

Wildlife and Domestic Animal Rabies
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OP033

Potential economic benefits of eliminating canine rabies

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Although canine rabies has been eliminated from industrialized countries, infected dogs remain the primary source of human and livestock exposures in Asia, Africa and much of South America. Human deaths are the most important direct economic impact of canine rabies, followed by livestock losses and the cost of PEP, while expenses associated with dog vaccination and control are major indirect impacts. The global burden of rabies disproportionately affects Asia, which experiences more than half of human rabies deaths and approximately 65% of livestock losses, and performs more than 90% of post-exposure prophylaxis (PEP). Africa is second to Asia in terms of human deaths and livestock losses, but administers the least number of PEPs of the three regions. Recent experience in Latin America shows that efforts to reduce human deaths from rabies through expanded dog vaccination and improved access to PEP result in significant monetary savings. The elimination of canine rabies would lead to major economic benefits in developing countries that are often the least capable of dealing with the disease.

OP034

Insight into a Rabid Brain

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Interactions between hosts and pathogens play a crucial role in their adaptation, evolution and persistence. These interactions have been extensively studied in model organisms, yet it is unclear how well they represent mechanisms of disease response in primary vectors in natural settings. In this study we examined host-pathogen interactions in natural host populations exposed to raccoon rabies virus (RRV). RRV is endemic to North America, which results in acute encephalopathies in mammals and is commonly regarded as 100% lethal if untreated; however variable immune responses have been noted in natural reservoirs. In order to further understand variable immune responses to RRV we evaluated, (i) the nature of immune responses triggered in raccoons after infection, and (ii) viral expression and variation to provide insight into factors that may influence RRV virulence. Our results indicate that RRV activated components of the innate immune system, with transcript levels correlated with the presence of the virus. These data indicate that the timing of an immune response is crucial in pathogenesis. Expression patterns of viral genes suggested they are tightly controlled until reaching the central nervous system (CNS), where replication increases significantly. Furthermore, a nucleoprotein mutation was associated with viral challenge outcomes and pathogenicity of RRV. Overall this study enhances our understanding of the immunological factors that contribute to the pathogenesis of RRV in a natural system. Results of this study highlight the need for further studies in primary hosts of rabies, to obtain a thorough understanding of host-pathogen interaction.

OP035

Detection of rabies virus antibodies in free-living jaguars (*Panthera onca*) in the pantanal of Mato Grosso, Brazil

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The proximity to domestic animals has been considered an important cause of disease of wildlife, and has led to recent epidemics in endangered species around the world. In this study, exposure to rabies virus in eleven free-living jaguars (*Panthera onca*) captured from July 2010 to November 2012 in two protected areas in the Pantanal/MT/Brazil, was screened by Simplified Fluorescent Inhibition Microtest (SFIMT) and Rapid Fluorescent Focus Inhibition Test (RFFIT). Serum sample from each jaguar was analyzed twice in different days. Considering the presence of virus neutralizing antibodies (VNA) in samples with titers = 0,10, three jaguars had low positive titers for each test performed, for a frequency of 27.3%, but only a jaguar showed rabies-neutralizing antibodies on both SFIMT and RFFIT (0,19/0,12 and 0,14, respectively). Low titers of VNA have been detected in other species of wild carnivores, including apparently healthy free-living jaguars, suggesting a non-lethal infection. In our study, we could not correlate or presumed the cause of death of a jaguar that showed the highest rabies-neutralizing antibodies and reacted on both tests, therefore it was not possible to infer about the possible effects of the virus in this animal health. Although some species of wild animals are known to serve as rabies reservoirs, nothing is known about wild felids as reservoirs, precluding any conclusion about the role of wild cats in the circulation of the rabies virus. Prevalence in free-living jaguars require further investigations, as this feline specie is protected through an epidemiological peculiarity, the viral strain that occurs in jaguars must be better evaluated and epidemiological characteristics as the way of infection must be determined. Genotypic and phylogenetic analysis of rabies virus that occurs in jaguars could be interesting to clarify issue of rabies in the Brazilian pantanal.

