïve patient recently described with an acute, self-limited neurological syndrome following possible animal exposure, with serological responses fitting diagnostic criteria for human rabies. No cross-reacting lyssaviruses are known in North America. Virus was not isolated in these patients, but has from some survivors of rabies who later developed high titers of neutralizing antibodies. Attempts to better define innate and cell-mediated responses in this patient and other rabies survivors are underway.

AN EVALUATION ON SAFETY AND POTENCY OF EQUIRABTM (ERIG) THRU INDIRECT MEASUREMENT ON SUPPRESSION OF VACCINE INDUCED ANTIBODY PRODUCTION AMONG HEALTHY VOLUNTEERS

Efren M.Dimaano, Eumelia P. Salva, Jose Benito R. Villarama

The availability and cost of rabies immunoglobulins (RIGs) in the management of category III rabies exposure is a growing concern in the Philippines. This study evaluated the potency of a new highly purified equine RIG (EquirabTM) through indirect measurement of its suppression on vaccine induced antibody production using the rapid fluorescent focus inhibition test (RFFIT). We performed a prospective, randomized, open label clinical trial,

enrolling 45 subjects which were randomly assigned to three different groups of 15 subjects in each group. Group A received EquirabTM at same site with purified chick embryo cell (PCEC) vaccine using the 2-1-1 regimen, group B received EquirabTM at a different site from the PCEC vaccine and group C received only PCEC vaccine. Group A demonstrated immunosuppression with only 36% of the subjects achieving seroconversion by day 14 versus group B (80%) and Group C (93%). The comparative analysis of the RVNA titers on day 14 in the three groups yielded a significant difference ($p < 0.0014$). Likewise, safety analysis revealed 13.3% (4/30) of subjects had mild adverse reactions. Our study showed that EquirabTM is potent, safe and can be utilized for passive immunization in category III rabies exposures.

GLOBAL EVALUATION OF NEUTRALIZING ACTIVITY OF CL184, A MONOCLONAL ANTIBODY COMBINATION AGAINST RABIES

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Rabies remains a neglected problem in many countries and causes in excess of 55,000 deaths every year. In the tropics and canine rabies enzootic areas children and adolescents are particularly at high risk for exposure. We have developed CL184, a 1:1 protein mixture of two human IgG1 monoclonal antibodies (mAbs), CR57 and CR4098, which are directed against non-overlapping rabies virus (RV) glycoprotein epitopes. To evaluate full coverage of RV by CL184 we have designed a global epidemiology plan that allows for testing of representative primary RV isolates. By modifying existing RV neutralization assays, primary brain or salivary gland homogenates from rabid animals can be tested directly for in vitro neutralization. Here, we report an update on our global epidemiology plan and present US, Indian, Philippine, and Chinese data generated to date on the RV glycoprotein sequences and the neutralizing activity profile of CL184 against street RV. Interim test results of RV isolates from natural reservoirs (e.g., Procyonidae, Canidae, Chiroptera, etc.) support the in vitro neutralization activity of CL184, as well as the in vivo efficacy of the mAbs in a Syrian hamster model.

THE NEXT GENERATION PURIFIED VERO RABIES VACCINE IS SAFE AND AS IMMUNOGENIC AS THE REFERENCE VACCINE VERORAB™

Sylvie Pinchon, Françoise Guinet-Morlot, Maria Minutello, Yves Donazzolo, Victor Hou

Background: The purified Vero cell rabies vaccine next generation (PVRV-NG) is a highly purified vaccine developed with innovative technology in a medium free from all components of human or animal origin. Extensive characterization and preclinical studies have demonstrated its comparability to Verorab™. Safety and immunogenicity in pre-exposure prophylaxis were evaluated in a phase II clinical study in adults in France designed to demonstrate the immunological non-inferiority of PVRV-NG to Verorab™. Methods: This was a randomized, blind-observer, controlled, multicentric study in 18 to 60 year olds. Participants received a three dose primary series of PVRV-NG or Verorab™ (ratio 2:1) at D0, D7 and D28 and

a booster dose one year later. Participants who had received Verorab™ for the primary series were randomized to receive either PVRV-NG or Verorab™ for the booster dose. Immunogenicity was evaluated at D0, D28, D42, M6, M12 and M12 +14 days by measuring the level of rabies virus neutralizing antibodies (RVNA) using the rapid fluorescent focus inhibition test. Safety was evaluated with a list of predefined solicited injection site and systemic reactions during the seven days after each dose and any adverse events until 28 days after the final dose. Results: 384 participants were enrolled, 256 in PVRV-NG group and 128 in Verorab™ group. The predefined criterion for non-inferiority in terms of proportions of participants with RVNA titers ≥0.5 IU/mL at D42 (14 days after the primary vaccination series) was met in the per-protocol analysis set and confirmed in ITT population. While antibody levels gradually decreased over the 12 months period, most participants in both groups still had titers ≥0.5 IU/mL 6 and 12 months after the vaccination. A PVRV-NG booster dose induced a strong immune response, irrespective of the primary series vaccine received. PVRV-NG was safe and well tolerated after each vaccination and its safety profile was similar to Verorab™ in terms of unsolicited adverse events and solicited systemic reactions for primary and booster vaccinations. The incidence of solicited injection site reactions was lower with PVRV-NG than with Verorab™ after the primary series and the booster dose. Conclusions: PVRV-NG was at least as immunogenic as Verorab™ and presented a similar safety profile. This data supports the use of PVRV-NG for rabies prophylaxis.

CHARACTERIZATION OF A PLANT-DERIVED MONOCLONAL ANTIBODY COCKTAIL FOR HUMAN RABIES PROPHYLAXIS

Leonard Both, Craig van Dolleweerdm Ashley Banyard, Anthony R. Fooks, Julian K.-C. Ma

Background: Antibodies directed against the rabies virus glycoprotein are able to neutralize the virus and to prevent clinical disease in individuals exposed to rabid animals. A cocktail of murine monoclonal antibodies (mAbs) for rabies post-exposure-prophylaxis (PEP) has recently been recommended by the WHO Rabies Collaborating Centres. The aim of this project is to adopt this mAb cocktail for production in transgenic plants, which constitute an inexpensive alternative to existing mammalian production platforms. Methods: The two mAbs 62-71-3 and E559 were expressed in Nicotiana benthamiana as chimeric IgG1 antibodies. The mAbs were purified from plant leaves and their neutralization was evaluated with a Lentiviral Pseudotype Neutralization assay and with the Fluorescent Antibody Virus Neutralization (FAVN) assay.Results: Lentiviruses pseudotyped with the rabies virus glycoprotein, as well as live rabies virus, were shown to be potently neutralized by the two plant-derived mAbs. By using pseudotype viruses containing mutations in their antigenic sites, we established that mAb 62-71-3 recognizes a neutralizing epitope distinct from the neutralizing epitope recognized by mAb E559, confirming that these mAbs are suitable in combination. Conclusion: The large-scale production of the two mAbs in plants would constitute an economically feasible alternative to currently used

polyclonal sera for rabies post-exposure-prophylaxis in developing countries.

TECHNOLOGY AND DEVICES FOR RELIABLE AND SIMPLE INTRADERMAL (MANTOUX) INJECTION FOR RABIES VACCINATION

Izzy Tsals, Zach Marks, Hillit Mannor Shachar, Darin Zehrung, Courtney Jarrahian

Interest in intradermal injection is growing due to the positive dose sparing and immunogenic properties of this delivery method. Realizing the need for simpler, more reliable intradermal injections for delivery of vaccines such as rabies vaccine, we developed technology minimizing the complexity of the Mantoux technique, thereby providing predictable, reproducible results. It can be applied to either special adapters which are fitted to fixed needle syringes, either empty or prefilled, needle assemblies for luer-lock syringes, or other products. In either case, the technology employs geometry designed to gently deform the skin surface and the subcutaneous tissue, providing the ideal angle and depth of needle insertion for consistently successful intradermal injections. All versions share the same proprietary dermal interface technology. Devices instituting this design can be easy to use, require minimal training, and employ conventionally molded parts and cannula. Our findings were confirmed by positive results in a user study. A majority of registered nurses felt this technology simplified intradermal injections. Additionally, using prototypes of the design they were able to get successful blebs nearly twice as often as when using Mantoux. The technology has been evaluated on live pigs, by injecting 0.1ml saline/dye. The Mantoux method was used as a comparator, with all effective injections producing blebs of similar size. Minimal injectite leaks were observed. Histology studies confirmed successful injections of dye in the dermis for both the Mantoux method, and our prototypes. A Phase I clinical trial was conducted in 2011 to further evaluate the ID adapter. Twenty adult volunteers were included with each study participant receiving two injections of 0.1mL of sterile saline solution in the upper deltoid region of the arm using the investigational ID adapter with a commercially available BD insulin syringe.
The ID adapter was successful at delivering 100% of study injections intradermally as confirmed by external visual observation of injection site wheals and by ultrasound imaging. The ID adapter performed similarly with the needle bevel oriented either up or down relative to the surface of skin. Based on the results of this preliminary study, the ID adapter appears to be safe and effective, and suitable for use in future studies evaluating intradermal delivery of vaccines.