OP036

Positivity and classification of bats submitted for rabies diagnosis at Pasteur Institute over the period from 2007 to 2012

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The bats are a major reservoir of rabies virus and have a relevant importance in the disease transmission. The aim of this study was to evaluate the bat population submitted for rabies diagnosis. Data were analyzed from 18.805 bats received by the diagnostic section of the Pasteur Institute of São Paulo, originated from several counties of the state of São Paulo over the period from 2007 to 2012. These specimens were morphologically classified according to their family, at the time of execution of the rabies diagnosis technique. The central nervous system from these animals was submitted to direct immunofluorescence test and, when viable, to viral isolation. From the total of bats received, 76.92% bats belonged to the Molossidae family, 12.14% to the Phyllostomidae family, 9.52% to the Vespertilionidae family, 0.02% to the Noctilionidae family and 1.37% to a group of bats whose identification was not possible to establish. Regarding positivity, 261 (1.44%) bats were diagnosed positive, 94 (36.01%) were from Vespertilionidae, 87 (33.33%) were from Phyllostomidae, 79 (30.27%) were for Molossidae and 01 (0,38%) was unable to classify. We also observed a total of 197 (1.26%) bats that were not submitted to the diagnosis due to poor preservation of the samples. These results show that Molossidae was the main family received for rabies diagnosis; however, the positivity was higher in the Vespertilionidae family. The dynamic population investigation of the species is necessary in order to promote a better understanding of rabies seasonality in bats. These data reinforce the importance of an active search for suspect animals in order to establish new control strategies of these animals considering the epidemiologic surveillance of rabies and other zoonosis.

OP037

A historical perspective of raccoon rabies control in the United States (1992-2012)

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Public health officials, wildlife biologists, medical and veterinary professionals collaborate to diagnose and prevent the spread of rabies, a fatal yet preventable zoonotic disease. Post-exposure prophylaxis in humans and vaccination of domestic animals create an immune barrier but do not remove the source of rabies infection. Rabies virus survives by transmission in reservoir species such as raccoons, foxes, skunks and bats. By the mid-1990's a raccoon rabies epizootic had spread to all east coast states. Significant increases in rabies cases reported in wildlife and unvaccinated domestic animals created a public health need to address raccoon rabies at its source. In 1992, the first raccoon oral rabies vaccination (ORV) program using RABORAL V-RG® began in Cape May, New Jersey. Additional raccoon rabies control programs followed at the federal, state, and county levels. Coordinated ORV proved successful in preventing the western spread of the epizootic beyond the Appalachian Mountains, while smaller programs demonstrated reduced prevalence of raccoon rabies. From 1992-2012, ORV zones were strategically created across the eastern US landscape. State budgets were reduced or eliminated for ORV during the recent economic downturn in spite of these successes. Federal funds were stretched to try to fill the void. After 20 years, ORV has become accepted to target rabies at its source in wildlife. Important questions remain unanswered to take raccoon rabies management in the U.S. to the next level. Reducing the burden of raccoon rabies provides substantial economic benefit and remains a worthy goal; however, a timeline and cost estimate to eliminate or substantially minimize the threat of raccoon rabies looms important. Selection of field parameters to measure, report, and demonstrate program success are critical to inform the scientific community and governmental decisions-makers if increased financial support of U.S. ORV programs is to be secured to meet these goals.

OP038

Immunogenetic comparisons of Arctic fox across multiple rabies zones in Alaska

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In Alaska, Arctic rabies is endemic in Arctic foxes in the northern and western regions of the state, where each of three viral variants is geographically restricted in its distribution (Arctic-2 is restricted to the Seward Peninsula, Arctic-3 the North Slope, and Arctic-4 to Southwest Alaska). These data contrast with other Arctic regions, where a single variant predominates across a vast landscape. It is presumed that the panmictic genetic structure of Arctic fox best explains the patterns of Arctic fox distribution, however it is unclear if these contrasting patterns of geographically restricted rabies variants in Alaska are reflective of, 1) the genetic structure of Arctic fox in Alaska that differs from other Arctic regions as a matter of specific landscape or habitat preferences, or 2) if the endemic nature of Arctic rabies in Alaska is suggestive of patterns of local adaptation to specific variants of this disease. We used the major histocompatibility complex (MHC Class II DRB exon 2) as a tool to elucidate functional genetic structure for this species to determine if patterns of fox gene flow or local adaptation to rabies best explain the geographic distribution of Arctic rabies. Preliminary data suggest that functional genetic variants are geographically restricted in Alaska, and neutral genetic data are currently being collected to provide context to these results. These data provide insight into the mechanisms of disease spread as a matter of the competing forces of natural selection, genetic drift, and gene flow of the host species and are suggestive of landscape features having an important influence on rabies spread in this region.

OP039

The North American rabies management plan—the first five years and a look toward the future

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The North American Rabies Management Plan (NARMP), signed at RITA XIX, established a collaborative framework to advance rabies management on the North American Continent. The four NARMP cornerstones—information transfer, surveillance, research and control serve as a backdrop to discuss accomplishments and challenges. The NARMP has established an environment for exchange of information on rabies along our borders. Timely information transfer has resulted in coordinated management to prevent re-emergence of raccoon rabies in Quebec and Ontario from the U.S. and canine rabies in Texas from Mexico. Application of a direct rapid immunohistochemistry test (dRIT) for enhanced rabies surveillance by all partners has led to more-informed rabies management decisions. ORV research between the U.S. and Canada led to ONRAB field trials in the U.S. to evaluate this vaccine-bait for use in raccoon rabies elimination. SAG₂ vaccine has also been evaluated in Mexico. Safety and immunogenicity research on the simultaneous administration of the immunocontraceptive GonaCon™ and rabies vaccine in captive dogs has been completed on Navajo lands and in Mexico, with fertility studies warranted. Handout models for oral vaccine delivery have been evaluated on the Navajo Nation, advancing our understanding of optimal ways to apply this strategy. The formation of Arctic Ecological Group for Infectious Studies has led to an initial study of climate change in relation to rabies dynamics in foxes and public and animal health in the Arctic. The potential to control mongoose rabies in the Caribbean has gained renewed energy and is being seriously explored. The first five years of NARMP has established a track record against which future progress can be weighed. However, maintaining an open dialog to facilitate the exchange of ideas that lead to meaningful collaborative projects though changing personnel, political and economic environments will be critical for continued success under this living, one-health model.

PP036

Monitoramento de morcego não hematófago e a raiva no espaço urbano - pernambuco - Brasil

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Introdução: A raiva é uma antroponose letal que acomete os mamíferos. A transmissão se dá através da saliva do animal infectado e envolvem nos seus ciclos diferentes grupos de mamíferos entre eles animais domésticos e silvestres. Uma das propostas de monitoramento é o encaminhamento de amostras para análise rábica de diferentes espécies de mamíferos a fim de se estudar a circulação do vírus nos diferentes ciclos (urbano, silvestre, aéreo e rural). O Diagnóstico da Raiva no Estado de Pernambuco- Brasil, é realizado em parceria entre o LANAGRO/PE do Ministério da Agricultura, Pecuária e Abastecimento – MAPA e o Laboratório Central – LACEN/PE. Objetivo: Monitorar a incidência do vírus rábico em área urbana, a partir de amostras enviadas pelos Municípios do Estado de Pernambuco – Brasil. Método: Os animais foram submetidos às análises para Diagnóstico da Raiva na prova de Imunofluorescência Direta e na Prova Biológica para Raiva, onde é feita inoculação intracerebral em camundongos albinos de 21 dias. Resultados: O espaço urbano tem marcante presença de espécies de quirópteros. As amostras com resultados positivos neste espaço, pode caracterizar que a raiva silvestre está cada vez mais próxima da população, visto que, com a iminente prática de desmatamento e, a oferta de alimentos para estes animais está cada vez mais escassa, podemos concluir que o monitoramento de colônias de espécies de morcegos não hematófagos, se faz necessário para o controle de circulação do vírus rábico em áreas urbanas.