OVER A DECADE OF CLINICAL EXPERIENCE WITH FAVIRAB™, PURIFIED EQUINE RABIES IMMUNOGLOBULIN

Anvar Rasuli, Marie-Claude Bonnet, Jean Lang

Background: Favirab™, chromatographically purified, heat-treated equine rabies immunoglobulin (pERIG) manufactured by Sanofi Pasteur, was first registered in France in 2000 and is currently licensed in 35 countries including six in the European

Union. Favirab™ pERIG was clinically evaluated in The Philippines and Thailand, two countries where rabies is enzootic. Methods: During the development of Favirab™, an initial, prospective, randomized, controlled trial determined the safety and pharmacokinetics, while a second trial simulated post-exposure prophylaxis. Three post-license trials were carried out in field conditions. Firstly, a retrospective survey of rabies post-exposure prophylaxis (PEP) outcomes was conducted in The Philippines. This was a case series involving 7,660 patients (including 151 with laboratory-confirmed rabies exposure) given Favirab™ and a cell culture rabies vaccine. Secondly, two prospective prescription event monitoring (PPEM) trials were run in The Philippines and in Thailand. These studies determined the health status of patients who had received PEP for category III exposures to a laboratory-confirmed rabid animal. A total of 193 patients bitten by or exposed to laboratory-confirmed dFAT-positive animals were enrolled in the PPEM study in The Philippines. In Thailand, 178 exposed patients were enrolled. Results: No serious adverse events were reported in either clinical trial. In particular, no immediate (anaphylactic type) or delayed (serum sickness) allergic reactions were observed during 28 or 30 days follow-up. The Favirab™ pharmacokinetic parameters, geometric mean titer (GMT) profile, and the evolution of GMT values were similar with those of a standard ERIG product used as a control. The post-licensure trials, which also selected the patients on the basis of the severity of their wounds, confirmed the clinical efficacy of Favirab™ in the real conditions of clinical practice when appropriate PEP is correctly performed. In all three field effectiveness studies, Favirab™ administered along with the first dose of vaccine provided 100% survival rate during the follow-up periods (6—48 months for the case series, one year for the two PPEM trials) in patients who received PEP strictly in accordance with WHO recommendations. Conclusions: An extensive review of outcomes in patients with proven or suspected rabies exposure shows that the recommended PEP using Favirab™ and rabies vaccine is safe and efficacious.

EFFICACY OF SHORT-INTERFERING RNAS IN EXPERIMENTAL RABIES TREATMENT.

Camila Michele Appolinario Harary, Susan Dora Allendorf, Joao Marcelo Azevedo de Paula Antunes, Bruna Aparecida de Oliveira, Jane Megid

Even after a long period of studies about rabies pathogenesis, a lot of its aspects is unclear and find an effective therapy it's a challenge. Considering that the use of gene therapy in rabies virus infection showed promising results the aim of our study was to evaluate the efficacy of a RNA silencing therapy based on the use of Short-Interfering RNAs in experimental rabies in mice. Short interfering RNAs (siRNAs) is a small RNA involved in RNA silencing, a mechanism for sequence-specific gene silencing involving RNAs of 19–30 nucleotides (nt) in length. Results from many in vitro and in vivo studies (DeVincenzo et al., 2010; Brandão et. al. 2007; McCaffrey et al., 2003) provide the proof-of-concept for the use of an RNAi as a therapeutic agent. Experiment was performed with females, 6 weeks old C57/BL6 mice inoculated with a canine street rabies virus (variant 2) or with a bat rabies virus (variant 3) kindly provided by Instituto Pasteur (SP), by intramuscular route. Animals were separated in only inoculated and inoculated and treated group. A mix of three different sequences of siRNAs designed by Brandao et al (2007) against nucleoprotein mRNA associated with Lipofectamin 2000(Invitrogen®) was administrated in a single dose (3.3µM each sequence) by intraperitoneal route, 24

hours after inoculation. Animals were evaluated during 30 days daily for the onset of clinical signs that included hunching, limb paresis and ataxia. The percentage of mice showing clinical signs and lethality rate was recorded for each group. Statistical significant difference was found in lethality rate as well as in the cumulative clinical signs between the groups, both with variant 2 and variant 3 , when the treatment started 24h after infection. In the first one, siRNA lethality rate 30% lower than only inoculated group was observed. By the other way, when the same therapy was applied in animals inoculated with variant 3, lethality rate was 20% higher than non-treated group. The loss of efficacy may be explained by possible viral mutations that can be observed in wild rabies virus strain what could decrease siRNA efficiency once it must have a perfect matching with the target sequence for a properly activity.

Considering the results obtained in post exposure treatment and the importance of a symptomatic therapy in rabies, another study was run using the same mix of siRNA in mice already presenting clinical signs. The observational data showed that therapy was unable to avoid dead or prolong evolution period of the disease regardless rabies virus variant demonstrating absence of efficacy in this condition.

ANIMAL RABIES

DEVELOPMENT OF THERMOSTABLE RABIES VIRUS VACCINE FORMULATIONS

Victor Bronshtein, Marina Siirin, Xianfu Wu, and Charles E. Rupprecht

Viral vaccines intended for the oral immunization of free-ranging carnivores need to be stable in the environment. The goal of our studies was the development of thermostable formulations of rabies virus vaccines, suitable for delivery per os and via the intestinal mucosa, without a need for reconstitution. A corner stone for thermostabilization of rabies virus was a recently developed method: Preservation by Vaporization (PBV) Technology. The PBV technology initially dries the vaccine under a vacuum by vaporization (simultaneous sublimation, boiling, and evaporation) from a partially frozen state, several degrees below 0°C, and finishes in mechanically stable foam. The thermostability is reached by subsequent drying at elevated temperatures (>40°C). Our investigation using rabies virus ERA formulated by PBV presented no loss of virus titers after drying, and less than 0.5 log of loss after 23 months of storage at room temperature, less than 0.5 log of loss after 2 months at 37°C, and no loss after 3 hours at 80°C. Short term (~3 hours) stability at 80°C is important for PBV formulations, embedded in quick dissolvable polymeric films, for delivery to the oral mucosa. However, this method of vaccine delivery could be limited in canine species that typically swallow baits. To overcome the potential limitation, we formulated PBV-preserved vaccine encapsulated in alginate gel microparticles (~ 100µ size), to target intestinal mucosa. The PBV preserved alginate gel microparitiicles were optimized by (i) replacing $CaCl_2$ with calcium D-gluconate for the gel particle preparation, (ii) by using a novel cryo-encapsulating procedure for gel particle preparation, and (iii) by optimizing a PBV protocol and the amount of calcium D-gluconate utilized in the formulation. Our results showed less than 0.3 log loss of ERA virus titer after drying, less than 0.3 log activity loss after 2 weeks of storage at 37°C and one month at room temperature. Such preliminary results with viral stability are encouraging towards future immunogenicity and efficacy studies in relevant species.

RABIES VIRUS DISTRIBUTION IN TISSUES OF NATURALLY INFECTED CATTLE

Susan Dora Allendorf, João Marcelo A. P. Antunes, Didier Quevedo Cagnini, Acácia Ferreira Vicente, Rafaela Mastrangelo Risseti, Claudenice Batista de Barros, Isabella Belletti Mutt Perrotti, Anna Paula Vitirito Martinho, Camila M. Appolinário Harary, Clóvis Reynaldo da Fonseca, Antônio Carlos Paes, Jane Megid.

In Brazil rabies in cattle is endemic through the country and controling the disease is still a challenge to government authorities. In Latin America rabies in herbivores is mainly transmitted by the hematophagous bat, *Desmodus rotundus*. The economic losses in the livestock industry and public health impact of the disease in those animals had lead to indiscriminate actions to exterminate vampire bat population. During a recent outbreak in a city, located in southeast region of São Paulo state, a calf with neurological symptoms and a cow from the same region was also brought to the veterinary hospital of UNESP- School of Veterinary Medicine and Animal Science (Botucatu) by a veterinary showing paralysis and evident sings of vampire bat aggression. Both animals had clinical suspect of rabies and were euthanized. Necropsy was realized in both animals. Fragments of organs, tissues and liquor sample were aseptically collected. The rabies diagnosis was confirmed by the fluorescent antibody test (FAT) and intracerebral mouse inoculation. The

mice inoculated with brain sample started to present symptoms after 12 days of the intrecerebral inoculation. The presence of the RABV in tissues was investigated by FAT and by heminested RT-PCR (hnRT-PCR). The viral RNA of samples were extracted using TRIzol® reagent and hnRT-PCR using primers to the nucleoprotein-coding gene were performed. The amplified fragment was sequenced and the viruses detected from both animals were genetically characterized as been a lineage from *Desmodus rotundus*. The FAT was realized in different tissues and in a smear of liquor using specific anti rabies conjugate produced by CCZ (SP). The virus was identified in brain and medula by both techniques in the bovines. The presence of the RABV was detected by hnRT-PCR and FAT in saliva by testing tissue fragments from the cow salivary glands and from an oral lesion collected from calf´s mouth. The liquor samples, lungs and liver resulted negative by both techniques in all tested samples. The heart, spleen, intestine, submandibular limph node and abomasum collected from the calf resulted negative by hnRT-PCR and FAT, however the kidney resulted positive only in hnRT-PCR. And finally the RABV was detected in cow´s kidney and spleen by both techniques. The detection of the virus in brain, medula and mouth demostrated the expected pathogenesis of RABV neutropism and centrifugal spreed to other tissues similar to already reported in several animal species.