PP037

Different clinical manifestations observed in mice inoculated with several rabies virus variants in Southeast Brazil

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Antigenic and genetic studies were realized in 11 samples of the rabies virus isolated from central nervous system samples from bovines, equines and different non-hematophagous bat species from Southeast Brazil. After virus titration through intracerebral mouse inoculation, 20% suspensions with 1000 DL50 were prepared. These suspensions were then inoculated in groups of 10 mice per sample, 0.1mL of the suspension per animal through intra-femoral via. During the observation period important differences were observed in the mice behavior; mainly regarding the incubation period of the different virus samples and in the symptoms observed in the inoculated mice. These observed differences can be suggestive of different pathogeneses caused by different virus variants and the host from where this variant was isolated.

PP038

Phylogeography of the rabies viruses circulating in the recent Italian epidemic and evaluation of the affected fox population dynamics through microsatellite analysis

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Fox rabies re-emerged in North-eastern Italy at the end of 2008 and circulated to early 2011. As with previous rabies epidemics, the Italian cases were due to the epidemiological situation in adjacent affected regions. To obtain a comprehensive picture of the dynamics of the recent Italian epidemic, we performed a detailed evolutionary analysis of RABVs circulating in the recent epidemic and genetically characterised the affected fox population in the North-eastern Italian regions involved by the disease. Sequences were obtained for the hyper-variable region of the nucleoprotein gene, the complete glycoprotein gene, and the intergenic region G-L from 113 selected rabies cases. We also analyzed 21 microsatellite markers from 379 fox samples collected from 2006 to 2011. These were genotyped using GeneMapper4.0 and Micro-Checker; the genetic and spatial characterization of the fox population structure was carried out by using GenAlEx 6.5, Structure 2.3.3 and Geneland 4.0.2. We identified two viral genetic groups, here referred to as Italy-1 and Italy-2. Phylogenetic and phylogeographical analyses revealed that both groups had been circulating in the Western Balkans and Slovenia in previous years and were only later introduced into Italy, further occupying different areas of the Italian affected territories. We examined the spatial structuring and the gene flow of the red fox subpopulations which suggested the presence of four genetically homogeneous groups, referred to as pop-1 to -4. The genetic populations are localised in defined geographic areas, with spread of rabies possibly occurring through fox movement along corridors. Understanding the ecological dynamics of the fox populations, their distribution and putative corridors on the territory may greatly contribute to modelling surveillance and disease monitoring programmes. As well, this would implement control activities better targeted at a local level in case of re-introduction of the disease.

PP039

Canine and Wildlife Enhanced Rabies Prevention in Israel (2009-2013)

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Israel is experiencing a unique public health situation. Wildlife rabies is under control while canine rabies is present in the northern area of the Golan Heights since 2004. Abandoned or feral dogs entering Israel from areas impacted by political instability are the source of the rabies virus. From 2009-2012 there were 167 rabies cases in Israel of which 77 (46%) were in dogs in the North while the vast majority of the Israel territory (90%) remains rabies free. The wildlife oral rabies vaccination program is successful as evidenced by only one rabid red fox and two golden jackals detected at the borders in 2012. This transition from fox-mediated rabies to dog-mediated rabies is of great concern to Israeli public health officials. There are 400,000 registered and vaccinated dogs in Israel of which 320,000 receive an annual booster resulting in a compliance rate of 80%. In the rural area of the Golan Heights, 3,867 dogs (<1% of dogs in Israel) are registered of which 65% are recorded as receiving an annual booster. Due to numerous rabies cases reported from this rural area a higher level of dog rabies prevention will be implemented. Enhanced rabies prevention includes: enforcement of compulsory pet dog vaccination, increased frequency and density of wildlife oral vaccine bait distribution in this area, and initiation of a cooperative program for wildlife rabies control in the Jordan Valley. Retrospective analysis revealed that non vaccinated dogs or <1 year of age and receiving one rabies vaccine dose are more likely to be at risk for rabies. In northern Israel, free dog rabies vaccination, micro-chipping, and registration by the municipality veterinarian plus door-to-door inventory are expected to further reduce dog rabies cases. The recent canine rabies intrusion in Israel is being addressed by governmental support and community participation.

PP040

Diversity of bats species sent for rabies diagnosis to Pasteur Institute over the period from 2009 to 2012

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Bats found in Brazil are classified in 9 families, 64 genus and 168 species. They are important rabies reservoirs and all species can be infected by and transmit rabies virus, independently of their specie classification or feeding habits. This work aimed a survey of bats species originated from counties of São Paulo state, which were sent to the diagnostic laboratory of the Pasteur Institute – São Paulo. Between August/2009 to October/2012, 9.109 bats specimens were received and submitted to recommended techniques for rabies diagnosis by World Health Organization. From these specimens, 8.421 (92,45%) were classified in family, genus and specie, by morphological and morphometric parameters using bats identification keys. Furthermore, they also were characterized by feeding habits. The classified bats belonged to 4 families, 27 genus and 52 species. From the total of classified bats, 102 (1,21%) were diagnosed positive for rabies, belonged to 14 different species. According to the feeding habit, the insectivorous bats were the most numerous, totalizing 68 specimens among the positives, predictable fact due to the bigger demographic population of these bats in our environment, which the most prevalent species were *Myotis nigricans*, *Nyctinomops laticaudatus* and *Eptesicus furinalis*. Among the frugivorous, 28 positive specimens were detected as positive for rabies, which 100% belonged to *Artibeus* genus. The hematophagous bats (4) were identified as *Desmodus rotundus*. Nevertheless, it was not possible to identify the specie and the feeding habit of 2 specimens. These results observed in this study emphasize the importance of these species in the circulation of rabies virus in the state of São Paulo. Thus, a deeper knowledge of the chiropterofauna could contribute to improve the actions for epidemiologic surveillance employed in the rabies control.

PP041

Recombinant ERAG3G strain constructed by reverse genetic system induces protective immunity in raccoon dogs

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The raccoon dogs (*Nyctereutes procyonoides koreensis*) are known to play a key role transmitting rabies to domestic animals in Korea. The raccoon dogs were used to evaluate safety and immunogenicity of recombinant ERAG3G strain constructed by reverse genetic system. One group of raccoon dogs (n=4) was inoculated with the ERAG3G strain via oral route and the other group (n=2) was inoculated via intramuscular (IM) route. Safety and immunogenicity of ERAG3G were evaluated using clinical signs and virus neutralizing (VN) antibodies until 28 days after inoculation. All raccoon dogs inoculated with the ERAG3G IM or orally did not show the typical signs of rabies. The VN antibody titer of raccoon dogs inoculated via oral route (7.9 to 41.6 IU/ml) was higher than those of raccoon dogs via IM route (0.87 to 1.51 IU/ml). The ERAG3G strain will be used as effective oral rabies vaccine strain in raccoon dogs.

PP042

Primer aislamiento de virus rabico variante antigenica 3 en un perro de la zona urbana de guayaquil, Ecuador en 2012