LINEAR FLOW ASSAYS FOR RABIES DIAGNOSIS LACK SENSITIVITY AND SPECIFICITY

Lillian A. Orciari, Dillon Hightower, Pamela A. Yager, Ivan Kuzmin, James A. Ellison, Felix R. Jackson, Beverly Dew, William C. Carson, Melvin M. Berry, Romulo T. Morales, Bonny Mayes, Mahin Park, Vicki Lambert, Charles E. Rupprecht

Accurate and timely diagnosis of suspect animals is critical for proper administration of rabies prophylaxis. Linear flow assays (LFA) are used for rapid real-time disease diagnosis in clinical settings, with potential field applications. In this investigation, three LFA Kits for rabies diagnosis were examined (approximately 500 tests in total). Samples included saliva swabs from normal and rabid domestic animals and wildlife, but also included virus titrations, CNS tissues, and as a control, media. All tests were performed according to the manufacturer's recommendations at the time the kit was obtained. In general, directions for the kits were clear. Saliva swabs from animals, usually dogs or cats, were placed in a buffer to elute saliva (and any potential agent). A measured amount of solution was transferred to a cassette with a nitrocellulose strip, containing rabies virus antibody. The validity of each test was determined by examination of the test cassette, packet desiccant, and the presence of a control band after the incubation period. Kits A and B were direct test methods, meaning a positive result was the development of a band in the test portion of the cassette, along with the presence of the control band. Kit C was an indirect test, and was considered positive if no band, or a test band weaker than the control, was observed. Comparing basic features, Kit B was not manufactured well. Physical examination of many cassettes demonstrated warping, which could alter the sample diffusion rate. Additionally, Kit B lacked sensitivity, and evaluation was terminated when the kit failed to detect 14/56 rabies virus -positive samples, including a predominant rabies virus variant represented in the geographical region where this Kit was manufactured. In contrast, LFA Kit A seemed to work well regarding sensitivity and specificity. No false positives were detected, from a comparison of 80 samples of non-rabid bats. However, Kit A did not detect a particular lineage of Lagos bat virus. Kit C resulted in numerous

CANINE RABIES VACCINE AVALIABILITY AND POST-EXPOSITION PROPHYLAXIS: ANALYSIS FOR RABIES CONTROL IN LATIN AMERICA AND CARIBBEAN REGION

Vigilato, Marco Antonio Natal; Tamayo, Hugo; Cosivi Ottorino

The Pan American Health Organization - PAHO consider four major components to a successful control program for human rabies transmitted by dog in a country level: A) Disease Surveillance in humans and animals, B) Educational continued programs, C) Availability and opportune post-exposition prophylaxis, and D) Systematic dog mass vaccination program. In the last five years (2006-2010) there were 79 cases of human rabies transmitted by dogs, which represents no more than 16 cases by year. In dogs, in 2006 were 1,171 rabid dogs, and 268 by 2010, which represents 77% of reduction. From the 35 countries and territories that belongs to Latin America and Caribbean Region (LAC), 18 with about 553 millions habitants, realizes systematic dog mass vaccination program. With the information available from these countries they estimated that each year more than 52 million of dogs were vaccinated in those countries in 2009, which represents a coverage 68% (52,174,793/76,783,380). The other component is the availability and efficiency of post-exposition prophylaxis (PEP). With the information from 17 countries from LAC, was identified that 1,284,502 people were attacked in 2008 and 865,569 in 2009, which means an incidence ratio of 2.3 and 1.6/1000 inhabitants, respectively. Another observation was the complete PEP that was 35% (446,707/1,284,502) in 2008 and raised to 52%(453,223/865,569) in 2009, even with less cases of dog attacks. Those results may indicates in the case of dog mass vaccination campaigns the need of a strategy for dog population management and local procedures to estimate these population. For PEP it is necessary to review and maintaining the health professional training to indicate the applicable protocol, considering the epidemiological status.

RABIES IN THE CARIBBEAN

Special presentation
Dr.Amy Turmelle

THE IMPACT OF CLIMATE CHANGE ON THE DISTRIBUTION OF VAMPIRE BATS AND RABIES

Amy Turmelle, Shahroukh Mistry, Rodrigo Medellin, Sergio Recuenco and Charles Rupprecht

Over the last century the average global temperature has risen by 0.74°C and is expected to rise by 1.1-5.4°C in the next 100 years. The impacts of climate change on ecosystems, species distributions, as well as agricultural, socioeconomic and human health issues are of considerable concern. In Latin America, common vampire bats (*Desmodus rotundus)* are the main vector for the transmission of rabies to livestock and humans, with significant public health and economic impacts. In Mexico, field studies have revealed that vampire bat populations may be expanding into northern latitudes and higher elevations, and GIS modeling predicts that vampire bats may enter the United States in the coming decade. The objectives of this project are to investigate the impact of climate change on the geographic distribution of vampire bats, rabies infection dynamics, and the associated economic and public health implications of a range expansion in a key reservoir host. We propose to use Geographic Information Systems (GIS) and ecological niche modeling (ENM) to predict the range expansion of vampire bats under several climate change scenarios. Individual variation in the dispersal behavior of bats is a critical aspect of this model and can be inferred from telemetry data. A recent telemetry project completed in Guatemala suggests that vampire bats display high fidelity to local feeding resources, and exhibit relatively small home ranges across an agricultural landscape. An upcoming telemetry study in Mexico will further investigate and compare the foraging dynamics and home ranges of vampire bats, to elucidate population dispersal patterns for application to our GIS and ENM models. When combined with seasonal rabies surveillance data, we can evaluate the public health and agricultural impacts of climate change-mediated expansion of vampire bats and rabies in North America.

DOG-MONGOOSE RABIES VIRUS VARIANTS POSE A CHALLENGE FOR THE ELIMINATION OF ENZOOTIC DOG RABIES IN THE CARIBBEAN

Andrés Velasco-Villa, Mang Chi, Susan A Nadin-Davis,Rafael González, Ramón Flores, Myriam García Negrón, Enid Garcia, Yira Tavares Villaman, Raquel Pimentel, Maria de los Angeles Ribas, Charles E Rupprecht

The elimination of enzootic dog rabies has been attained in several developed and developing countries, by vaccine-induced herd immunity in dogs and control of stray dog populations, in concert with a sensitive laboratory-based surveillance network for animal rabies detection. The USA achieved this goal twice during its history, eliminating the last rabies virus (RABV) variant (dog-coyote) with documented dog-to-dog transmission potential in 2004. A dog-mongoose RABV variant, with similar features, circulates in the Caribbean. To characterize this variant, descriptive epizootiologic and phylogenetic analyses using complete nucleoprotein sequences were conducted on samples obtained from rabid animals in Puerto Rico, the Dominican Republic, and Cuba (2000-2008). Paraphyletic RABV lineages were

found in both dogs and mongoose across the Caribbean delineating a clear phylogeographic pattern. No positive selection was detected in any of the lineages found. Purifying selection, linked to functional constraints, limited significant changes in this gene across the viral population analyzed. Nonetheless, conserved amino acid signatures at positions 377, 378 and 379 within the nucleoprotein were found associated with these different RABV lineages, which were not species-specific, but were partitioned geographically within and among the countries. The dog-mongoose RABV variant circulating in the Caribbean presented a cysteine at position 40 consensus for all dog-related RABVs, and a glutamic acid at position 110, consensus for the cosmopolitan dog and the Arctic/Arctic-like fox variants. Our results suggest that RABV circulating in both dogs and mongoose in the Caribbean have a rooted dog origin as a consequence of the European colonization of the New World (a common ancestor with the cosmopolitan dog-variant). The rabies epizootic dynamics inferred via phylogenetic and phylogeographic approaches reflect different control scenarios among countries. These variables have impacted ongoing RABV diversity, both in dog and mongoose populations. These species serve as reservoirs of several canine-related RABV lineages circulating in the region and present challenges for rabies elimination in the Caribbean. Comprehensive rabies control programs targeting both species simultaneously should be undertaken to prevent cross-circulation between domestic and sylvatic species, which otherwise will continue to complicate rabies elimination attempts throughout the region.

SPECIAL PRESENTATION

Dr. Dennis Slate

THE EXOTIC SMALL ASIAN MONGOOSE IN THE CARIBBEAN: RABIES MANAGEMENT OPPORTUNITIES AND CHALLENGES

Dennis Slate, USDA, APHIS, Wildlife Services

Established populations of the exotic small Asian mongoose on at least 29 islands in the Caribbean have had profound adverse impacts on native fauna and agriculture, and led to the emergence of this species as a significant reservoir for rabies in Cuba, Grenada, the Dominican Republic (and Haiti by extension) and Puerto Rico. Perpetuation of rabies in mongoose populations on these islands represents a continuing threat to public and animal health. The risk of rabies translocation leading to a broader distribution of mongoose rabies within the Caribbean Basin is probable in the absence of intervention toward rabies elimination. The limitations and advantages of existing and prospective methods of control for rabies in mongooses will be discussed. Strategies with ORV as the central tactic offer the best promise for elimination of rabies in island environments. Conservation of rare species requires consideration in rabies management strategies and may provide an opportunity to work collaboratively toward mutual goals. This overview should provide basic context for the field trip to El Yunque National Forest, Puerto Rico.

ASSESSMENT OF MONGOOSE DENSITY ON PUERTO RICO: PRELIMINARY REPORT

Are R Berentsen, Shylo R Johnson, Pedro F. Quinones, Kurt C VerCauteren

In Puerto Rico, the small Indian mongoose (Herpestes auropunctatus) is the primary rabies vector and comprises 79% of all rabies cases. Currently, a rabies vaccination program for mongooses does not exist in Puerto Rico and no vaccines are yet proven and registered for mongoose. When a vaccine becomes available and if vaccination of mongoose is deemed an appropriate management action, the most likely method will be through the distribution of oral rabies vaccine (ORV) baits. Information about the population density of the target species is necessary for determining the application rate of ORV baits. Our objective is to proactively assess a method of determining mongoose density and apply it in the two primary habitat types on Puerto Rico. We established two study sites at opposite ends of the island: El Yunque National Forest and Cabo Rojo National Wildlife Refuge. We are estimating density using a modified National Rabies Management Program raccoon density protocol and grid-based camera trapping. Additionally, we will collect a sample for DNA testing from each trapped mongoose to evaluate whether mongooses at the two research sites are isolated populations. We started research in April 2011 at El Yunque National Forest,

trapping 47 mongooses; 19 were trapped during the first two days before the development of an effective marking technique and the following six days yielded 28 unique mongooses. Preliminary results conservatively suggest a density of approximately 0.28 mongooses/ha. Our efforts are ongoing and in this presentation we will provide an update of our progress and findings.