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La rabia en Ecuador ha sido un problema endémico que ha provocado desde su aparición en 1942, grandes pérdidas humanas como la que ocurrió en 1996 causando más de 65 fallecimientos humanos y 1250 caninos muertos por rabia, según el Programa Nacional de Control de la Rabia, MSP. En junio del 2012 se presentó el caso de un perro que fue atropellado en una zona urbana de la ciudad de Guayaquil, y después de ser atendido en una Clínica Veterinaria, murió. Como esa mascota agredió al personal de esa clínica, el animal fue trasladado a los Laboratorios de salud animal, INSPI-MSP donde se realizó el diagnóstico de rabia por las técnicas de Inmunofluorescencia Directa y Aislamiento viral por inoculación en ratones lactantes, resultando el mismo positivo a rabia. Como la rabia canina ya está controlada en esta ciudad, y debido a que en los últimos siete años se había producido un alentador silencio epidemiológico del ciclo terrestre, se notificó a las autoridades de salud y se procedió a enviar las muestras a la Universidad de Sao Paulo, Brasil, con la cooperación de la OPS donde fue confirmada la presencia del virus mediante la caracterización antigénica con anticuerpos monoclonales antinucleocapside y secuenciamiento genético, resultando positivo a la Variante Antigénica 3 del virus de la rabia, perteneciente al género *Lyssavirus*, genotipo 1 que corresponde a la transmitida por el murciélago *Desmodus rotundus*. El hallazgo de la Variante Antigénica 3 en una ciudad implica un reto para el control de la rabia en nuestro país, por cuanto se desconoce como fue el mecanismo de transmisión si, murciélago– perro, o murciélago-gato-perro lo que deja abierta la sospecha de si hubo o no otros posibles contactos o la existencia de nichos cercanos a la ciudad donde circulando esta variante.

PP043

A raiva em morcegos no ambiente urbano de pernambuco, Nordeste do Brasil

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Introdução: Os morcegos nos últimos anos têm-se destacado como um dos principais transmissores da raiva humana no Brasil, com inúmeros registros de positividade no ambiente urbano. Este trabalho objetiva averiguar a situação da raiva em morcegos na Região Metropolitana do Recife (RMR), para isso foram levantadas as amostras recebidas para diagnóstico de raiva pelo Laboratório Nacional Agropecuário em Pernambuco (LANAGRO/PE)

provenientes da RMR, entre 2002 e 2011. Métodos: Nesse período o LANAGRO - PE recebeu 412 amostras de morcegos, com 26 positivas, provenientes de onze municípios da RMR. Destas, 180 foram identificados até espécie e/ou família (17 espécies e cinco famílias), destacando-se os Molossidae, com 108 espécimes e três espécies, seguido dos Phyllostomidae (n = 72; 10) e Vespertilionidae (n = 16; 2). Resultados: Sete municípios apresentaram morcegos positivos entre frugívoros, insetívoros e hematófagos e dentre estes positivos, cinco espécies foram identificadas, com *Molossus molossus* (n = 6) apresentando o maior número de casos. Conclusão: Apesar de um crescente aumento no índice de envio de amostras de quirópteros a partir de 2008, outras atividades voltadas para monitoramento desses animais na RMR são pouco desenvolvidas, reforçando a necessidade de ações contínuas de monitoramento das populações das espécies sinantrópicas e capacitação de profissionais para a identificação e manejo dos quirópteros para melhor subsidiar a criação de ações eficientes voltadas para as espécies.

PP044

Paradigm shift in wildlife rabies surveillance to support oral rabies vaccination

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Laboratory-based surveillance is a cornerstone of effective wildlife rabies management. Enhanced rabies surveillance includes the submission and testing of wildlife samples from: road kills, those observed with unprovoked aggressive behavior, those humanely euthanized as part of a local population reduction during an outbreak, and on limited occasions those collected from nuisance wildlife control officers. Enhanced surveillance occurs in proximity to established oral rabies vaccination (ORV) zones, or in related areas where rabies research and management is conducted. Such surveillance, in concert with public health activities, better defines the spatial distribution of specific rabies virus variants. A direct, rapid immunohistochemistry test (dRIT) was developed for rabies diagnosis and allows real time virus detection in the field by use of light microscopy. Wildlife Services (WS) has conducted the dRIT since 2005, through a cooperative program with the Rabies Team at CDC to improve sample turnaround time and reduce the burden of increased samples on the public health system. From 2005-2012, WS tested 57,868 enhanced surveillance samples using the dRIT, representing 82% of USDA-collected surveillance specimens during that period. A total of 1,031 rabid animals were confirmed in 15 states that would not likely have been tested through exposure-based public health surveillance. Enhanced surveillance samples tested by WS using dRIT comprised 6-8% of all rabies surveillance specimens tested in the U.S. annually. Rabid animals comprised 1.8% of all samples tested using the dRIT. This paradigm shift has enabled WS and cooperators to better delineate the distribution of rabies and the predicted direction of disease spread for strategic ORV baiting, as well as establish a system to better evaluate program success by sampling a broader spectrum of suspect rabid animals.

PP045

The evolving history of Texas gray fox rabies contingency actions: Texas Wildlife Services Rabies Responses

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Throughout the history of the Texas gray fox strain management program there have been a number of incidents where cases erupted outside of the Oral Rabies Vaccination (ORV) zone. Beginning in 2006, a significant number of cases were identified at McCamey, TX, followed in 2007 with cases in Rankin and near Balmorhea, TX. The 2006 cases were addressed through public health monitoring followed by ORV in 2007. The cases in 2007 were addressed through localized depopulation, enhanced surveillance and ORV. The history of each contingency, including subsequent year cases is described. Costs for both depopulation and ORV are also presented. Recognizing that local area depopulation may have differing effects on territorial and non-territorial species, the authors present some of the rationale to help decision makers evaluate contingency response.

PP046

Rabies in New Mexico: Recent epizootics and historical perspectives.

Rolan Davis, Kansas State University

Rolan Davis, Gary Roemer, Todd Atwood, Kurt VerCauteren, Adam Aragon, Mike Moore and Cathleen Hanlon.

New Mexico is one of only a hand full of states that deals with more than a single species of terrestrial reservoir of rabies virus infected with multiple viral variants. The southwestern portion of the state is affected by rabies primarily in gray foxes (*Urocyon cinereoargenteus*). The first case in this species was reported in 2007 with a total of 9 cases that year. Phylogenetic analysis of viral isolates shows that this viral variant is an eastern extension of the Arizona Gray Fox variant, which has origins in canine variant rabies. Rabies within striped skunks (*M. mephitis*) has been reported in many regions of the state with the most recent foci of disease centered near Carlsbad, which is located in Eddy County in very southern New Mexico. Phylogenetic analysis of viral isolates shows that this viral variant is classified as South Central Skunk variant (SCSK). Sequence analysis of nucleoprotein (N) gene sequences of gray foxes from Texas and New Mexico along with skunks from New Mexico and other neighboring states will be presented. Variation within each viral variant will be discussed with emphasis on introduction, maintenance and spread of rabies within the state.

PP047

Phylogeographic analysis of rabies virus circulating in bovines in the State of Mato Grosso, Brazil

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In Brazil, rabies is enzootic throughout the entire territory, representing not only a serious public health issue but also an economic concern for livestock losses. Dogs and vampire bats are the principal rabies virus (RABV) transmitters to both humans and livestock. To investigate the genetic diversity and the spatial and evolutionary processes of RABVs circulating in bovines in the State of Mato Grosso (Brazil) we sequenced the partial nucleoprotein (N) gene of 162 rabies positive brain specimens collected between 2007 and 2011. Phylogenetic analysis reveals that all cases in terrestrial animals were caused by viruses of the American Indigenous lineage, closely related to those known as circulating in vampire bats (*Desmodus rotundus*). To determine the spatial dissemination of the RABVs in bovines in Mato Grosso, the sequences of the N gene were grouped into five geographic regions: North, South, West, Central, and East. Our Bayesian phylogeographic analysis, implemented by using the BEAST software, suggests that sequences cluster together according to the geographic origin of the samples and indicates the Northern region (Amazon biome) as the main source of vampire rabies for bovines in the State of Mato Grosso. Indeed, we observed that the gene flows from the Northern area to all the other regions considered in this study. Our investigation confirms the role of vampire bats as RABV reservoirs for domestic herbivores in Brazil. The identification of the forest-covered area of Mato Grosso (Northern area) as the main source of the rabies viruses confirms that the presence of forests/deforestation fronts may act as one of the potential risk factors for the dissemination of vampire bats and the associated rabies infection in Brazil. The identification of these risk factors may help in defining more effective prevention and control measures to minimize the impact of rabies in livestock and related human health issues.