ELIMINATION OF HUMAN RABIES TRANSMITTED BY DOGS IN LATIN AMERICA AND CARIBBEAN: ACHIEVEMENTS

Tamayo, Hugo, Vigilato, Marco Antonio Natal, Cosivi Ottorino

In the governing bodies mandates framework from PAHO/WHO, the Inter-American Meeting at Ministerial Level on Health and Agriculture (RIMSA) and the Meeting of Directors of National Programs for Rabies Control in Latin America and Caribbean (REDIPRA), is inscribed the Elimination Program of Human Rabies transmitted by dog in Latin America and Caribbean.

By the year 2010 has been achieved 95% cases reduction in humans and canines. Approximately 350 human cases and 25000 canine in the early 1980s have been reduced to 5 and 268 cases, respectively by 2010.The disease is focused on a few countries, and the promotion of the declaration and countries and territories international recognition of free of rabies, variants 1 and 2, according to the OIE Sanitary Code for the Terrestrial Animals. The program key success is related to the epidemiological surveillance system supported by a network of more than 100 diagnostic laboratories, ease of access and free medical treatment to more than 1,200,000 people are attacked by dogs each year, the high coverage and systematic immunization of approximately 52,000,000 dogs and cats annually, as well as the information training dissemination and community participation. The Region of Latin America and Caribbean is very close to reach the goal of elimination of human rabies transmitted by dog, and in this final level the member countries need to engage their commitment for the Regional Program and continuing the support the activities for the rabies control and elimination programs in its territories.

WILDLIFE RABIES

A RETROSPECTIVE QUANTITATIVE ASSESSMENT OF ORV OF FOXES IN EUROPE BETWEEN 1978 AND 2010

Müller Thomas F, Freuling Conrad M, Selhorst Thomas,Kliemt Anke, Meslin Francois X

Since the first fox oral rabies vaccination (ORV) field trials conducted in Switzerland in 1978 this strategy has become the method of choice for fox rabies control in Europe. During the past 33 years, 24 European countries had or have implemented ORV programs on their territories. As a result, fox rabies has been successfully controlled in 7 and 2 (countries of Western and Central Europe, respectively. The number of animal rabies cases in Europe decreased from 18,588 cases in 1980 to 7,589 cases in 2010.
In order to assess ORV programs in Western, Central and Eastern Europe we collected data for each country for the time period 1978-2010 on (i) size and location of vaccination areas, (ii) oral rabies vaccine virus strain used, (iii) bait density used, and (iv) modalities of bait distribution for each campaign. Spatial information data was subsequently digitized and geo-referenced and entered into the European (ArcGIS) database for further analysis. A spatial-temporal analysis revealed that since 1978 the overall size of the area under vaccination steadily increased and reached maxima of 600,000 and 1,100,000 km2 in 1996 and 2007, respectively. The maximum total area ever covered at least once with vaccine baits in Europe between 1978 and 2010 encompassed almost 1,911,900 km2.
A total of 9 different commercial modified live rabies virus vaccines were used in ORV campaigns, e.g. SAD Bern, SAD B19, SAD P5/88, SAG1, SAG2, SAD VA1, V-RG, Vnukovo, and RV_97. The maximum size of the area in which a single rabies virus vaccine strain was ever used during this time period ranged between 25,000 and 1,019,100 km2. The time period needed to control fox rabies using ORV ranged between 5 and 26 years for individual countries and depends on many factors. The influence of (1) the initial epidemiological situation (rabies incidence) at the beginning of ORV campaigns, (2) the size of the affected area and (3) the length of borderline to neighbouring rabies endemic regions on the duration and costs of rabies elimination will be presented. The results obtained can be used to further improve ORV campaigns in Europe.

EPIDEMIOLOGIC STUDY OF RABIES VIRUS IN WILD MAMMALS FROM A RAINFOREST AREA, NORTH COAST OF SÃO PAULO STATE, BRAZIL

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Rabies is a zoonotic viral disease that causes thousands of humans and animals deaths each year worldwide. In Brazil there are two different cycles of the disease. The terrestrial, characterized by the virus variant maintained by dogs is controlled due to

vaccination and animal control campaigns, but the aerial cycle is still a challenge to the disease control. The most important virus variant of the aerial cycle is the one related to the haematophagous bat *Desmodus rotundus*, but variants related to different bat species and other two, one related to wild canines, *Cerdocyon thous* and one related to marmosets, *Callithrix jacchus*, were also identified in our country. The emerging importance of wild animals in rabies epidemiology points out to the necessity of a continuous vigilance to establish adequate strategies of prevention and control of the disease. The use of central nervous system (CNS) and blood samples from several species of wild mammals from a rainforest release area named Acarau Farm, in Bertioga County, north coast of São Paulo State, Brazil, provides an excellent opportunity for the study of rabies epidemiology. The aim of the present study was the identification of rabies virus in CNS samples from animals founded dead in the area with Mouse Inoculation Test (MIT), Fluorescent Antibody Test (FAT) and Reverse transcriptase Polymerase Chain Reaction (RT-PCR) techniques and the detection of rabies virus neutralizing antibodies (VNA) with the simplified fluorescent inhibition microtest for the titration of rabies neutralizing antibodies – SFIMT - technique in sera of animals captured and released in a monitored area. The studied animals belonged to the Didelphimorphia, Primata, Carnivora, Rodentia, Cingulata, Artiodactyla and Pilosa Orders. None of the 57 samples submitted to FAT, MIT and RT-PCR presented positive results; while 52 (33%) out of 158 blood sample presented positive titers (≥ 0,16UI/mL) and 15 (09%) presented titers ≥ 0,5UI/mL (considered as protective titers for humans). Even with the negative results for virus detection, the presence of VNAs indicates virus circulation in wild mammals at the studied area. The presented results provides a valuable information that can be used for the establishment of better strategies of prevention and control of the disease, especially when considering the potential risk of rabies transmission from wild to domestic animals and humans and the lack of epidemiological surveys in Brazilian wild life.

FORECASTING THE SPREAD OF RACCOON RABIES USING A PURPOSE-SPECIFIC GROUP DECISION MAKING PROCESS

Aaron Anderson, Stephanie A. Shwiff, Richard Chipman, Dennis Slate, Charles Rupprecht, Roland Tinline , Forecasting Group

Raccoon rabies is enzootic throughout much of the eastern U.S. Negative impacts in the region result from high rates of human post-exposure prophylaxis use and increased risk to humans, pets, and livestock. USDA Wildlife Services and cooperators have been actively involved in an oral rabies vaccination (ORV) program since the late 1990's that has prevented further westward spread of the disease. To understand the benefits of ORV zones to prevent spread to the west, it is necessary to understand how raccoon rabies would spread in its absence. Thus, the objective of this study was to forecast the spread of raccoon rabies if the zone is not maintained and ceases to exist. To do this, a group decision making process was designed to address the unique nature of the forecasting problem. The process included a combination of several established methods of group decision making and incorporates some of the advantages of each while making concessions to the complexity of the forecast. The forecasting problem is complex because the spread of the disease must be forecast over a 20 year period while accounting for the fact that the rate of spread is influenced by many factors such as land use, rivers, human population density, habitat, and climate. The decision making process was implemented using a group 15 experts and four support personnel at a two day meeting at the USDA National Wildlife Research Center in

March 2011. A consensus forecast was produced that predicts the disease will spread at 15 to 60 km/year and will reach as far as east Texas and western Iowa 20 years after the ORV barrier is removed. Additionally, 10 expansion regions are constructed that describe the spread of the disease in two year time intervals.

USING HOST POPULATION STRUCTURE TO FORECAST THE SPREAD OF RABIES IN NORTHERN OHIO

Samantha M. Wisely, Are R. Berensten, Chadd E. Fitzpatrick, Mike R. Dunbar, Andrew J. Gregory

In the United States, unique rabies strains are maintained in distinct wildlife species, including the raccoon (*Procyon lotor*). Raccoon variant rabies (RVR) has spread throughout the eastern US and is moving west. To combat the westward spread of the disease, oral rabies vaccines (ORV) are distributed along natural barriers that may restrict raccoon movement, such as mountain ranges and rivers. The spread of rabies into Ohio is prevented by an ORV program in the eastern portion of the state and utilizes the mountains of western Pennsylvania; however, in 2004 a rabid raccoon breached the barrier. To better understand and control the spread of rabies into northeast Ohio from Pennsylvania, we used genetic tools and resistance modeling to investigate dispersal dynamics and land use patterns within the urban footprint of northeastern Ohio. These results can be used to forecast the potential spread of RVR and design control and ORV programs. We collected genetic samples from 317 raccoons in 8 counties in and around Cleveland, Ohio. We found high levels of diversity and low levels of genetic structure (global F_{ST} = 0.01) in the population suggesting that this population is large, highly admixed, and mobile. We did not find evidence of sex-biased dispersal, but found spatial structuring of kin groups. Two populations (metropolitan Cleveland and rural outskirts) separated by 100 km exchanged 5 migrants over 1 to 2 generations. Isolation by resistance modeling suggested greenbelts within the urban footprint may provide enhanced dispersal opportunities that maintain genetic diversity, but could be effective targets where ORV distribution could be concentrated. More detailed analysis of population density is warranted to determine the quantity of ORV baits needed to vaccinate a sufficient proportion of the host population.