PP048

Current research on rabies at the National Wildlife Research Center of USDA/APHIS/Wildlife Services

Kurt VerCauteren, USDA/APHIS/WS/National Wildlife Research Center

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The goal of the Rabies Project at the National Wildlife Research Center (NWRC) is to provide research support to the National Rabies Management Program (NRMP) to aid in the control of rabies. Research conducted by the Rabies Project improves the overall understanding of rabies and helps the NRMP optimize management by the development of better strategies, methods and tools for controlling infection and spread in wildlife for the benefit of humans, livestock and wildlife. Project research is performed with a broad breadth of interdisciplinary collaborators from other agencies, universities, and the private sector. Studies are conducted in lab, captive animal, and wild settings at NWRC Headquarters and across the country. A current primary focus is on determining methods that will make oral rabies vaccine baits more attractive to the diversity of wildlife reservoir species in the US and Puerto Rico. We are also assisting the NRMP in conducting field trials with ONRAB, a candidate vaccine for potential widespread use in the US that has shown promising results in Canada. Additional research has focused on the ecology of reservoir species and the epizootiology of rabies. In this presentation we will provide updates on current research efforts as well as directions for future project research.

PP049

Novel insights into the genetic composition of attenuated oral rabies vaccines

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Live attenuated oral vaccines have been developed and have been successfully used for the control of wildlife (fox-mediated) rabies in Europe and North America. In the frame of requested assistance for the responsible regulatory authority for marketing authorization in Germany, PEI, a vaccine batch of SAD-B19 (Fuchsoral ©) as well as SAD-Berne as the parent virus, were analysed for their genetic identity. Additionally, vaccine strains that had been studied before using conventional Sanger's sequencing were also included. Next generation sequencing (NGS, Genome Sequencer FLX, 454 Life Sciences) and deep sequencing was applied for the full genomes with a mean depth of 500 reads per position. To analyse the amount of raw data a program was specifically developed in R. Interestingly, both in the vaccine batch and in the parental stock we found a diversity of viral viruses discovered, i.e. at least 5 variants were found in the batch and 8 were found in the parental stock. This unprecedented finding of virus variants was also discovered in other commercially available samples, indicating selection pressure as a driving force behind the process of SAD-vaccines. The fact that vaccine virus strains may contain a composition of various variants opens the floor for discussion, e.g. on their true genetic identity, the effect on the biological properties, and regulatory issues.

PP070

Experimental infection of *Artibeus* spp with rabies virus

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Artibeus spp. bats are abundant in tropical and sub-tropical regions of Mexico and other parts of the world; they are synanthropic as the *Desmodus rotundus* vampire bat. Furthermore, it has been observed that both species may occupy the same roosts. Rabies virus (RABV) variants circulating in *Artibeus* spp. in Mexico are closely related to vampire bat variants. In a study of seroprevalence of rabies antibodies in bats our team found 6 out of 51 positive sera (11.8%) from *Artibeus* spp., suggesting that they have been exposed to RABV. The aim of this work was to study the pathogenesis and distribution of RABV in *Artibeus* spp. We performed an experimental infection

of *Artibeus* spp. with vampire bat isolates (V3). High ($1 \times 10^{5.34}$ FFU) and low (1×10^3 FFU) doses of RABV, inoculated by two routes, were compared. All animals were tested for rabies antibodies before the infection. Only one bat out of 38 died at day 14 post inoculation and was positive to FDA, but no clinical signs were observed. The bats in this experiment do not showed clinical signs of the disease, but high titers of antibodies were determined since 7th day post-inoculation. These data suggest pre-exposure to RABV and fall of antibodies titers below detection levels previous this study and the inoculation could act as a booster. RABV semi-nested PCR was positive in organs such as brain, stomach, liver and heart in animals that did not show clinical signs of disease and were negative by immunofluorescence. These results suggest that previous non-lethal exposure to RABV somehow protected *Artibeus* spp. from rabies infection in this study, independently of doses and inoculation routes, but the epidemiological role of frugivorous species in the maintenance of rabies virus in the wild remains to be studied.