BAT RABIES

RABIES VARIANTS WITHIN BATS IN THE CENTRAL UNITED STATES, 2007-2011

Rolan Davis, Mike Moore, Cathleen Hanlon

Rabies isolates from several central states were collected by the Rabies Diagnostic Laboratory at Kansas State University. Genetic sequences from over 250 bats collected during 2007-2011 were generated and analyzed. Isolates were assigned to variant according to convention taken from Streicker, et al. (2010) and mapped to their site of origin. Distinct ranges were discovered for several of the variants when multiple clades of rabies virus are defined within the same species (E. fuscus and L. borealis). Data was mined from GenBank within the National Center for Biotechnology Information to find locations of other similar viruses for each clade. Utilizing these locations, along with species ranges we have attempted to determine ranges for each unique virus found within mid-western bats. Continued advancement of rabies diagnostic laboratories skills at variant analysis and sharing of data will continue to add to our knowledge of bat associated rabies variants.

BOKELOH BAT LYSSAVIRUS - PATHOGENICITY AND VACCINE CROSS-NEUTRALIZATION

Conrad Freuling, Jens P. Teifke, Stefan Finke, Claudius Malerczyk, Thomas Müller

Recently, a novel lyssavirus designated Bokeloh bat lyssavirus (BBLV) was isolated from a rabid Natterer's bat (Myotis nattererii). Antigenic and molecular characterizations suggest that this distinct virus belongs to phylogroup I of the lyssaviruses (Freuling et al., 2011). Since the Natterer's bat is a widespread and abundant bat species in Europe the public health importance of this new virus needs to be elucidated. To assess the pathogenicity of BBLV in comparison with the two other European Bat Lyssaviruses (EBLV-1 and 2), 3-week old mice were inoculated at different doses and routes of infection and the mortality of infected mice was compared.

In a second part, using a panel of human sera (N=100) from individuals previously vaccinated with PCECV, individual VNA concentrations against BBLV were measured with a modified RFFIT. Briefly, instead of CVS other viruses, i.e. BBLV but also DUVV, LBV, and MOKV were used as testvirus as done previously with ABLV, EBLV-1 and 2. (Malerczyk et al., 2009). As expected, sera neutralized viruses of the phylogroup I, including BBLV whereas in general no neutralizing activity was measured against members of phylogroup II. Interestingly, though, single individuals showed relevant VNA concentrations against MOKV. These results suggest that

vaccination with PCECV leads to adequate VNA concentrations against the novel BBLV, and also DUVV. This confirms the cross-neutralization ability of PCECV in humans after vaccination in a PEP regimen against other non-classical lyssaviruses of phylogroup I (ABLV, EBLV-1, and EBLV-2, Malerczyk et al., 2009) and thus provides relevant information for risk mitigating measures.

VAMPIRE BATS DVD: MÉXICO, UNITED STATES OF AMÉRICA, BRAZIL

María Dolores Manzano, Roberto Ramírez Hernández, Luis Leucona, Silvana Regina Favoretto.

Introduction: Vampire bats are represented by three species found only in Latin America. Desmodus rotundus is the most common and studied, feed mainly on the blood of mammals. Diphylla ecaudata and Diaemus youngi are less frequent and feed on blood of birds.The common vampire bat Desmodus rotundus was the most favored by the introduction of livestock in Latin America would represent not only an economic problem, but also public health because humans are usually used as food source in several places such as Amazonian region. The Pan American Health Organization says that the trend of rabies transmitted by vampire bats in Latin countries is increasing on a recurring basis, which is a difficult challenge to solve. Therefore, experts in bat and rabies from Mexico, Brazil and United States pool their knowledge and experience to the development of a scientific-educational DVD on vampire bats species. The filming set in Mexico received Best Cinematography Award at the film festival "The Dark Carnival Film" in Bloomington, Indiana. Objectives: Combine the experience of experts in vampire bat rabies in three countries for the production of a DVD and provide tools for better understanding of these bats and bring better marketing and control of rabies. Methodology: The filming in Mexico was conducted by industry-renowned high-tech production of 3D movies Pasmore Lab in coordination with the CFSAEPUE, Mexico and APHIS-USDA, USA and the filming set in Brazil was conduced by Dr. Uieda team. Additional cooperation to the project was done by BUAP and UNAM from Mexico and USP, UNESP and CCZ-SP from Brazil. Results: The present DVD describes the anatomy, habitat, reproduction, feeding, behavior, social organization and population structure of vampire bats. This DVD would be more a tool to infer the behavior and distribution of rabies at the regional level, applied to any American countries. It also seeks to engage with the DVD in the dissemination of the International Year of bats, giving educational and scientific approach to the issue of bats and rabies.

EXPERIMENTAL RABIES VIRUS INOCULATION IN WILD AND CAPTIVE BORN BATS

April D Davis, Jodie Jarvis, Craig Pouliott, Richard A. Bowen, Paul Gordy, Robert J. Rudd

The study of rabies virus in bats can be challenging due to quarantine requirements, husbandry concerns, genetic differences among animals, and lack of medical history. To date, all rabies virus (RV) studies in bats have been performed in wild caught animals. Determining the RV exposure history of a wild caught bat based on the presence or absence of viral neutralizing antibodies (VNA) may be misleading. Previous studies have demonstrated that the presence of VNA following natural or experimental inoculation is often ephemeral. With this knowledge, it is difficult to determine if a seronegative, wild caught bat has been previously exposed to rabies. The influence of prior rabies exposure in healthy, wild caught bats is unknown. To compare the pathogenesis of RV infection between wild caught bats and bats born in captivity (naïve bats), wild caught and naïve bats were inoculated intramuscularly with one of two Eptesicus fuscus rabies virus variants, EfV1 or EfV2. To determine the host response to

a heterologous RV, a separate group of naïve bats were inoculated with a Lasionycteris noctivagans rabies virus variant (LnV). Six months following the first inoculation, all bats were challenged with EfV2. Our results indicate similar susceptibility of naïve and wild caught bats to intramuscular RV inoculation. Additionally, naïve bats inoculated with the LnV demonstrated the lowest clinical infection rate of all groups. However, primary inoculation with EfV1 and LnV did not appear to be protective against a challenge with the more pathogenic EfV2.

VIRUS DE LA RABIA EN MURCIÉLAGOS INSECTÍVOROS DE MONTERREY, MÉXICO

Efren Jaramillo Reyna, Jesús Zacarías Villarreal Pérez, Edgar Iván Galindo Galindo, Ma. Isabel Tavitas Aguilar, Pilar Carranza Rosales

En México se han registrado 154 especies de murciélagos, de estas, en Nuevo León se distribuyen 37 especies, entre insectívoros, frugívoros, hematófagos y polinívoros. El murciélago más común en Nuevo León es Tadarida brasiliensis mexicana, es insectívoro y es el más pequeño de los murciélagos de cola libre. Se distribuye en tres zonas fisiográficas del estado de Nuevo León, donde se han localizado grandes colonias de murciélagos en la cueva La Boca en Santiago y en cueva el Tío Bartolo en Santa Catarina. Existen otras especies de murciélagos insectívoros que son útiles en el mantenimiento del equilibrio ecológico, ayudan a la diseminación de las semillas y se alimentan de insectos que dañan la agricultura. Sin embargo, en 1984 se detectó el virus rábico en la subespecie Tadarida brasiliensis mexicana y en 1998 se reportó el primer deceso de rabia humana transmitida por murciélagos insectívoros. Estudios moleculares realizados en el Centro de Control y Prevención de Enfermedades (CDC), han determinado que la especie transmisora fue Tadarida brasiliensis mexicana. A partir de esa fecha, se han estado vigilando colonias de murciélagos que se establecieron en edificios, árboles huecos, palmas, techos de fábricas y casas abandonadas de la ciudad de Monterrey, NL, México y algunos municipios colindantes. Por lo anterior, el objetivo del presente trabajo fue determinar las especies de murciélagos existentes y sus variantes antigénicas. Utilizando las técnicas de inmunofluorescencia directa y caracterización antigénica se analizaron 33 murciélagos. Los resultados para la identificación de las especies correspondió a 24 Tadarida brasiliensis mexicana, 2 Nycticeius humeralis, un Myotis sp, Lasiurus ega, Eumops perotis, Antrozous pallidus, Corynorhinus townsendii, Leptonycteris nivalis y Lasiurus cinereus respectivamente. Los resultados para el diagnóstico del virus de la rabia fueron sorprendentes: 7 murciélagos resultaron positivos y posteriormente se les realizó la caract

AN OUTBREAK OF RABIES IN INSECTIVOROUS BATS IN CAICÓ, RIO GRANDE DO NORTE, BRAZIL, 2010

Maria Luiza Carrieri, Helvecio Evandro de Castro Martins, Luiz Umberto Sales,
Rafael de Novaes Oliveira, Carla Isabel Macedo, Ivanete Kotait
José Eduardo Ungár de Sá

Following the reduction in the number of cases of canine and feline rabies in the last decade, the Southeastern region of Brazil has started an epidemiologic surveillance program for urban bat rabies. This has identified an increasing number of cases in insectivorous bats of various species in São Paulo. Bat rabies was rarely diagnosed in the states of Northeastern Brazil, where canine and feline rabies is still present. Rabies-positive insectivorous bats from Molossus spp. began to be identified in 2010 in the municipality of Caicó, in the south of the state of Rio Grande do Norte (RN), by the Central Public Health Laboratory (LACEN) in that state using direct immunofluorescence and viral isolation in mice. The municipality has a population of 60,000 inhabitants and the primary industry is ore mining. In 2010, 42 positive cases distributed across different neighborhoods in the town were identified among the 204 samples analyzed (a positivity rate of 20%). The viral isolates were sent to the Pasteur Institute of São Paulo for antigenic and genetic analysis. The samples were analyzed by RT-PCR for the viral nucleoprotein (21G-304 primers) and after DNA sequencing generated viable 791 bp sequences (nt 203 to nt 993 of the viral genome) corresponding to 263 amino acids (aa 45 to 307 of the viral nucleoprotein).

The results of the antigen tests revealed a variant with a reaction pattern different from those defined by the CDC panel of monoclonal antibodies. Phylogenetic analysis revealed that most of the isolates formed a single cluster with a mean intra-species identity of 99.6% for nucleotides and 99.8% for amino acids, varying between 99.3% and 100%, and 99.2% and 100%, respectively. The interspecies identities calculated for the other clusters on the phylogenetic tree and the amino acid substitution analysis for the region analyzed indicate that this cluster is phylogenetically close to the lineage for which insectivorous bats from species Nyctinomops laticaudatus are the reservoir in the state of São Paulo.

RABIES SPATIAL MODELING & ECONOMIC MODELS

FACTORS INFLUENCING THE SUCCESS OF VACCINATION FOR CONTROLLING CANINE RABIES

Katie Hampson, Sunny Townsend, Tiziana Lembo, Sarah Cleaveland, Dan Haydon

Background: Considerable success has been achieved in the control of rabies through the mass vaccination of domestic dogs. However, there are no practical guidelines on how to strategically roll out vaccination campaigns and we know little about what key operational aspects either compromise or consolidate the success of canine rabies vaccination programmes. Methods: here we draw on detailed epidemiological data to inform models of rabies spread. Using a stochastic, spatially-explicit simulation we explore the effectiveness of alternative mass dog vaccination strategies that incorporate different spatial and temporal allocations of vaccination effort. We further analyze the impacts of ongoing vaccination efforts in Northern Tanzania to show how these modeling insights translate into practical scenarios. Results: we show that several important factors should be considered in the design and implementation of canine rabies control programmes. Specifically, we demonstrate how human-mediated dog transport can compromise control efforts, how omission of small portions of the population can jeopardize the success of vaccination programs, and how population isolation affects the likelihood of achieving and maintaining freedom from disease. We discuss how the time horizon, geographic scope and spatial roll out of control programs should be considered to improve the chances of success. Conclusion: our findings offer practical guidance on how to allocate vaccination effort most effectively to have the greatest impact on controlling rabies in a wide variety of settings.

POTENTIAL FOR RABIES CONTROL BY DOG VACCINATION IN TANZANIA

Meagan C Fitzpatrick, Stephen Davis, Katie Hampson, Sarah Cleaveland, Lauren Ancel Meyers, Jeffrey P. Townsend, Alison P. Galvani

Canine vaccination has been successful in controlling rabies in diverse settings worldwide. However, doubts remain regarding the potential effectiveness of vaccinating only domestic dogs in systems where transmission occurs both between and within domestic dogs and other carnivore hosts. To evaluate control

strategies targeted at domestic dogs when secondary species also contribute to transmission, we applied a next-generation matrix model. The matrix elements were sampled based on contract-tracing data from two districts in northwest Tanzania, Ngorongoro and Serengeti. We estimated transmission rates between and within different host types, calculated corresponding values of R0, and determined, for policy purposes, the probabilities that various annual vaccination targets would control the disease. Transition rate estimates and corresponding probabilities of vaccination-based control indicate that rabies transmission in this region is indeed driven by transmission within domestic dogs. Based on our calculations, annual canine vaccination at the 70% level recommended by the WHO would be more than sufficient to control rabies in Serengeti District. In Ngorongoro District, this coverage level would only be adequate with a probability of 85%. We also demonstrate that the removal of rabid dogs may contribute substantially to successful rabies control.

INFLUENCE OF HABITAT STRUCTURE ON VACCINATION EFFECTIVENESS OF RACCOON RABIES

Erin E. Rees, Bruce A. Pond, Rowland R. Tinline, Denise Bélanger

Few studies have examined how the texture (grain) and configuration of the habitat at finer scales affect infectious wildlife disease control. We explored these questions through stochastic simulation using the Ontario Rabies Model (ORM). The use of the ORM on a high performance computing network (Réseau québécois de calcul de haute performance; http://rqchp.qc.ca) enabled us to run many thousands of experimental trials to explore interactions between multiple landscape grain and configuration combinations and vaccination barriers with varying widths and immunity levels. Our results show that numbers of rabies cases and breaches of the vaccination barrier respond to population immunity, landscape texture and configuration and interactions among these main effects. Low levels of population immunity are more effective in coarser than finer grained landscapes, but this trend is reversed at higher levels of immunity. Disease breaches of the vaccination barrier are less likely in coarser grained landscapes at lower levels of population immunity; however, these landscapes have the most breaches at the highest levels of immunity. Increasing barrier width decreases the likelihood of breaches at a slower rate in the structured landscapes. Furthermore, mid-levels of population immunity can be counterproductive resulting in disease persistence rather than control. The disease dynamics observed in our experiments are analogous to metapopulation dynamics observed in animal populations. This modelling approach has the flexibility and capacity to explore a wide range of questions about infectious wildlife disease control in order to optimize efficacy.

BENEFIT-COST ANALYSIS OF RACCOON ORAL RABIES VACCINATION IN THE UNITED STATES

Stephanie A. Shwiff, Aaron Anderson, Katy N. Kirkpatrick, Tyler W. Cozzens, Kurt C. VerCauteren

Raccoon rabies is endemic throughout much of the eastern U.S. Negative impacts in the region result from high rates of human post-exposure prophylaxis use and increased mortality risk to humans, pets, and livestock. USDA Wildlife Services has been actively involved in an oral rabies vaccination (ORV) zone since the late 1990's that has prevented further westward spread of the disease. Maintaining this ORV zone of immunity is costly, with over 38 million vaccine-laden baits distributed between FY2006 and FY2010. However, the benefits are significant. Kemere et al. 2000 conducted the only previous benefit-cost analysis of the raccoon rabies ORV program and estimated a present value of net benefits of $138 million to $628 million. This study will improve previous estimates by using an updated and more thorough forecast of the disease spread over a twenty year period. Improved assumptions will also be made about post-exposure prophylaxis (PEP) rates and costs by incorporating indirect costs of PEP, such as lost work time, and accounting for the spike in PEP rates that occurs during the first several years of an epizootic. Benefits that are considered include lower costs associated with human PEP, pet and livestock vaccination and mortality, animal testing, and surveillance.

ANIMAL POPULATION CONTROL

DIRECT AND INDIRECT COST ASSOCIATED WITH RACCOON RABIES IN TWO CATTLE HERDS IN HAMPSHIRE COUNTY, WV AND GUERNSEY COUNTY, OH USA.

Richard B. Chipman, Rita Biswas, Tyler Cozzens, Stephanie Shwiff, Charles Rupprecht, Dennis Slate

Diseases transmitted among humans, domestic animals and wildlife impact public health and agriculture. Direct and indirect costs associated with wildlife rabies in livestock are not well documented. Moreover, a better understanding of the economic impacts of rabies on livestock in relation to the high annual reported public health costs associated with rabies in the U.S. is warranted. Cattle and calf production is an important agricultural industry in the U.S., involving 92.6 million head with a value of production of $37 billion dollars. From 1990-2008, 2,240 rabid cattle were reported to the CDC from 49 U.S. states and Puerto Rico, a mean of 118 rabid cattle per year. A single rabies incident in a cattle herd can result in substantial costs that are borne by cattle producers and the public in the form of government agencies response to prevent human disease, ensure food safety and protect agricultural interests. To better understand the economic impact of raccoon rabies to cattle operations, an ex post cost analysis was conducted to estimate the total direct and indirect costs associated with livestock operations in Hampshire County, West Virginia in 2008 and Guernsey County, Ohio 2010. Seven cost variables were used to characterize the economic impact in these cattle herds, including: salary and benefits for personnel involved in the case; human post-exposure prophylaxis and indirect patient costs; rabies diagnostics testing; cattle carcass disposal; market value of euthanized cattle and enhanced rabies surveillance. Data were collected for each incident through phone and email interviews with 16 federal, state and county agency personnel involved in these case investigations and coordinated responses. Estimates of direct costs were based on agency records and other relevant data gleaned from informal notes and reports made by agency staff at the time of the incident and the primary literature. Primary cost drivers for these cases were market value of euthanized cattle ($12,561 in OH; $51,461 in WV), human post exposure prophylaxis ($16,446 in OH; $25,837in WV) and government personnel costs ($5,452 in OH; $19,791 in WV). These incidents are noteworthy because they are the first cases in which the costs associated with raccoon rabies in cattle are well documented. Moreover, the results of this analysis provide a basis to help inform a broader, more detailed economic analysis of the impacts of wildlife rabies in the U.S. and the benefits and costs

of oral rabies vaccination to prevent the spread of wildlife rabies.

RECOMBINANT RABIES VIRUS ERAG3P/2GNRH AS AN IMMUNOCONTRACEPTIVE IN EITHER ATTENUATED OR INACTIVATED FORMULATIONS

Xianfu Wu, Todd Smith, Richard Franka, Charles E. Rupprecht

Live attenuated recombinant rabies virus ERAg3p/2GnRH, expressing 2 in-tandem copies of gonadotropin-releasing hormone (GnRH) in a rearranged ERA backbone, induced 100% protection against rabies and 80% immunocontraception in a mouse model. The ERA glycoprotein (G) genes with 3 to 20 copies of GnRH were constructed, but only G/3GnRH and G/4GnRH constructs expressed detectable GnRH by indirect fluorescent antibody staining (IFA). However, the attempts to recover ERAg3p/3GnRH and ERAg3p/4GnRH were unsuccessful. Thereafter, the ERAg3p/2GnRH at 107 focus forming units, in either live or inactivated (by gamma-irradiation) formulations, was inoculated intramuscularly into two-month-old CBA/CaJ mice. The animals received 2 additional boosters at 3-week intervals. Two weeks after the last boost, immunized mice were matched with a treated opposite gender mice for breeding. In the control group without any treatment, 15/15 paired mice became pregnant within 3 to 4 weeks. In the live ERAg3p/2GnRH immunized group, 1/14 (9%) paired mice became pregnant. In the inactivated ERAg3p/2GnRH immunized group, 2/10 (20%) of paired mice became pregnant. Mice that did not become pregnant, 13/14 in the live virus immunized group and 8/10 in the inactivated virus immunized group, remained infertile during the observation period. Overall, more weight gain was observed in the immunized male mice than the immunized female mice, but did not represent a significant difference (P=1.00). To date, the ERAg3p/2GnRH worked as well as an immunocontraceptive using either live or inactivated virus formulations in mice, and should be a potential vaccine candidate for both rabies and immunocontraception studies in dogs in the near future.

A MULTIVARIATE ASSESSMENT OF ORAL VACCINATION CAMPAIGNS WITH ONRAB® BAITS FOR THE CONTROL OF RABIES IN RACCOONS AND STRIPED SKUNKS IN SOUTHERN QUÉBEC, CANADA

Julien Mainguy, Erin Rees, Pierre Canac-Marquis, Denise Bélanger, Christine Fehlner-Gardiner

Background: A retrospective analysis is used to evaluate the success of oral rabies vaccination (ORV) campaigns with ONRAB vaccine baits against the raccoon variant of rabies in southern Québec, Canada. The objectives were to estimate rabies seroprevalence in raccoons (Procyon lotor) and striped skunks (Mephitis mephitis), and to identify factors influencing seropositivity at the individual level in these species, following the aerial and hand distribution of ONRAB baits from 2007 to 2009.
Methods: Five to seven weeks after baiting, raccoons and striped skunks were live-captured, blood-sampled, sexed and aged. Serological responses were determined by a competitive enzyme-linked immunosorbent

assay (cELISA). The probability of seropositivity was examined using a generalized linear model given individual characteristics, vaccine bait densities, estimated raccoon density, number of past ORV campaigns and landscape factors. The age structure of the captured animals was also assessed. Results: The annual percentage of animals considered positive for rabies antibodies after the aerial distribution of 43 to 155 ONRAB baits/km2 varied between 35 and 56 % in raccoons, and 11 and 17 % in striped skunks. Seropositivity in raccoons was positively associated with vaccine bait density, number of previous ORV campaigns and age. Conversely, seropositivity was negatively associated with raccoon density and the proportion of residential areas found near the raccoon capture location. None of the variables examined explained variation in the seropositivity of striped skunks. The annual percentages of juveniles live-captured were 45 ± 3 (SE) % for raccoons and 66 ± 2 (SE) % for striped skunks. Conclusions: Results indicate that the oral vaccination strategy using ONRAB baits in southern Québec is an appropriate tool to immunize raccoon populations against rabies, but apparently not striped skunk populations. The serological results obtained indicate that bait densities should be adjusted to account for variations in raccoon density and the presence of residential areas, in an effort to create an effective immunological barrier. The high percentages of juveniles in both species also suggest that ORV campaigns should be conducted at least annually to account for the recruitment of naïve individuals in the populations.

IDENTIFICATION OF ADVERSE EFFECTS CAPTIVE DOGS IN MEXICO FROM SIMULTANEOUSLY INTRAMUSCULAR ADMINISTERED INJECTION AND RABIES VACCINE

Fernando Vargas-Pino, Verónica Gutiérrez-Cedillo, Jorge Felipe Islas-Fuentes, Ana María Tavares-Jiménez, Erick José Canales-Vargas, Luis René Gress-Ortega, Lowell A. Miller, Kathleen A. Fagerstone, Charles E. Rupprecht, Dennis Slate, Scott Bender, José Manuel Méndez García , Patricia García-Reyna, Juan Ocampo-López, Luis Lecuona.

Mexico serves as global model for achieving important advances in rabies control in dogs. The Mexican Ministry of Health applies approximately 16 million doses of parenteral canine rabies vaccine each year for dog rabies control. The reduction in dog rabies cases reported during the past 20 years demonstrates the effectiveness of these campaigns in protecting against rabies. A collateral effect of rabies protection is dog overpopulation. Taking this into consideration, it is necessary to enhance public awareness to reinforce the role dog owners play in the reduction of the risks associated with dog overpopulation to human and animal health. An initiative of mutual interest is to conduct a captive dog trial in Mexico to test the immunocontraceptive vaccine GonaCon™ developed (initially for Cervids) by WS, National Wildlife Research Center (NWRC). This product has been tested in captive free roaming dogs on the Navajo Nation, Chinle, Arizona. The Mexican Ministry of Health and USDA/APHIS/WS, with the technical support of the Rabies Section of the CDC, collaborated on an experimental design to test GonaCon™ in captive dogs in Mexico. This trial was conducted in Hidalgo State with the participation of the State Health Services as well as the local institutions and organizations during 2010-2011. This paper presents the preliminary results of this trial. Three groups of 6 female dogs were used in this study. The first group received rabies vaccine, the second GonaCon™ and the last group received GonaCon™ and rabies vaccine. All vaccine was delivered by IM injection. All animals were placed under observation and evaluated clinically during 61 day period. Blood and serum samples for CBC, BCP, VNA, THR and GnRH were obtained on days 0, 31 and 61. Results of the medical and clinical evolution of the animals, as well as the results of the CBC, BCP, VNA, THR and GNRH measurements and

comparisons on D0, D31 and D61 will be presented. The preliminary conclusions show that adverse effects of Gonacon™ were less frequent and in lower intensity than reported in previous dog studies. The immune responses to the rabies vaccine and GonaCon™ were not limited by the simultaneous use of these products. Also, some macro and microscopic lesions will be presented that are consistent with findings in previous studies in dogs that received GonaCon™.

COMMUNICATIONS

METODOLOGÍAS PARTICIPATIVAS EN LA PREVENCIÓN DE MORDEDURAS POR MURCIÉLAGOS EN ESCOLARES AGUARUNAS

José Luis Daza Arévalo, Víctor Luis Osorio Pretel, Liliana Reátegui Angulo, Yamina Chunga Nunton

Objetivo.- Diseñar y ejecutar metodologías participativas en el sector educación como una de las formas de mejorar la prevención de mordeduras de murciélagos en los niños y niñas en edad escolar de las comunidades nativas. Material y Métodos.- el estudio comprendió las fases de identificación de aliados estratégicos, elaboración de documentos de gestión educativos, elaboración de guía del maestro, medición del nivel de conocimientos, actitudes y prácticas mediante la aplicación de encuestas semi estructuradas a la población. La segunda fase se inició con el uso por parte de los docentes del nivel primario de la guía de enseñanza, se continuo con las labores de abogacía y acuerdos por parte de los padres de familia para adoptar en forma voluntaria y bajo su propia responsabilidad algún un método de barrera física para la protección de sus menores hijos ante las mordeduras de murciélagos, en toda la fase del proyecto se registró los casos de mordeduras, estos datos se utilizó para calcular las tasas de incidencia, indicador que evaluó la efectividad de la intervención. Resultados.- el estudio mostró que el uso de las metodologías participativas de forma conjunta entre los sectores de educación y salud, si influyen en los padres de familia de las comunidades nativas y contribuyen a mejorar la prevención de las mordeduras en los niños y niñas de las comunidades nativas en edad escolar.

AREB: 7 YEARS OF COLLABORATIVE WORK TO IMPROVE RABIES AWARENESS, PREVENTION AND CONTROL IN ASIA

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Established in 2004, the Asian Rabies Expert Bureau (AREB) is an informal network of experts in rabies. At the outset, 9 countries formed the network. In 2010, experts from 3 additional Asian countries joined AREB bringing the overall total representation to twelve countries: Bangladesh – Cambodia – China - India – Indonesia – Laos – Myanmar - Pakistan – Philippines – Sri Lanka – Thailand – Viet Nam.

AREB members meet annually to review the state of the art in rabies prophylaxis and management, define the best clinical management practices in local situations and discuss how to increase rabies awareness and improve access to quality immunobiologicals (rabies vaccine and immunoglobulin). During their last meeting in Goa (India) in November 2010, AREB acknowledged the recent switch from nervous tissue to modern cell culture rabies vaccines in Bangladesh (2010), and the progress in rabies control in the Philippines. They reviewed the rabies situation in Bali, Indonesia, and in the countries that recently joined AREB (Cambodia, Laos and Myanmar). They also discussed the risks of and limitations to a non-strict observance of WHO-recommended protocols for post-exposure prophylaxis (PEP). Finally, they reviewed the national guidelines for rabies prophylaxis and administration of cell culture rabies vaccines and the PEP options

best adapted to a given rabies situation. For intramuscular (IM) PEP vaccination, due to difficulties in determining the immunocompetence of patients in Asian countries, AREB still recommends the use of either the complete 5-dose Essen regimen (rather than the shortened 4-dose Essen regimen), or the 4-dose (Zagreb) regimen. In order to reduce the total quantity of vaccine administered per patient, intradermal (ID) rabies vaccination regimens have been introduced in several Asian countries as an alternative to the reference-standard IM administration with the result that more people will have access to modern cell culture vaccines for PEP. In order to ensure the protection of all patients, countries that have used ID vaccination for over a decade have defined a minimum vaccine potency (antigen amount) per ID dose. AREB members reiterate their concern that the ID vaccine dose should be pharmaceutically defined by its potency, as is the case for the IM vaccine dose. A meta-analysis published in Human Vaccines in 2010 confirms that an antigen content of ≥0.50 IU per ID dose is efficacious. The next AREB meeting is going to be held in Bangkok, Thailand in November 2011.

IMPLEMENTING GLOBAL RABIES PREVENTION THROUGH INNOVATIVE PROGRAMS

Louise Taylor, Peter Costa, Deborah Briggs

Five years ago the Global Alliance for Rabies Control (GARC) was established to find new solutions to the ancient neglected disease of rabies, against a background where funding for major rabies prevention projects was difficult if not impossible to secure.

The strategy that GARC used was first to build global awareness of the issues, utilizing electronic communications to establish a global network which included everyone (from individuals to international organizations) with an interest in improving rabies control efforts in their communities and worldwide. This network promotes and supports local champions and small organizations to implement rabies control programmes in their own communities. By providing information and materials necessary to educate the general public, medical and veterinary professionals about rabies prevention and control, we seek to reduce the impact of rabies in all communities. Through initiatives such as World Rabies Day we can demonstrate how much global commitment there is to rabies control. Next, GARC successfully brought together international stakeholders, under the umbrella of the Partners for Rabies Prevention, to pool ideas, resources and advocacy to have a larger impact on global rabies control policy. One of this group's outputs was the online Blueprint for Canine Rabies Control, available free to anyone needing technical advice on canine rabies control projects. A reassessment of the global burden of rabies is now underway as a vital part of gathering evidence of how many avoidable deaths still occur due to rabies.

Another part of GARC's strategy has been to help fund initiatives to demonstrate the value of educational and canine vaccination programmes in practice, keeping sustainability in mind. Over the last 5 years, GARC has made considerable progress towards our mission of eliminating human deaths from rabies, and relieving the burden of rabies in animals. As a result of

World Rabies Day, and other GARC initiatives, millions of people worldwide are more aware of the risks of rabies, millions of animals have been vaccinated, communities have been empowered to start rabies control projects, and several countries have instituted or re-instituted rabies prevention programmes, and rabies has more prominence on the global health agenda.

THE VERMONT RABIES HOTLINE: A COOPERATIVE PROGRAM TO ADDRESS RABIES CONCERNS SPANNING TWO DECADES

Parker Hall, Jamie Recore, Robert Johnson, Mark Scott, Dennis Slate

The public's need for accurate, timely information during wildlife rabies epizootics places increased demands on state and federal health, agriculture and wildlife management agencies. In 1992, in response to public concern over rabies in red foxes (arctic fox rabies virus variant) in northern Vermont and in anticipation of raccoon rabies entering Vermont from the south, the U.S. Department of Agriculture, Animal Plant Health Inspection Service, Wildlife Services program established a toll free rabies hotline. Cooperating agencies included the Vermont Department of Health; the Vermont Agency of Agriculture, Food and Markets; and the Vermont Fish and Wildlife Department. During 1992-2010, 45,529 calls (average of 2,678 calls/ year) were fielded on the Vermont Rabies Hotline and call volume peaked in 1995 at 4,090 calls. From 2001-2009, when referrals to other sources of expertise were tracked, 69% of calls were addressed by the Vermont Rabies Hotline staff, representing a cost-savings to cooperating agencies. The primary rabies related concerns were potential human exposures (12%) and domestic animal exposures to wildlife (8%). All calls related to known or suspected human exposures were referred directly to the Vermont Department of Health, following agreed upon protocol. Although WS continues to assume a greater proportion of costs for staffing and management of the Hotline as the collective funding from the three state cooperators has remained level at $34,600/year, WS remains confident that this one-on-one attention to rabies is a value added public service in VT. Public response to the toll-free Vermont Rabies Hotline strongly suggests that it is an important complement to other rabies prevention and control education efforts. We provide two decades of Vermont Rabies Hotline data trends and make recommendations for others planning to use this model.

AFROREB: 3 YEARS OF COLLABORATIVE WORK TO IMPROVE RABIES AWARENESS, PREVENTION AND CONTROL IN FRANCOPHONE AFRICA

Dr Mathurin Tejiokem, Betty Dodet

The African Rabies Expert Bureau, AfroREB, created in 2008, is an informal group of rabies experts from francophone countries throughout the African continent. AfroREB brings together over 40 scientists from 15 countries in North Africa (Morocco, Algeria, Tunisia) and sub-saharan Africa (Benin, Burkina Faso, Cameroon, Congo, Ivory Coast, Gabon, Madagascar, Mali, Niger, The Central African Republic, Senegal and Togo).

AfroREB members meet biannually to discuss the rabies situation in their region, exchange experience, and identify the most urgent and appropriate initiatives to fight rabies. While rabies is endemic in Africa, accurate data on rabies incidence is scarce in most of AfroREB sub-saharian countries. This lack of data has become a major factor in the low level of political commitment to rabies control. Consequently, ensuring the proper collection of rabies data in their own countries has been identified by AfroREB members as top priority. During their 3rd meeting that was held in May 2011 in Casablanca, Morocco, and in an open letter that was published as a follow-up to the meeting, AfroREB called on Ministries of Health and Agriculture of sub-saharan countries to take drastic measures for rabies control. They urged the Ministries: (a) to implement surveillance of human and animal rabies cases in order to evaluate the rabies burden; (b) to make rabies PEP accessible and affordable to the population through a network of rabies prevention centers with freely available quality immunobiologicals; (c) to implement programmes for dog rabies control; and (d) to set up communication and education programmes to raise rabies awareness in both, healthcare workers and the lay population. They also called for a reinforcement of rabies control programmes in North Africa, as well as increased cooperation between the Ministries of Health, Agriculture and Education of Morocco, Algeria and Tunisia, and a harmonization of their strategies in order to eliminate rabies. As a result of this meeting, AfroREB members from this region are preparing a document on the rabies situation in the region and a common action plan for 2012-2016. As partners of the Global Alliance for Rabies Control, AfroREB members will be the driving forces in the organization of events for the World Rabies Day.

PREVENTING HUMAN RABIES IN INDIA THROUGH COMMUNITY EMPOWERMENT

Sira Abdul Rahman

India has the highest reported number of human Rabies cases in the world with approximately, 20,000 human rabies deaths and 17 million animal bites occurring every year. The situation is further unique in India due to diversity of religion, culture, languages and a large dog population. Rabies is endemic throughout the country except for the islands of Andaman & Nicobar and Lakshadweep. The dog (97%) is the principal vector followed by cats (2 %) and others such as cattle, sheep, goats, horses, pigs, camels and monkeys. India has increasing number of regulations and laws preventing culling of stray dogs which number 28 million, with an equal number of pets. It is often animal welfare organizations supported by Animal Welfare Board of India, through the Municipal corporations, which are involved in vaccination of dogs as a part of catch neutering release programmes. These programmes however are very expensive, costing 20 to 30 dollars per dog and thus prohibitive to implement across India. A novel approach of preventing human rabies through community empowerment was initiated as a pilot project in 2009 in selected villages around Bangalore with help from Global Alliance for Rabies Control, Rabies in Asia Foundation and Commonwealth Veterinary Association. This Adopt a Village program is a new and unique approach to providing a more sustainable and locally adapted and conducted rabies control program. The objectives of the project were to

- Assess the knowledge, attitude and practice (KAP) of people regarding rabies and its prevention and improving awareness of rabies.
- Provide vaccination in study villages and estimation of rabies virus neutralizing antibodies in vaccinated animals and conduct surveillance in animals by dRIT an providing post exposure prophylaxis to all bite victims.

- Determine the cost benefit of educational programme in rabies prevention.
- Ultimately to develop a model for rabies prevention programme that will empower local public health experts around the world to prevent rabies in their own communities. After two years of the programme, successful outcomes include a reduction in incidence of rabies deaths in human and animal population. An increase in the KAP of the population regarding rabies and its prevention in humans and animals and pet care practices.

DEVELOPING AN EFFECTIVE RABIES COMMUNICATIONS PLAN

Peter Costa, Abbigail Tumpey, Tiziana Lembo, Deborah Briggs, Louise Taylor

Communicating health information to at-risk populations is essential for reducing morbidity and mortality and improving our quality of life. To be effective, rabies prevention messages must be communicated to diverse audiences, many of which consist of individuals in the general population and beyond the scientific community. World Rabies Day is a global health communication initiative aimed at raising awareness on rabies prevention and control by educating the world that rabies is 100% preventable, yet continues to kill one person every ten minutes. Through the planned coordination and strategic application of proven evidence based health communication techniques -- such as Diffusion Theory, Health Belief Model and Hierarchy of Effects – a rabies communications plan provides a framework to assist public health practitioners in more effectively assessing the science, defining purpose of the communication, identifying and understanding audiences, developing and testing messages, choosing media and channels for the message, determining the best timing for delivering the message and implementing and evaluating the health promotion program. When properly planned and implemented, a well-structured rabies communications plan can serve as a powerful tool to help increase knowledge and change attitudes and/or behaviors conducive to health. This presentation will serve as a train-the-trainer course for RITA participants to learn how to apply the eight inter-related steps for developing an effective rabies communications strategy as outlined in the Blueprint for Rabies Prevention and Control. These steps are essential to ensuring a well thought-out strategy that can be implemented in any locale and adapted to the local culture.

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